LIVE POLIOVIRUS VACCINES

Papers Presented and Discussions Held at the Second International Conference on Live Poliovirus Vaccines

PAN AMERICAN HEALTH ORGANIZATION
Pan American Sanitary Bureau, Regional Office of the
WORLD HEALTH ORGANIZATION
Washington, D. C.
1960

ACKNOWLEDGMENT

The holding of the Conference and the printing of these proceedings have been made possible through the generous cooperation of the Sister Elizabeth Kenny Foundation, of Minneapolis, Minnesota.

$5.00

SECOND INTERNATIONAL CONFERENCE ON LIVE POLIOVIRUS VACCINES

(Washington, D. C., 6–10 June 1960)

Sponsored by the Pan American Health Organization and the World Health Organization, with the cooperation of the Sister Elizabeth Kenny Foundation

Papers Presented and Discussions Held

Scientific Publication
No. 50

PAN AMERICAN HEALTH ORGANIZATION
Pan American Sanitary Bureau, Regional Office of the
WORLD HEALTH ORGANIZATION
1501 New Hampshire Avenue, N.W.
Washington 6, D.C., U.S.A.
1960

Editorial Note

The present volume contains all the papers delivered by the participants at the Conference, arranged in the order of their presentation, by session, together with the discussions on each paper or group of papers. The transcripts of the discussions have been edited for the sake of brevity and consistency of style; all statements related to the opening or closing of sessions, and other non-substantive remarks, have been deleted in order to condense the presentation as far as possible.

The texts of the papers have been reproduced as submitted by the authors, with a minimum of editorial changes. For the most part, tables and illustrations have been reproduced from the original material submitted by the authors, either in slides or prints. This made possible an early publication of the proceedings but precluded the possibility of standardizing format for tables and nomenclature in all the papers.

Preface

The accumulation of information on the use of attenuated live polioviruses as immunizing agents against poliomyelitis has progressed at such a rapid pace since the PAHO/WHO Conference on this subject in June 1959 that the need for further evaluation of the data became evident.

The Pan American Health Organization and the World Health Organization, again with the cooperation of the Sister Elizabeth Kenny Foundation of Minneapolis, Minnesota, therefore agreed to convene a Second International Conference on Live Poliovirus Vaccines, which was held in Washington, D. C., 6-10 June 1960. The new information presented at this meeting, through formal papers and discussions, by 85 distinguished scientists from 20 nations, is recorded in the following pages.

We wish to express our thanks to all concerned with the preparation of this volume—especially to the participants for promptly correcting the transcripts of their remarks—for their efforts in making possible its early publication. The collaboration of Georgetown University in making available the excellent facilities of its Edmund A. Walsh School of Foreign Service is also gratefully acknowledged.

ABRAHAM HORWITZ
Director
Pan American Sanitary Bureau
Regional Office, World Health Organization

Table of Contents and Program of the Conference

FOURTH SESSION, Tuesday, 7 June 1960, 2:00 p.m.

Chairman:
DR. JAMES H. S. GEAR

TOPIC II. SAFETY. (B) FIELD EVIDENCE OF SAFETY
(*continuation*)

(2) SPREAD OF VIRUS IN THE COMMUNITY

(3) SAFETY IN PREGNANCY AND FOR THE NEWBORN

TOPIC III. EFFICACY. (A) LABORATORY EVIDENCE

(1) ANTIBODY RESPONSE

(a) *Methodology*

(b) *Influence of Age*

FIFTH SESSION, Wednesday, 8 June 1960, 9:00 a.m.

Chairman:
DR. HERMAN E. HILLEBOE

TOPIC III. EFFICACY. (A) LABORATORY EVIDENCE
(*continuation*)

(1) ANTIBODY RESPONSE

(b) *Influence of Age*

List of Participants

Dr. E. Russell ALEXANDER
Chief, Surveillance Section
Epidemiology Branch
Communicable Disease Center
U.S. Public Health Service
Atlanta, Georgia, U.S.A.

Dr. Gaylord W. ANDERSON
Director, School of Public Health
University of Minnesota
Minneapolis, Minnesota, U.S.A.

Dr. Charles ARMSTRONG
Medical Director (ret.), U.S. Public Health Service
Laboratory of Infectious Diseases
National Institutes of Health
Bethesda, Maryland, U.S.A.

Dr. Samuel BARON
Division of Biologics Standards
National Institutes of Health
Bethesda, Maryland, U.S.A.

Dr. Robert N. BARR
Secretary and Executive Officer
Minnesota State Board of Health
Minnesota State Department of Health
Minneapolis, Minnesota, U.S.A.

Dr. Randolph BATSON
Professor of Pediatrics
Vanderbilt University School of Medicine
Nashville, Tennessee, U.S.A.

Dr. Henry BAUER
Director, Division of Medical Laboratories
Minnesota State Department of Health
Minneapolis, Minnesota, U.S.A.

Dr. Joseph A. BELL
Chief, Epidemiological Section
Laboratory of Infectious Diseases
National Institute of Allergy and Infectious Diseases
Bethesda, Maryland, U.S.A.

Dr. Matilda BENYESH-MELNICK
Assistant Professor of Virology and Epidemiology
Baylor University College of Medicine
Houston, Texas, U.S.A.

Dr. David BODIAN
Director, Department of Anatomy
The Johns Hopkins University School of Medicine
Baltimore, Maryland, U.S.A.

Dr. Theodore E. BOYD
Assistant Director of Research
The National Foundation
New York, N.Y., U.S.A.

Dr. Samuel R. BOZEMAN
Director, Biological Laboratories
Pitman-Moore Company
Indianapolis, Indiana, U.S.A.

Sir F. Macfarlane BURNET
Director, Walter and Eliza Hall Institute of Medical Research
Melbourne, Australia

Dr. Victor J. CABASSO
Head, Virus Immunological Research Department
Lederle Laboratories
Pearl River, New York, U.S.A.

Professor Mikhail P. CHUMAKOV
Director, Institute for Poliomyelitis Research
Academy of Medical Sciences
Moscow, USSR

Dr. Ernest T. CONYBEARE
Principal Medical Officer
Ministry of Health
London, England

Dr. Ghislain COURTOIS
Director, Princess Astrid Institute of Tropical Medicine
Leopoldville, Belgian Congo

Dr. Herald R. COX
Director of Virus Research
Lederle Laboratories
American Cyanamid Company
Pearl River, New York, U.S.A.

Dr. James G. CRAWFORD
Charles Pfizer and Company
Terre Haute, Indiana, U.S.A.

Dr. George W. A. DICK
Professor of Microbiology
The Queen's University of Belfast
Belfast, United Kingdom of Great Britain and Northern Ireland

Dr. Renato DULBECCO
Professor of Virology
California Institute of Technology
Pasadena, California, U.S.A.

Dr. Carl M. EKLUND
Rocky Mountain Laboratory
National Institutes of Health
Hamilton, Montana, U.S.A.

Dr. Paul M. ELLWOOD, Jr.
Assistant Medical Director
Sister Elizabeth Kenny Foundation
Minneapolis, Minnesota, U.S.A.

Dr. Juan A. EMBIL
Director, Viral and Rickettsial Department
Municipal Children's Hospital
Havana, Cuba

Dr. George M. ERICKSON
Dade County Health Department
Miami, Florida, U.S.A.

Dr. Martin Eugene FLIPSE
Chief, Section of Preventive Medicine and Public Health
University of Miami School of Medicine
Miami, Florida, U.S.A.

Dr. Sven GARD
Professor of Virus Research
Karolinska Institute
Stockholm, Sweden

Dr. James H. S. GEAR
Director, South African Institute for Medical Research
Johannesburg, Union of South Africa

Dr. Henry M. GELFAND
Chief, Enterovirus Unit
Communicable Disease Center
U. S. Public Health Service
Chamblee, Georgia, U.S.A.

Dr. Natan GOLDBLUM
Director, Virus Laboratory
Ministry of Health
Tel Aviv-Yaffo, Israel

Dr. Eduardo C. GUEVARA
Ministry of Public Health
San José, Costa Rica

Dr. William McD. HAMMON
Head, Department of Epidemiology and Microbiology
Graduate School of Public Health
University of Pittsburgh
Pittsburgh, Pennsylvania, U.S.A.

Dr. Albert V. HARDY
Assistant State Health Officer
Florida State Board of Health
Florida State Department of Health
Jacksonville, Florida, U.S.A.

Dr. Herman E. HILLEBOE
Commissioner of Health
New York State Department of Health
Albany, New York, U.S.A.

Dr. Maurice R. HILLEMAN
Director, Merck Institute for Therapeutic Research
Merck and Company, Inc.
West Point, Pennsylvania, U.S.A.

Dr. Horace L. HODES
Pediatrician-in-Chief
The Mount Sinai Hospital
New York, New York, U.S.A.

Dr. Dorothy M. HORSTMANN
Associate Professor of Preventive Medicine and Pediatrics
Yale University School of Medicine
New Haven, Connecticut, U.S.A.

Dr. George A. JERVIS
Director of Research
Letchworth Hospital
New York State Department of Mental Hygiene
Thiells, New York, U.S.A.

Dr. Anne C. KIMBALL
Chief, Section of Special Laboratory Studies
Minnesota State Department of Health
Minneapolis, Minnesota, U.S.A.

Dr. Ruth L. KIRSCHSTEIN
Division of Biologics Standards
National Institutes of Health
Bethesda, Maryland, U.S.A.

Dr. Masami KITAOKA
Chief, Department of Viral and Rickettsial Diseases
National Institute of Health
Shinagawa-ku, Tokyo, Japan

Dr. Herman KLEINMAN
Chief, Section of Chronic Diseases
Division of Disease Prevention and Control
Minnesota State Department of Health
Minneapolis, Minnesota, U.S.A.

Dr. Hilary KOPROWSKI
Director, The Wistar Institute
Philadelphia, Pennsylvania, U.S.A.

Dr. Saul KRUGMAN
Professor of Pediatrics
New York University School of Medicine
New York, New York, U.S.A.

Dr. Alexander D. LANGMUIR
Chief, Epidemiology Branch
Communicable Disease Center
U.S. Public Health Service
Atlanta, Georgia, U.S.A.

Dr. André J. G. LEBRUN
Director, Marcel Wanson Institute of Hygiene
Leopoldville, Belgian Congo

Dr. Pierre R. LÉPINE
Chief, Virus Research Division
Pasteur Institute
Paris, France

Dr. John O. MacFARLANE
Assistant Director, Biological Development
Eli Lilly and Company
Indianapolis, Indiana, U.S.A.

Dr. Joseph L. MELNICK
Professor of Virology and Epidemiology
Baylor University College of Medicine
Houston, Texas, U.S.A.

Dr. Malcolm H. MERRILL
Director, California State Department of Health
Berkeley, California, U.S.A.

Dr. Arden W. MOYER
Head, Virus Biological Research Department
Lederle Laboratories
American Cyanamid Company
Pearl River, New York, U.S.A.

Dr. Roderick MURRAY
Director, Division of Biologics Standards
National Institutes of Health
Bethesda, Maryland, U.S.A.

Dr. Frederick P. NAGLER
Chief, Virus Laboratories
Department of National Health and Welfare
Ottawa, Ontario, Canada

Dr. Nils C. OKER-BLOM
Professor of Virology
University of Helsinki
Helsinki, Finland

Dr. Joseph S. PAGANO
Research Associate
The Wistar Institute
Philadelphia, Pennsylvania, U.S.A.

Dr. John R. PAUL
Professor of Preventive Medicine
Yale University School of Medicine
New Haven, Connecticut, U.S.A.

Dr. Frank T. PERKINS
Biological Standards Control Laboratory
Medical Research Council
Holly Hill, Hampstead
London, England

Dr. Edith PETTE
Institute for the Research of Poliomyelitis and Multiple Sclerosis
Hamburg, Germany

Dr. Heinrich PETTE
Professor of Neurology
Institute for the Research of Poliomyelitis and Multiple Sclerosis
Hamburg, Germany

Dr. Stanley A. PLOTKIN
Epidemic Intelligence Service Officer
U.S. Public Health Service, assigned to The Wistar Institute
Philadelphia, Pennsylvania, U.S.A.

Dr. Konald A. PREM
Assistant Professor
Department of Obstetrics and Gynecology
University of Minnesota School of Medicine
Minneapolis, Minnesota, U.S.A.

Professor Feliks PRZESMYCKI
Professor of Epidemiology of the Medical School, and Director, State Institute of Hygiene
Warsaw, Poland

Dr. José Manuel QUIRCE
Minister of Public Health
San José, Costa Rica

Dr. Manuel RAMOS ALVAREZ
Chief, Virus Laboratory
Children's Hospital
Mexico, D.F., Mexico

Dr. Andrew J. RHODES
Director, School of Hygiene
University of Toronto
Toronto, Ontario, Canada

Dr. Frederick C. ROBBINS
Professor of Pediatrics
Western Reserve University School of Medicine
Cleveland, Ohio, U.S.A.

Dr. Manuel ROCA-GARCÍA
Viral and Rickettsial Research Section
Lederle Laboratories
American Cyanamid Company
Pearl River, New York, U.S.A.

Dr. Albert B. SABIN
Professor of Research Pediatrics
University of Cincinnati College of Medicine
Cincinnati, Ohio, U.S.A.

Dr. Alexis SHELOKOV
Director, Middle America Research Unit
National Institutes of Health
U.S. Public Health Service
Balboa Heights, Canal Zone

Dr. Vilém SKOVRÁNEK
Department of Epidemiology
Ministry of Health
Prague, Czechoslovakia

Dr. Joseph E. SMADEL
Associate Director
National Institutes of Health
Bethesda, Maryland, U.S.A.

Professor Anatol A. SMORODINTSEV
Chief, Virus Department
Institute of Experimental Medicine
Academy of Medical Sciences
Leningrad, USSR

Dr. Fred L. SOPER
Director Emeritus of the Pan American Sanitary Bureau
4104 Rosemary Street
Chevy Chase, Maryland, U.S.A.

Dr. Alex J. STEIGMAN
Chairman, Committee on Control of Infectious Diseases
American Academy of Pediatrics
Louisville, Kentucky, U.S.A.

Dr. Charles H. STUART-HARRIS
Professor of Medicine
University of Sheffield
Sheffield, England

Dr. Howard TINT
Director, Product Development
Wyeth Laboratories
Philadelphia, Pennsylvania, U.S.A.

Dr. John O'Hara TOBIN
Biological Standards Control Laboratory
Medical Research Council
Holly Hill, Hampstead
London, England

Dr. C. E. VAN ROOYEN
Professor of Bacteriology
Dalhousie University School of Medicine
Halifax, Nova Scotia, Canada

Dr. Oscar VARGAS-MÉNDEZ
Director General of Health
Ministry of Public Health
San José, Costa Rica

Dr. Milton V. VELDEE
Medical Director (ret.)
U.S. Public Health Service
Menlo Park, California, U.S.A.

Dr. Jacobus D. VERLINDE
Professor of Microbiology
University of Leiden Faculty of Medicine
Leiden, Netherlands

Dr. Vladimir VONKA
Virus Department
Institute of Sera and Vaccines
Prague, Czechoslovakia

Dr. Marina K. VOROSHILOVA
Head, Laboratory of Immunology
Institute for Poliomyelitis Research
Academy of Medical Sciences
Moscow, USSR

Dr. William G. WORKMAN
Chief, Laboratory of Control Activities
Division of Biologics Standards
National Institutes of Health
Bethesda, Maryland, U.S.A.

Professor Victor M. ZHDANOV
Academic Secretary
Academy of Medical Sciences
Moscow, USSR

Staff Members of the Pan American Health Organization and the World Health Organization

Dr. Juan José ALCOCER, Medical Officer, PAHO/WHO, Guatemala City, Guatemala

Dr. Alfredo N. BICA, Chief, Communicable Diseases Branch, PAHO/WHO, Washington, D.C.

Dr. Hanna B. DOANY, Virology Consultant, PAHO/WHO, Cali, Colombia

Dr. Carlos Luis GONZÁLEZ, Assistant Director, PAHO/WHO, Washington, D.C.

Dr. Abraham HORWITZ, Director, PAHO/WHO, Washington, D.C.

Dr. Mauricio MARTINS DA SILVA, Poliomyelitis Adviser, Communicable Diseases Branch, PAHO/WHO, Washington, D.C.

Dr. Juan A. MONTOYA, Epidemiology Consultant, PAHO/WHO, San José, Costa Rica

Dr. Anthony M.-M. PAYNE, Chief Medical Officer, Virus Diseases, WHO, Geneva

Dr. Myron E. WEGMAN, Secretary General, PAHO/WHO, Washington, D.C.

FIRST SESSION

MONDAY, 6 JUNE 1960, 9:30 a.m.

Chairman

DR. GAYLORD W. ANDERSON
Director, School of Public Health
University of Minnesota
Minneapolis, Minnesota

INTRODUCTORY REMARKS

Dr. Abraham Horwitz, Director
Pan American Sanitary Bureau
Regional Office, World Health Organization
Washington, D. C.

Dr. Raymond D. Ritts
Department of Microbiology
Georgetown University
Washington, D. C.

TOPIC I. GENERAL CONSIDERATIONS

Presentation of Paper by:

Dr. Hilary Koprowski

TOPIC II. SAFETY. (A) LABORATORY EVIDENCE OF ATTENUATION AND SAFETY

Presentation of Papers by:

Dr. Joseph L. Melnick

(DISCUSSION)

Dr. Victor J. Cabasso

(DISCUSSION)

Dr. Horace L. Hodes

(DISCUSSION)

INTRODUCTORY REMARKS

DR. ABRAHAM HORWITZ (*Director, Pan American Sanitary Bureau, and Regional Director for the Americas, World Health Organization*): A year has passed since we met in this room at the First International Conference on Live Poliovirus Vaccines to analyze what was known about them, to see what still remained to be learned, and to determine the course which must be followed to reach our objectives. A year is a short time in scientific endeavor, and usually one could not expect very much to have happened in that period. However, there comes a time when mounting experience demands that crucial decisions be taken, a moment when it seems that even though we may recognize that many problems remain unsolved, enough may be known for a major step forward in the application of a new procedure to be seriously considered. We have, I believe, reached that moment in the story of live poliovirus vaccines.

The story began quietly 10 years ago, and for several years it was overshadowed by news of the progress in the development of the inactivated vaccine. That that vaccine has proved very useful when properly administered is generally accepted. But it is also widely felt that it has disadvantages arising from its high cost and the need for repeated injections for maximum effectiveness, which preclude its application in many parts of the world. The live vaccine seemed to offer hope of overcoming these disadvantages, and also to raise the possibility of a great reduction in the circulation of fully virulent viruses. During the last three or four years, therefore, interest in the development of the vaccine increased greatly, and this has culminated, during the past year, in studies on a scale undreamt of a few years ago. However, aside from their magnitude, one of the most striking features of these studies has been their international nature. As an example of international cooperation, this is indeed difficult to better, and, as Director of the Pan American Sanitary Bureau, and on behalf of the Director-General of the World Health Organization, I should like to pay tribute to this spirit which is so close to the heart of our Organizations. Political differences which cause us so much anxiety take on a less gloomy tint when viewed in the light of this spirit. These are no empty words, but are based on an impressive array of facts of which we shall hear the details this week.

As you all know, the development of this vaccine has not always been plain sailing. We have had stormy passages, and it is natural to expect more in a subject on which such strong views are held. I believe that the First Conference last year did much to clear the air by providing an opportunity for frank and open discussion in an atmosphere conditioned by the freshness of scientific observations. I am confident that this week will bring further improvement because of the mass of new information which will be placed before you. The history of the development of medical science is filled with examples from which we can draw confidence. New procedures on which data are scanty are at first criticized on the basis of pre-existing knowledge—that is right and inevitable. As fresh information accumulates, preconceived ideas need to be revised, and judgments can be made on the basis of directly observed facts, and not on extrapolations from observations in other fields. Furthermore, the real meaning of laboratory tests in terms of the effect of the product in man—which is, of course, what these tests are intended to estimate—may only become clear after rather extensive experience in man itself. The fallacies that may arise in applying to man the results obtained in mice or monkeys are well known. However, these field tests are very much more difficult than laboratory experimentation. Only too often in the past, field studies have not been conducted in accordance with scientific principles, with the result that there may be little confidence in the validity or reproducibility of the findings. If we are to base our public health policies on field studies, we need to examine them meticulously in this respect. Mere numbers alone mean nothing.

When estimating the efficacy of a new procedure, the incidence of the disease in question must be compared with that in some other group. When such a group does not exist, the incidence may be compared with that in other areas in the same year, or in the same area in other years, but in a disease with such an extremely variable incidence as poliomyelitis our confidence in an estimate of efficacy obtained in this way is low. There are too many other possible explanations of the observed differences. Several years of careful observations may be needed before firm conclusions can be drawn.

On the other hand, answers to scientific questions are not enough. The achievements of science must be translated into practice if they are to have their full effect in mankind. Smallpox has not been eradicated, although we have had a highly effective vaccine for 150 years—a good expression of human indifference when risk of death and disease diminish because of an outstanding scientific achievement. When we are sure that we have an effective live poliomyelitis vaccine, we must not rest until its full potential benefits are given to the whole population. But to reach this end, the population must understand and demand vaccination.

I shall not attempt to detail all the many other problems which will certainly be discussed at this Conference, nor will I try to forecast the answers which will be brought forward. We certainly have a mass of new data to help us. Over 40 papers will be delivered, and many valuable opinions and further information will come from the discussions. As was done last year, a drafting group has been selected and will follow the proceedings with special care, so as to draw up the Summary of the Conference for your consideration and approval on Friday. As last year, we will publish the proceedings as quickly as possible.

This year, however, the Conference fills an additional role. The World Health Organization is convening an Expert Committee on Poliomyelitis immediately after the Conference, and all members of the Committee are with us. Thus, they will have the rare advantage of hearing all the latest data and opinions immediately prior to their deliberations, and this can only be of incalculable value to them in coming to their conclusions and making their recommendations.

As last year, the Pan American Health Organization and the World Health Organization are indebted to the Sister Elizabeth Kenny Foundation for the generous financial assistance which has made this Conference possible. I wish to express, on behalf of Dr. Candau and myself, our deep appreciation. Our sincere thanks are also due to Georgetown University for making this excellent accommodation available. I would also like to thank the scientists present today for coming to this meeting, many from long distances and at considerable inconvenience.

I am sure that all of us look forward to an instructive and rewarding week.

DR. RAYMOND D. RITTS (*Department of Microbiology and Tropical Medicine, School of Medicine, Georgetown University*): As Chairman of Microbiology at Georgetown University, I have fallen heir to many strange administrative duties, and this year, because Dean Hussey is ill—fittingly, with a viral infection—on this occasion he has asked me to express the welcome of Georgetown University to the Second International Conference on Live Poliovirus Vaccines.

I do not have any Palladian to deliver to you. I am usually impressed, when I have attended or participated in international meetings of this kind, that the gentleman who delivers the words of welcome usually has a series of truisms that are better known to the participants than to the person who delivers the welcome.

So it is with honor that the Pan American Health Organization and Georgetown bid you welcome.

DR. HORWITZ: I shall turn the chair over to Dr. Gaylord Anderson, who will preside at our meeting this morning.

CHAIRMAN ANDERSON: Thank you very much, Dr. Horwitz.

I appreciate the honor of serving as your Chairman for this opening session.

The first paper this morning is under the heading of General Considerations: The Tin Anniversary of the Development of Live Poliovirus Vaccine, by Dr. Hilary Koprowski of The Wistar Institute in Philadelphia. This will be followed by the first paper under Topic II: Safety, Laboratory Evidence of Attenuation and Safety, to be presented by Dr. Melnick of Baylor University, Houston, Texas. The title of that paper is "Problems Associated with Live Poliovirus Vaccine and Its Progeny after Multiplication in Man."

TOPIC I. GENERAL CONSIDERATIONS

THE TIN ANNIVERSARY OF THE DEVELOPMENT OF LIVE POLIOVIRUS VACCINE

DR. HILARY KOPROWSKI

The Wistar Institute
Philadelphia, Pennsylvania

DR. KOPROWSKI: Ten years ago we found that the oral administration of live attenuated poliovirus to a small group of non-immune children gave rise to an asymptomatic intestinal infection, followed by the appearance of antibodies in the blood.[1] The reports to be given at this Second International Conference on Live Poliovirus Vaccines will show that over 60 million people throughout the world have been fed live attenuated poliovirus for immunization purposes.

These two events frame a decade of progress in research extending from a small and hopeful beginning to undertakings of this magnitude. The purpose of this paper is to examine the canvas within this frame—the background sketched in 1950 and the figures now occupying the foreground, painted in during the rest of the decade. I shall be guided in this task by Salvador Dali, who said: "I do not paint a portrait to look like the subject, rather does the subject grow to look like the portrait."

Development of Attenuated Strains of Poliovirus. Looking first at the background, we see that the development of the attenuated poliovirus was a kind of game in which you tried to get something you did not have to begin with. After Armstrong's success in propagating Lansing virus in a non-primate host,[2] we adapted a poliovirus to mice and cotton rats, not knowing what would come out, and ended up with a comparatively non-virulent virus. This was the TN Type 2 strain, the first fed to man. But while this was a game of chance, it was not entirely haphazard, and the development of new strains became increasingly purposeful. The introduction of tissue-culture methods by Enders and his colleagues [3] advanced the development of attenuated strains and led to Sabin's efforts to select attenuated viruses by rapid passage in tissue-culture cells.[4] Another innovation, Dulbecco's plaquing technique,[5] made possible a more precise selection of progeny of single-virus particles. More recently it has been possible to single out an attenuated virus by a purposeful manipulation of the physiological conditions of cultivation, such as the choice of particular tissue-culture systems, changes in pH (Li; [6] Gard [7]), and, especially, cultivation at different temperatures of incubation (Dubes and Wenner [8]; Lwoff [9]). Today we have advanced to the point where it may be possible to achieve outright mutation of poliovirus particles by changing the composition of nucleic acid bases with nitrous acid.[10]

"Es steht doch alles im Faust" (Hans Zinsser quoting his father). Before events rush on, let us go back to 1952, to the first paper on the subject.[1] Some questions were raised in that paper and some problems posed—and, already, some of the answers were furnished. Let us see if we recognize them.

The first questions were whether feeding attenuated poliovirus led to true infection of the intestinal tract, whether infection was followed by serologic evidence of immunity, and whether the procedure of feeding living poliovirus could be asymptomatic. The results of this first series of observations provided affirmative answers to these queries. Another question was then raised: Does live virus induce a state of local intestinal

immunity and thus prevent the carrier state in poliomyelitis? Resistance of 10 out of the 12 vaccinated children to reinfection provided an affirmative answer. It was suggested then and later confirmed that immune children occasionally became infected, and then only transiently.

Yet another question was raised in 1950: would the administration of one type of vaccine provide protection against poliomyelitis of another type? At last year's Conference you heard about the ambiguous results of a trial in Singapore based on this speculation.[11]

The vaccine strain tried in 1950 was chosen on the basis of its relatively low pathogenicity for monkeys when inoculated intracerebrally. At the same time, however, it was recognized that it was "impossible to draw a definite . . . parallel" between "innocuousness" for monkeys and safety for human beings.

Sabin's introduction of intraspinal testing,[12] and Melnick's refinement of the technique[13]—still in monkeys—provided a more sensitive index of pathogenicity for monkeys, but the significance and relevance of this increased sensitivity were not clear. The results of intraspinal testing in the hands of different investigators are still contradictory, and the importance—indeed, the necessity—of acquiring comparative data which would define attenuated and naturally occurring polioviruses in terms of monkey neuropathogenicity, has been increasingly recognized.

A further question noted then has concerned many workers since: How stable is the vaccine strain after passage through the human alimentary canal? The initial observations on this point indicated that the virus excreted in the feces "retained about the same degree of virulence for monkeys" as did the original vaccine strain.

Finally, it was pointed out in the 1952 paper that the establishment of attenuated poliovirus as an immunizing agent hinged on larger trials, in which safety could be evaluated with statistical validity, allowing for the chance probability of unrelated illnesses in vaccinated persons.

Thus, as you can see, although attenuated live vaccine research has progressed to a stately round of international conferences, the questions and problems raised at them are not new concerns.

Choice of subjects. With the important exception of certain cases I shall mention, the multiplication of vaccine virus in the intestinal tract is regularly associated with antibody production. Moreover, the presence of circulating antibodies, except those produced by infection with living poliovirus, do not interfere with immunization.

Of practical and theoretical importance within this context were the observations by Lipson *et al.*,[14] by Horstmann *et al.*[15] and by Fox and Gelfand,[16] that previous vaccination with inactivated virus neither interferes with subsequent immunization by live virus nor has any substantial effect in preventing subsequent intestinal infection with natural poliovirus. Perhaps even more important for the future were our related observations, made in 1955 and 1956 and since confirmed by Martins da Silva and others[17] that homotypic maternal antibodies in the circulation of newborn infants do not interfere with the establishment of intestinal infection by live attenuated poliovirus.

Whether or not it will be possible to take the ultimate step of routinely immunizing children on the first or second day of life is an unsettled matter, for we will show later in this Conference that infants in the first few weeks of life are relatively deficient in their ability to produce antibodies in response to the stimulus of intestinal infection with attenuated polio vaccine.[18, 19] The situation in newborn infants represents the only case in which we have found a dissociation between infection with attenuated virus and antibody response. And while increasing the dosage of the virus may overcome the relative resistance of infants less than three months old to intestinal infection with poliovirus, it is doubtful that the intrinsic relative immunologic incompetence of the newborn infant can be overcome easily.

The subject of infectiousness brings us to another crucial area of investigation. On the basis of the evidence at hand, immunization with attenuated poliovirus may be accomplished without viremia. However, this area of research invites further study from the theoretical point of view, now that more sensitive methods for detecting circulating virus are available. Since some aspects of the pathogenesis of poliomyelitis infection in man are involved in an enigma, we are exploring the problem of viremia and pathogenesis by plotting the pathway of infection with attenuated strains in man by following the ap-

pearance of the virus in the feces, in the white blood cells of the thoracic duct, and in the lymph and serum.

Transmission. Transmission of attenuated virus from person to person was first demonstrated in studies at an institution for retarded children in California.[20] This aspect of the vaccine's action was later reinvestigated by Martins da Silva *et al*,[17] Horstmann and Paul,[15] Dick and Dane,[21] Gelfand and Fox,[22] and by Plotkin *et al.*[23] The extent of virus spread to susceptible persons in contact with vaccinated persons depends on the strain employed, the age of the vaccinated person, and the prevailing social and economic conditions. The route of transmission is apparently fecal-oral rather than by way of pharyngeal secretions, but this point is still disputed by some workers. In general, one can expect that up to 50 per cent of susceptible family contacts will become asymptomatically infected. What evidence there is suggests that the attenuated virus, although relatively easily spread in institutions, is not widely disseminated through a community into which it has been introduced. In any case, there is no evidence supporting the contention that immunization of part of a community will eventually bring about immunization of all of the community, and the contention that the viruses stay permanently in the community.

Stability. Two methods were employed at first to gauge the stability of transmitted viruses. One was the measurement of the intraneural pathogenicity of strains isolated from contacts. The other was the deliberate serial transfer of virus from one child to another, so as to duplicate artificially—but with proper safeguards, of course —what might happen in nature. Six serial passages in man were carried out with the SM strain,[24] and 10-12 similar passages of the LSc strains, P-712 and Leon KP-34, were made by Smorodintsev.[25] These passages failed to show evidence of significantly altered pathogenicity for man or monkey. Obviously, an enormous number of serial passages have occurred in the course of field trials, yet there has been no evidence of an increase in the number of paralytic cases among the contacts of the vaccinated subjects.

The discovery by Vogt and co-workers [26] of the differential growth of attenuated and virulent polioviruses in acid media was the first marker employed for characterization of attenuated strains. We now possess a whole series of techniques for the more or less precise appreciation of the stability or instability of transmitted strains. Differential growth of viruses on stable-line cells and on fresh monkey-kidney tissue culture, described by Kanda and Melnick,[27] is another genetic marker, but the most practical virus label in use today is the temperature marker developed by Lwoff.[9] Another marker under investigation is differential growth of attenuated viruses in a human tissue-culture system, as compared to monkey tissue.[7] The most promising advance in the identification of virus strains is the intratypic serodifferentiation test investigated by McBride,[28] Gard,[29] and Wecker.[30]

Since biology is an integrative science—in which Chargaff's dictum that we have to look at both sides of the street at the same time is always applicable—absolute stability of a replicating agent is a contradiction in terms. The requirement that a product remain absolutely stable after passing through the alimentary canal can be applied to magnesium sulfate or other chemical laxatives, but not to a self-multiplying agent such as poliovirus. We should therefore be clear in our minds what we mean when we apply the term "stability" to poliovirus strains. The two essential characteristics are non-pathogenicity for man and immunizing capacity for man, and we must not forget that the laboratory markers currently available characterize the end-product of attenuation, and not virulence. So it is a hazardous presumption to select one or more out of the many possible markers as being a sentinel for either human pathogenicity or immunizing potential. Change in these markers can conceivably occur without concomitant change in the two characters of paramount importance: safety and efficacy.

Extraneous Viruses. Protagonists of live virus vaccination often search in vaccine preparations for viruses other than polio and usually find them in the products of their colleagues but not in their own, thus subscribing to the dictum of the fourth Earl of Chesterfield that "most people enjoy the inferiority of their friends." They often tend to forget Benjamin Franklin's advice: "Clean your finger before you point at my spots."

If, indeed, somebody were to poke his nose into the live virus vaccine, he might find a non-polio virus in all the preparations currently available; but this should hardly deter anybody

from accepting the product. The idea of disqualifying a live vaccine because it contains an extraneous virus not known to be a human pathogen immediately suggests the following considerations:

1. Almost every attempt to isolate a viral agent from the living tissues of any organism has succeeded, and we see no end to this interminable path.

2. Smallpox vaccine has been used successfully for almost 200 years. Yet extraneous viral agents could doubtless be found if searched for in calf lymph. Do we call a halt to vaccination on this account?

3. Live vaccines prepared from chick-embryo tissue could very well contain a chicken-cancer virus. The existence of an agent of this type has already been demonstrated by Rubin [31] in cultures of presumably normal fertile hen's eggs. How, therefore, does the question of the injectable 17D vaccine or chick embryo smallpox virus enter into this picture?

The polio vaccine is administered orally, and many viruses find their way into the human body through the mouth. If one wishes to be a purist in this entire matter, then the Food and Drug Administration should be requiring that all food items which are eaten uncooked be tested for the presence of viral agents.

As a scientist, I support continued investigations into the meaning of the viral agents that lie dormant in the cells of all living organisms and which can emerge when such cells are cultivated under conditions freeing them from the restraining influence exerted by the organism as a whole. If anyone is looking for a self-perpetuating project, here is a good one for the next thousand years. Moreover, the particular non-polio viral agents that may have been uncovered from live polio vaccine have perhaps undergone more rigorous safety tests than any other biological agent through its extensive use in millions of individuals throughout the world. If an adequate number of persons exposed to these agents has been shown to develop specific antibodies without any clinical disease, the evidence should be regarded as overwhelmingly in favor of the harmlessness of these agents.

Interference. Early in our studies, we observed that the simultaneous administration of two types of attenuated virus led to interference between the types.[32]

These observations indicated that simultaneous immunization with all three types of poliovirus might not be feasible. Cox and his associates [33] later reported that the difficulty presented by simultaneous feedings had been overcome by the use of larger doses of virus. However, our recent observations and the large-scale observations of Smorodintsev with other strains [34] support the earlier findings that strains of virus administered simultaneously interfere with each other. The phenomenon of interference between strains also foreshadowed one of the problems of immunization with attenuated virus, namely, interference against poliovirus caused by pre-existing infection of the intestinal tract with other enteric viruses.[35, 36, 37] Whether the adverse action of interference can be changed into a beneficial one through the use of homotypic attenuated virus during a polio epidemic remains to be seen, although those who would approach such a project with enthusiasm should be warned that the antagonists of live virus vaccination have two pat comments on any outcome of such a trial: If the epidemic is substantially abated, the vaccine failed to stop it completely; if the epidemic ends following vaccination, it would have stopped anyway, even without the use of vaccine. *Tertium non datur.*

TABLE 1. PERSISTENCE OF ANTIBODIES TO POLIO 10 YRS. AFTER VACCINATION

SUBJECT	TYPE 2 TITER POST-VACC.	
	8 yrs.	10 yrs.
2	128	256
5	32	16
7	64	>1024
8	64	256
9	128	256
13	16	64
14	16	16

Perseverance or Obstinacy. Table 1 should bear the caption provided 200 years ago by Laurence Sterne: "'Tis known by the name of perseverance in a good cause, and obstinacy in a bad one." Here you see the Type 2 antibody levels in the sera of children who were fed the TN virus 10 years ago. Does the remarkable persistence of immunity classify this as "perseverance"?

Envoi. Finally, a parable, which we commend to the attention of the adherents of all schools, live or dead. The greatest detective of them all, Sherlock Holmes, was less impressed by the mysterious stranger on the premises than by the failure of the dog to bark in the night. Perhaps in "The Case of the Spiked Potion," too, the mysterious agents encountered in our laboratories are less significant than all those healthy children who never complain! As Holmes himself remarked: "In solving a problem of this sort, the grand thing is to be able to reason backwards. That is a very useful accomplishment, and a very easy one, but people do not practice it much." Reasoning backwards from the successful culmination of this first decade of experience with live vaccines, we can, I think, look forward beyond our tin anniversary to years of continuing success and safety, and to anniversaries even more deserving of celebration.

REFERENCES

1. Koprowski, H., Jervis, G. A., and Norton, T. W.: Immune responses in human volunteers upon oral administration of a rodent-adapted strain of poliomyelitis virus. *Am. J. Hyg.* **55:** 108-126, 1952.
2. Armstrong, C.: The experimental transmission of poliomyelitis to the eastern cotton rat, *Sigmodon hispidus hispidus. U. S. Pub. Health Rep.* **54:** 1719-1721, 1939.
3. Enders, J. F., Weller, T. M., and Robbins, F. C.: Cultivation of Lansing strain of poliomyelitis virus in cultures of various human embryonic tissues. *Science* **109:** 85-86, 1949.
4. Sabin, A. B., Hennessen, W. A., and Winsser, J.: Studies on variants of poliomyelitis virus. I. Experimental segregation and properties of avirulent variants of three immunologic types. *J. Exp. M.* **99:** 551-576, 1954.
5. Dulbecco, R. and Vogt, M.: Plaque formation and isolation of pure lines with poliomyelitis viruses. *J. Exp. M.* **99:** 167-182, 1954.
6. Li, C. P., Schaeffer, M., Nelson, D. B.: Experimentally produced variants of poliomyelitis virus combining *in vivo* and *in vitro* techniques. *Ann. N. York Acad. Sc.* **61:** 902-910, 1955.
7. Gard, S.: Field and laboratory experiences with the CHAT strain Type 1 poliovirus. (See this volume pp. 187-190.)
8. Dubes, G. R. and Wenner, H. A.: Virulence of polioviruses in relation to variant characteristics distinguishable on cells *in vitro*. *Virology* **4:** 275-296, 1957.
9. Lwoff, A. and Lwoff, M.: Remarques sur quelques caractères du developpement du virus de la poliomyélite. *C. rend. Acad sc.* **248:** 1725-1727, 1959.
10. Boeye, A.: Induction of a mutation in poliovirus by nitrous acid. *Virology* **9:** 691-700, 1959.
11. Hale, J. H., Doraisingham, M., Kanagaratnam, K., Leon, K. W., and Monteiro, E. S.: Large-scale use of Sabin Type 2 attenuated poliovirus vaccine in Singapore during a Type 1 poliomyelitis epidemic. *Brit. M. J.* **1** (5137): 1541-1549 (June 20), 1959.
12. Sabin, A. B.: Characteristics and genetic potentialities of experimentally produced and naturally occurring variants of poliomyelitis virus. *Ann. N. York Acad. Sc.* **61:** (4) 924-939, 1955.
13. Melnick, J. L., Benyesh-Melnick, M., and Brennan, J. C.: Studies on live poliovirus vaccine: its neurotropic activity in monkeys and its increased neurovirulence after multiplication in vaccinated children. *J. Am. M. Assn.* **171:** 1165-1172, 1959.
14. Lipson, M. J., Robbins, F. C., and Woods, W. A.: The influence of vaccination upon intestinal infection of family contacts of poliomyelitis patients. *J. Clin. Invest.* **35:** 722, 1956.
15. Horstmann, D. M., Niederman, J. C., Melnick, J. L., and Paul, J. R.: Poliomyelitis: Comparison of responses of vaccinated and naturally immune humans to ingestion of attenuated poliovirus. *Tr. Ass. Am. Physicians* **70:** 91-101, 1957.
16. Fox, J. P., Gelfand, H. M., LeBlanc, D. R., and Rowan, D. F.: The influence of natural and artificially induced immunity on alimentary infections with polioviruses. *Am. J. Pub. Health* **48:** 1181-1192, 1958.

17. Martins da Silva, M., Prem, K. A., McKelvey, J. L., Bauer, H., Cooney, M. K., and Johnson, E. A.: Studies of orally administered attenuated live virus poliomyelitis vaccine in newborns and infants under six months. *U. Minn. M. Bull.* **29:** 133-150, 1957.

18. Pagano, J. S., Plotkin, S. A., Cornely, D., and Koprowski, H.: Experimental infection with CHAT attenuated poliovirus in premature infants. (See this volume, fifth session).

19. Plotkin, S. A., Pagano, J. S., and Koprowski, H.: Vaccination of full-term infants with attenuated polioviruses. (See this volume, fifth session).

20. Koprowski, H., Norton, T. W., Jervis, G. A., Nelson, T. L., Chadwick, D. L., Nelsen, D. J., and Meyer, K. F.: Clinical investigations on attenuated strains of poliomyelitis virus. Use as a method of immunization of children with living virus. *J. Am. M. Assn.* **160:** 954-966, 1956.

21. Dick, G. W. A., Dane, D. S., Fisher, O. D., Connolly, J. H., and McKeown, F.: A trial of SM Type 1 attenuated poliomyelitis virus vaccine. *Brit. M. J.* **1**(5010): 65-70, 1957.

22. Gelfand, H. M., Potash, L., LeBlanc, D. R., and Fox, J. P. Intrafamilial and interfamilial spread of living vaccine strains of polioviruses. *J. Am. M. Assn.* **170:** 2039-2048 (Aug. 22) 1959.

23. Plotkin, S. A., Koprowski, H., Richardson, S., and Stokes, J.: Vaccination of families against poliomyelitis by feeding and by contact spread of living attenuated virus: Including studies of virus properties after human intestinal passage. *Acta Paediat.* (May) 1960.

24. Koprowski, H.: Discussion of properties of attenuated polioviruses and their behavior in human beings. *Spec. Pub. N. York Acad. Sc.* **8:** 128-133, 1957.

25. Smorodintsev, A. A., Davidenkova, E. F., Drobyshevskaya, A. I., Klyuchareva, T. E., Ilyenko, V. I., Chalkina, O. M., Vasiliev, K. G., Glynskaya, E. V., Votiakov, V. I., and Feldman, E. V.: Material for the study of the harmlessness of the live poliomyelitis vaccine prepared from Sabin strains. In: *Live Poliovirus Vaccines, First International Conference.* Scient. Pub. no. 44 of the Pan American Health Organization, pp. 324-332, 1959.

26. Vogt, M., Dulbecco, R., and Wenner, H. A.: Mutants of poliomyelitis viruses with reduced efficiency of plating in acid medium and reduced neuropathogenicity. *Virology* **4:** 141-155, 1957.

27. Kanda, Y. and Melnick, J. L.: *In vitro* differentiation of virulent and attenuated polioviruses by their growth characteristics on *MS* cells. *J. Exp. M.* **109:** 9-24, 1959.

28. McBride, W. D.: Antigenic analysis of polioviruses by kinetic studies of serum neutralization. *Virology* **7:** 45-58, 1959.

29. Gard, S.: Immunologic strain specificity within Type 1 poliovirus. *Bull. World Health Org.* **22:** 235-252, 1960.

30. Wecker, E.: A simple test for serodifferentiation of poliovirus strains within the same type. *Virology* **10:** 376-379, 1960.

31. Rubin, H.: Remarks at *Symposium on Phenomena of Tumor Viruses,* Virology and Rickettsiology Study Section of National Institutes of Health, New York City, March 1960.

32. Koprowski, H.: Immunization of man against poliomyelitis with attenuated preparations of living virus. *Ann. N. York Acad. Sc.* **61**(4): 1039-1049, 1955.

33. Cox, H. R., Cabasso, V. J., Markham, F. S., Moses, M. J., Moyer, A. W., Roca-García, M, and Ruegsegger, J. M.: Immunological response to trivalent oral poliomyelitis vaccine. *Brit. M. J.* **2:** 591-597, 1959.

34. Smorodintsev, A. A. Drobyshevskaya, A. I., Bulychev, N. P., Chalkina, O. M., Groisman, G. M., Ilyenko, V. I., Kantorovich, R. A., Kurnosova, L. M., Vasiliev, K. G., Votiakov, V. I., and Zhilova, G. P.: Material on the immunological and epidemiological effectiveness of live poliomyelitis vaccine. (See this volume, seventh session).

35. Benyesh-Melnick, M., Melnick, J. L., and Ramos Alvarez, M.: Poliomyelitis infection rate among Mexican children fed attenuated poliovirus vaccines. In: *Live Poliovirus Vaccines, First International Conference.* Scient.

Pub. no. 44 of the Pan American Health Organization, pp. 272-285, 1959.

36. Gelfand, H. M., Potash, L., LeBlanc, D., and Fox, J. P.: Revised preliminary report on the Louisiana observations of the natural spread within families of living vaccine strains of poliovirus. In: *Live Poliovirus Vaccines, First International Conference.* Scient. Pub. no. 44 of the Pan American Health Organization, pp. 203-217, 1959.

37. Plotkin, S. A., Lebrun, A., and Koprowski, H.: Vaccination with the CHAT strain of Type 1 attenuated poliomyelitis virus in Leopoldville, Belgian Congo. 2. Studies of the safety and efficacy of vaccination. *Bull. World Health Org.* **22:** 215-234, 1960.

TOPIC II. SAFETY. (A) LABORATORY EVIDENCE OF ATTENUATION AND SAFETY

1. PROBLEMS ASSOCIATED WITH LIVE POLIOVIRUS VACCINE AND ITS PROGENY AFTER MULTIPLICATION IN MAN *

JOSEPH L. MELNICK AND MATILDA BENYESH-MELNICK

Department of Virology and Epidemiology, Baylor University College of Medicine, Houston, Texas

DR. MELNICK (*presenting the paper*): The ultimate safety of live poliovirus vaccine rests on the long-term results of its use in the field. Safety must be evaluated on the basis of the vaccine being used in fully susceptible persons —those who have lost maternal antibodies, and who have never been infected naturally or who have never received inactivated vaccine.

However, regardless of the field experience, the laboratory serves an essential function in supplying information on the properties of the virus in the vaccine, particularly whether these properties remain constant in successive manufacturing lots, and whether they remain constant after multiplication in vaccinated human beings.

To be of value, the laboratory methods should allow us to discriminate among strains according to their degree of attenuation for monkeys. As minimal monkey neurovirulence was the *only* criterion of safety on which the vaccine strains were selected for use in man, this property should not now be glossed over by saying that it has little or no significance for evaluating the safety of a polio vaccine. We have no other *bona fide* way of measuring the degree of virus attenuation. Strains which show more neurovirulence than the most highly attenuated vaccine strains must be regarded as possessing a somewhat more dangerous potential for human beings. This holds if we compare one strain with another as candidates for incorporation in the vaccine, or if we compare the vaccine virus with its progeny after multiplication in the intestinal tract of a vaccinated child. Just how much of a departure, or reversion, from the highest degree of attenuation can be safely tolerated in man remains an unanswered question, but by using strains of the highest degree of attenuation and by using strains which are also stable genetically, I hope that we shall never have to answer the question.

Comparison of Candidate Strains for Monkey Neurovirulence. Two impartial laboratories have carried out extensive comparative tests with the Lederle-Cox and the Sabin strains,[1, 2] and their results were reported at the Conference which met here in Washington last year. The results are of importance for several reasons. First, despite many allegations to the contrary, they show a relatively high degree of reproducibility of neurovirulence tests in the two laboratories. Second, the tests in these two laboratories are more sensitive than those reported by Sabin and by Cox. Third, they discriminate between strains possessing different degrees of attenuation. It is readily apparent that the Sabin strains are more highly attenuated, for after intracerebral inoculation they are virtually without neurotropic activity, and after intraspinal inoculation they yield less than 10 per cent of the clinical disease produced by the Lederle-Cox strains at the same doses.

Other analyses of the neurovirulence data bear this out. When the intensity of the cord lesions are graded by our scoring method,[1] the lower scores of Sabin strains again show them

* Aided by a grant from The National Foundation.

TABLE 1. COMPARATIVE HISTOLOGICAL LESIONS OF CANDIDATE STRAINS

TYPE	STRAIN	MONKEY KIDNEY TCD_{50}	INTRACEREBRAL INOCULATION		INTRASPINAL INOCULATION	
			POLIO LESION SCORES IN SPINAL CORD*		POLIO LESION SCORES IN SPINAL CORD*	
			CERVICAL	LUMBAR	CERVICAL	LUMBAR
1	Lederle	$10^{7.3}$	54	78	101	191
	Sabin	$10^{7.3}$	0	3	15	100
2	Lederle	$10^{6.9}$	58	84	121	212
	Sabin	$10^{7.2}$	0	0	5	41
3	Lederle	$10^{7.2}$	21	28	70	150
	Sabin	$10^{7.5}$	0	0	42	101

* Undiluted through 10^{-2} used for calculation of polio lesion scores for monkeys inoculated intracerebrally, and undiluted through 10^{-4} for those inoculated intraspinally.
Score for lumbar cord with Sabin's Type 1 strain inoculated intraspinally is arbitrarily designated 100 units, and the other scores computed accordingly.

to be more attenuated than the Lederle strains. In the results shown in Table 1, the polio lesions produced in the lumbar cord by the intraspinal injection of Sabin's Type 1 virus are graded arbitrarily as 100 units. The scoring of lesions supplies additional information allowing us to discriminate further among strains which are virtually avirulent by intracerebral injection. Thus Sabin's Type 2 strain offers advantages over his Type 3 strain even though both are intracerebrally avirulent. The Type 2 strain not only produces less activity in the spinal cord near the lumbar site of injection, but it hardly spreads to involve the cervical areas of the spinal cord. The Type 3 strain which shows a higher degree of activity in the lumbar area—and perhaps more important, a higher degree of spread to the cervical area—is the Sabin strain which seems to be least desirable in the field. Thus there is an association of this measurable laboratory property with the ability of the virus to become more monkey neurovirulent in the field after it multiplies in vaccinated persons.

Comparison of Vaccine Virus and Its Progeny after Multiplication in Human Beings. Perhaps the most important question to be answered in regard to the public health acceptance of live poliovirus vaccine in this country hinges on the genetic stability of the virus strains in the vaccine. Our approach to this problem has been dependent upon the newly described *in vitro* tissue culture markers. We have determined whether the vaccine viruses have changed with regard to these markers after multiplication in man, and have selected the altered viruses for the expensive neurovirulence tests.

Recently, several markers of poliovirus have been described which can be followed by tissue culture methods and which tend to discriminate between neurovirulent and attenuated strains. Virulent wild strains usually possess *d*+, *MS*+, and *T*+ markers and thus differ from highly attenuated strains which are *d*−, *MS*−, and *T*−.[3-6]

Two newly described markers which have had little application as yet include the *E* marker and the antigenic marker. The *E* marker is dependent upon the differential adsorption and elution of virulent and attenuated strains on

cellulose columns.[7, 8] The antigenic marker recognizes that even within the same poliovirus type, strains differ in the rate at which they are neutralized by homologous and heterologous antisera.[9] In our laboratory the test has been modified by incorporating the serum in the agar overlay and then adding the virus in the form of a filter paper disc containing 1000 PFU (plaque-forming units). Heterologous strains break through at higher concentrations of serum and produce confluent plaques around the disc. Figure 1 illustrates the difference between the LSc vaccine strain and a virulent Type 1 Houston strain recovered from a fatal case. It has been suggested that the antigenic marker is more stable than the other markers, and might well prove useful in being able to identify a strain as the progeny of a vaccine virus regardless of whether or not the excreted virus shows changes in other markers, thus discriminating between it and wild viruses of the same type but with different antigenic markers. Much more work is needed in this area to see if such a generalization holds true.

The next part of this presentation will be concerned chiefly with the *d* and *T* markers of Sabin's attenuated Type 1, 2, and 3 polioviruses after multiplication in children, and the correlation of these markers with monkey neurovirulence. We reported on this in preliminary

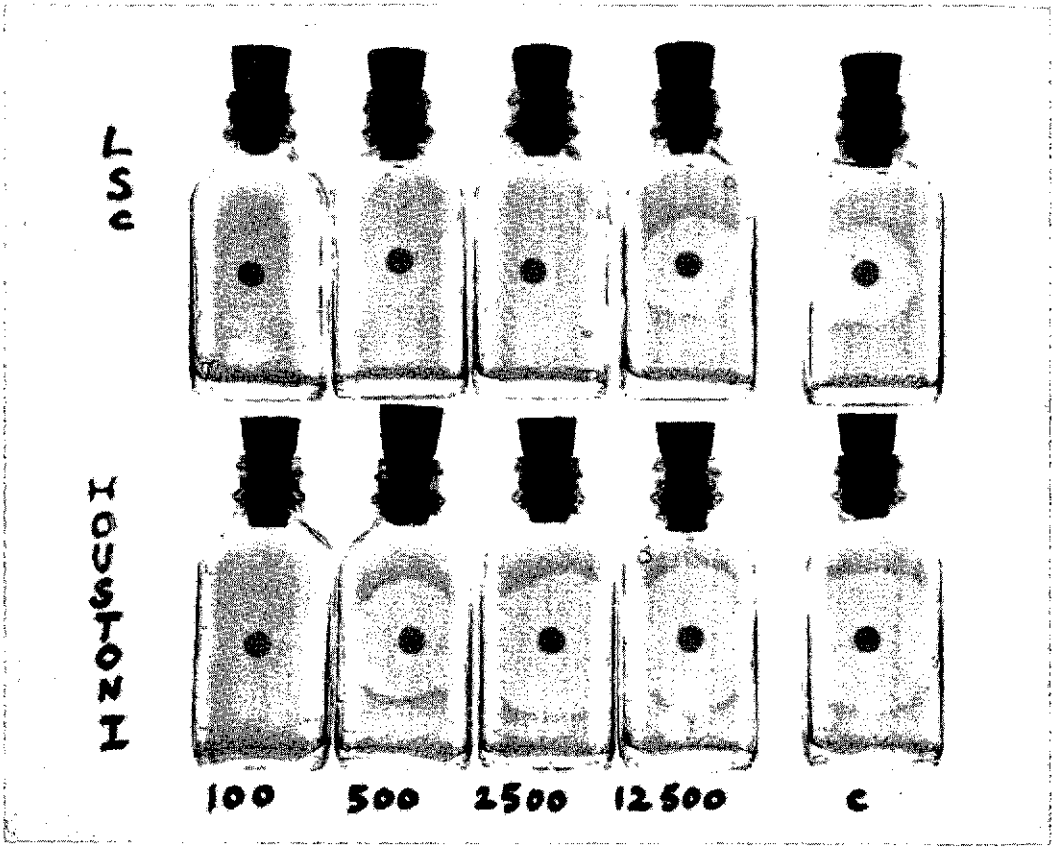


FIG. 1. Antigenic differences between attenuated LSc and Houston virulent Type 1 polioviruses as determined by the degree of neutralization with LSc guinea pig antiserum. LSc antiserum was incorporated in the agar overlay in five-fold dilution steps; discs containing 1000 plaque-forming units of each virus were then placed on top of the agar. At each serum dilution, the degree of neutralization was measured by the presence and size of the plaque area formed by the virus around the disc.

TABLE 2. REPRESENTATIVE RESULTS OF *d* AND *T* CHARACTER TESTS WITH HOMOTYPIC POLIOVIRUS EXCRETED BY ORALLY VACCINATED CHILDREN

CHILD NO.	TYPE AND CHARACTER OF VIRUS ISOLATED FROM SPECIMENS:								
	PRE-FEEDING	POST-TYPE 1			POST-TYPE 3			POST-TYPE 2		
		7 da	14 da	21 da	7 da	14 da	21 da	7 da	14 da	21 da
4	0	[P1 d−T−]	P1 d−T−	[P1 d+T−]	0	NP	[P3 d+T−]	P3 d+T−	—	—
268	0	P1 d−T−	P1 d+T−	0	0	NP	NP	P2 d−T−	P2 d+T−	0
107	0	0	0	0	P3 d+T−	P3 d+T−	NP	0	P3 d+T−	0
372	0	P1 d+T−	[P1 d+T−]	0	P3 d+T−	P3 d+T+	[P3 d+T+]	0	0	—
376	0	P1 d+T−	[P1 d+T−]	0	0	0	NP	—	—	P2 d+T−
126	0	P1 d+T−	0	NP	NP	0	NP	[P2 d+T−]	P2 d+T−	—
244	0	0	0	0	0	0	0	0	P2 d+T−	[P2 d+T−]

P1 = Poliovirus Type 1; P2 = Type 2; P3 = Type 3; NP = Non-poliovirus; 0 = negative; —— indicates no specimen.
[ ] indicates strain tested in monkeys.

fashion at last year's Conference. Homotypic strains isolated from test children at different periods after vaccination were subjected to the *d* and *T* tests and the results obtained are illustrated in Table 2.

When a strain reverted from $d- \rightarrow d+$, it was not always associated with a $T- \rightarrow T+$ reversion. However, a $T- \rightarrow T+$ reversion was usually linked with a $d- \rightarrow d+$ or a $d- \rightarrow d\pm$ reversion. An illustration of the degree of alteration in the homotypic polioviruses excreted by orally vaccinated children is shown in Table 2. Homotypic virus indicates poliovirus of the same type as that in the vaccine fed, and excreted after the feeding. The table includes the code numbers of the children, the types of virus isolated in their pre- and post-feeding specimens, and the *d* and *T* characters of the homotypic polioviruses excreted. The squares around some of the poliovirus isolates indicate those which were tested in monkeys.

It can be seen that the first two children in this table, who excreted poliovirus for several weeks, revealed a change from *d* to *d*+ character in their third and second isolates, respectively, with no change in the *T* character. On the other hand, children Nos. 372, 376, and 126 excreted *d*+*T*− viruses in the first week after feeding, thus indicating an early alteration of the vaccine virus in at least one character, during multiplication in the intestinal tract. The majority of the Type 3 isolates were of the *d*+*T*− character, and some underwent further reversion to *d*+*T*+, while the frequency of change in the Type 2 isolates was similar to that of the Type 1 strains isolated.

Summarized below in Table 3, are the results on 85 strains recovered from vaccinated children. In 23 strains, there was no detectable alteration in the virus, in 46 others, the recovered virus changed in the *d* but not in the *T* marker, and in 16, changes occurred in both markers. Thus in 19 per cent of the specimens isolated from children infected with *d*−*T*− strains, the virus had changed in at least two properties.

As indicated at last year's Conference, we selected strains with different degrees of alteration in these two markers for monkey neurovirulence tests. The strains had all been isolated in monkey kidney cultures maintained at pH 7.4. However, to insure that we were not selectively enhancing the proportion of monkey paralytogenic strains by passage of the human virus in tissue culture, a number of rectal swabs obtained from the vaccinated children were inoculated directly into monkeys by the intraspinal route. The dose of virus in such material which could be inoculated was very small indeed, varying from only 10 to 60 TCD_{50}, so that selection of virulent viruses from a tremendous excess of thousands or millions of avirulent virus particles by the monkey CNS does not play a role in these results.

A summary of the monkey tests to date on strains of the 3 types is shown in Table 4. It is apparent that excreted strains which retain the *d*−*T*− markers of the vaccine virus are not significantly more virulent for monkeys than the vaccines themselves, about 10,000 TCD_{50} being required to paralyze 50 per cent of the monkeys inoculated intraspinally, and no illness being produced by undiluted tissue culture fluid inoculated intracerebrally. The *d*+*T*− strains show increased activity, both intraspinally and intracerebrally, when compared to the *d*−*T*− vaccine virus. Thus only 10 TCD_{50} of the cultured virus produced paralysis and lesions in 6 of the 16 monkeys inoculated intraspinally, and some of the strains were also active after intracerebral inoculation. Furthermore, 10 to 60 TCD_{50} of virus in the rectal swabs themselves produced paralysis in a significant number of test monkeys inoculated intraspinally. The *d*+*T*+ strains showed even greater increases

TABLE 3. CHARACTER OF HOMOTYPIC POLIOVIRUS EXCRETED BY VACCINATED CHILDREN

VIRUS TYPE	d−T−	d±T−	d+T−	d+T±	d+T+	TOTAL
Polio—1	19	9	9	0	0	37
Polio—2	3	3	17	2	0	25
Polio—3	1	0	8	2	12	23
Total	23	12	34	4	12	85

TABLE 4. MONKEY NEUROVIRULENCE OF POLIOVIRUS TYPE 1, 2, AND 3 STRAINS AS RELATED TO THEIR *d* AND *T* CHARACTERS

STRAINS OF POLIO–1, 2, 3	NUMBER STRAINS TESTED	CHARACTER d	CHARACTER T	NUMBER OF MONKEYS WITH POLIOMYELITIS/NUMBER TESTED							
				Log_{10} TCD_{50}:							
				INTRASPINAL						INTRACEREBRAL	RECTAL SWAB IS
				6.0	5.0	4.0	3.0	2.0	1.0	7.0–6.0	1.8–1.0
Vaccines	3	d−	T−	21, 4*/27	10, 2*/16	8, 2*/16	2, 1*/8	2*/8		0/32	
After human passage (M)†	6	d−	T−				8/12	1*/9		0/3	1/7
	1	d±	T−					2/2	0/2		2/2
	8	d+	T−					16/16	6/16	5, 2*/10	5/11
	2	d+	T+					3/3	3/4	5/5	2/2
After human passage (C)‡	5	d+	T+					3/4	4/9		
Wild (M)	1	d−	T−	2/2	2, 1*/4	2*/4	0/2			0/3	0/2
	1	d+	T+					2/2	1/2	1/1	

* Nonparalytic poliomyelitis.
† M = Mexico
‡ C = Cincinnati

TABLE 5. HISTORY OF MEXICO AND CINCINNATI ISOLATES SELECTED FOR CHIMPANZEE NEUROVIRULENCE TEST

TYPE, CHARACTER OF VACCINE FED	MEXICO CHILD #372 (2 months old)		CINCINNATI CHILD KF (11 years old)	
	DAY AFTER FEEDING	TYPE, CHARACTER OF VIRUS EXCRETED	DAY AFTER FEEDING	TYPE, CHARACTER OF VIRUS EXCRETED
Polio 1 d−T−	7	Polio 1 d+T−	5	Polio 1 d−T−
	14	Polio 1 d+T−	17	Polio 1 d+T−
	21	Negative		
[Polio 3 d−T−]	7	Polio 3 d+T−	6	Polio 3 d+T+
	14	Polio 3 d+T+	20	[Polio 3 d+T+ Spec. S-33]
	21	[Polio 3 d+T+ Spec. 2576]		
Polio 2 d−T−	7	Negative	10	Polio 2 d+T−
	14	Negative	20	Polio 2 d+T−

[ ] Indicates strains tested in chimpanzees.

in neurovirulence, especially in the intracerebrally inoculated monkeys.

Cytopathogenic virus was recovered from the spinal cords of almost all monkeys sacrificed soon after the onset of paralysis, indicating that no selection of strictly neurotropic virus had taken place in the CNS of the monkeys.

Two Type 3 $d+T+$ strains shown in Table 5 (one recovered from a two-month-old vaccinated child in Mexico City, and one from an 11-year-old child vaccinated in the winter in Cincinnati by Sabin) were selected for inoculation not only in monkeys, but also into chimpanzees, as members of this species are known to be much less sensitive to intraspinal administration of attenuated poliovirus than monkeys. As shown in Table 6, we confirmed Sabin's results with the vaccine, in that the $d-T-$ vaccine itself was negative when undiluted material was injected intraspinally into the chimpanzee. The $d+T+$ isolates, however, produced paralysis and severe cord lesions.

Figure 2 is a diagrammatic expression of the intraspinal virulence of the vaccine strains and the isolates which were studied. The per cent of monkeys with polio is expressed as a function of the virus dose used. One can observe that the curve for the $d-T-$ isolates does not differ much from the curve obtained with the vaccine strains. On the other hand, there is a minimal difference between the $d+T+$ isolates and those which underwent change in the d character only ($d+T-$ isolates). Both types of isolates underwent a shift of about three logs towards increased neurovirulence, i.e., they are one thousand times more virulent for the monkey spinal cord than the vaccines which were administered. Data of this type bring up the question of whether the CNS of the monkey could act as a medium to encourage further changes in modified strains of the $d+T-$ type and only completely altered $d+T+$ particles would then continue to multiply and produce paralysis in the inoculated animals. If such were the case one would expect the spinal cords of such animals to yield only $T+$ progeny. If not, a question remains as to the correlation between the T marker and monkey neurovirulence.

Passage of T− Virus in Monkey Spinal Cord. To elucidate this question we undertook to study the character of isolates possessing a $T-$ marker linked to a $d+$ marker and pathogenic for monkeys, after propagation in the monkey spinal cord. Such $T-$ isolates were inoculated

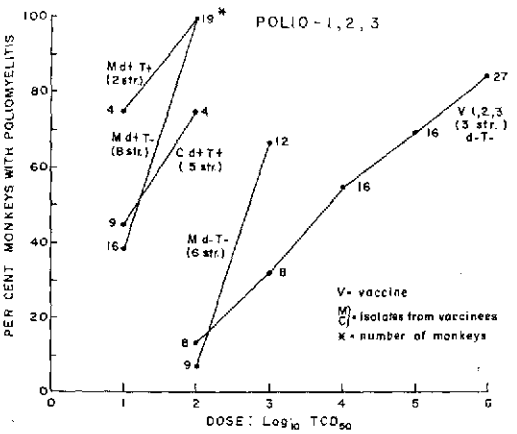


FIG. 2. Intraspinal monkey neurovirulence of poliovirus Type 1, 2, and 3 vaccine strains before and after multiplication in children. Horizontal coordinate represents log TCD_{50} dose administered; vertical coordinate indicates the per cent of the monkeys which developed poliomyelitis.

intraspinally in monkeys and then it was determined whether the virus which could be isolated from the cord of the paralyzed monkey was $T-$ or had changed to $T+$ in the course of its multiplication in the central nervous system. In Table 7, we can follow the passage of a Type 2 virus obtained from a vaccinated child. The initiating virus in this series was actually that present in the rectal swab of the child. As shown at the left of the table, the virus had an initial titer of 1.8 logs at 37° C. but < 1 at 40° C. This titer was too low to allow one to assess its T character. However, the first kidney culture passage, designated as K_1, had a titer of 6 logs at 37° C. and < 1 at 40°, the pattern of a $T-$ virus.

When the rectal swab was inoculated at a dose level of only 60 tissue culture doses intraspinally into Cynomolgus No. 502, the monkey became paralyzed on the fifth day, and developed severe lesions not only in the lumbar cord, but also in the cervical cord. The letters C and L refer to the cervical and lumbar regions of the cord; the number refers to the intensity of the lesion with the scale being 0 to 80 (for maximum lesions possible in 10 sections).[1] As one can see, the cord material had a titer of 2.9, and was $T-$, as was the kidney tissue culture passage, there being an inhibition of over 6.5 logs when the virus was titrated at 40°. Subsequent passage with only 10 tissue culture doses into Rhesus No. 726 produced paralysis in this animal on the sixth day, again with severe lesions in both the cervical and the lumbar parts of the cord. Again, the virus isolated was $T-$. A further passage with only 30 tissue culture doses into Cynomolgus No. 706 again produced paralysis in this animal and the virus isolated from the cord retained its $T-$ character.

Thus we have here a virus which has been passed from spinal cord to spinal cord through three serial transfers, and after the three passages the virus still retains the $T-$ character, the character which is usually associated with a virus that is not supposed to grow in the central nervous system.[6] Obviously this is a virus marker, whatever its usefulness may be, that does not always indicate whether or not a virus has a capacity to grow and destroy cells of the central nervous system.

Table 8 is concerned with a Type 1 virus, again a $T-$ strain. As shown in the top part of the table, this virus was passed serially by direct spinal cord to spinal cord passage at very low tissue culture doses, between 30 and 100. For the first two passages the virus was completely $T-$; at the end of the third passage, a $T-$ virus was isolated from the spinal cord, but this apparently was already a mixed population of particles for the virus was $T\pm$ in the first kidney culture passage.

After the second passage, the virus in the cord of Rhesus No. 722 was passed to Rhesus No. 728 by the intramuscular route, again producing paralysis. Here again a $T-$ virus was isolated from the spinal cord. The first kidney culture passage yielded a $T\pm$ virus.

Subsequent intramuscular inoculation in Cynomolgus No. 839 again produced paralysis, this time with the virus reverting to a fully active $T+$ state both in the cord and in its kidney culture passage.

Similarly, when the virus from the third nervous system passage was further passed, as shown in the right-hand part of the table, to another monkey by the intraspinal route, it yielded at $T\pm$ virus. But when the $T\pm$ tissue culture K_1 material was passed (see Rhesus No. 867), it yielded at $T+$ virus. So here we have evidence that the $T-$ character of a virus may be

TABLE 6. CHIMPANZEE INTRASPINAL NEUROVIRULENCE OF SABIN'S TYPE 3 VACCINE STRAIN BEFORE FEEDING AND AFTER MULTIPLICATION IN CHILDREN

CHIMPANZEE	STRAIN INOCULATED	DISEASE	POLIO LESIONS IN SPINAL CORD			NEEDLE TRACT LESIONS IN LUMBAR CORD*
			CERVICAL	THORACIC	LUMBAR	
Gamma	Type 3 Vaccine	Neg.	0	0	5 (0/++)	1, 8
Delta	Mexico Specimen No. 2576	Complete paralysis of both legs, and right arm	40 (0/+++)	26 (0/+++)	68 (++/++++)	2, 10
Zeta	Cincinnati Specimen No. S–33	Complete paralysis of both legs	24 (0/+++)	10 (0/++)	49 (0/++++)	8

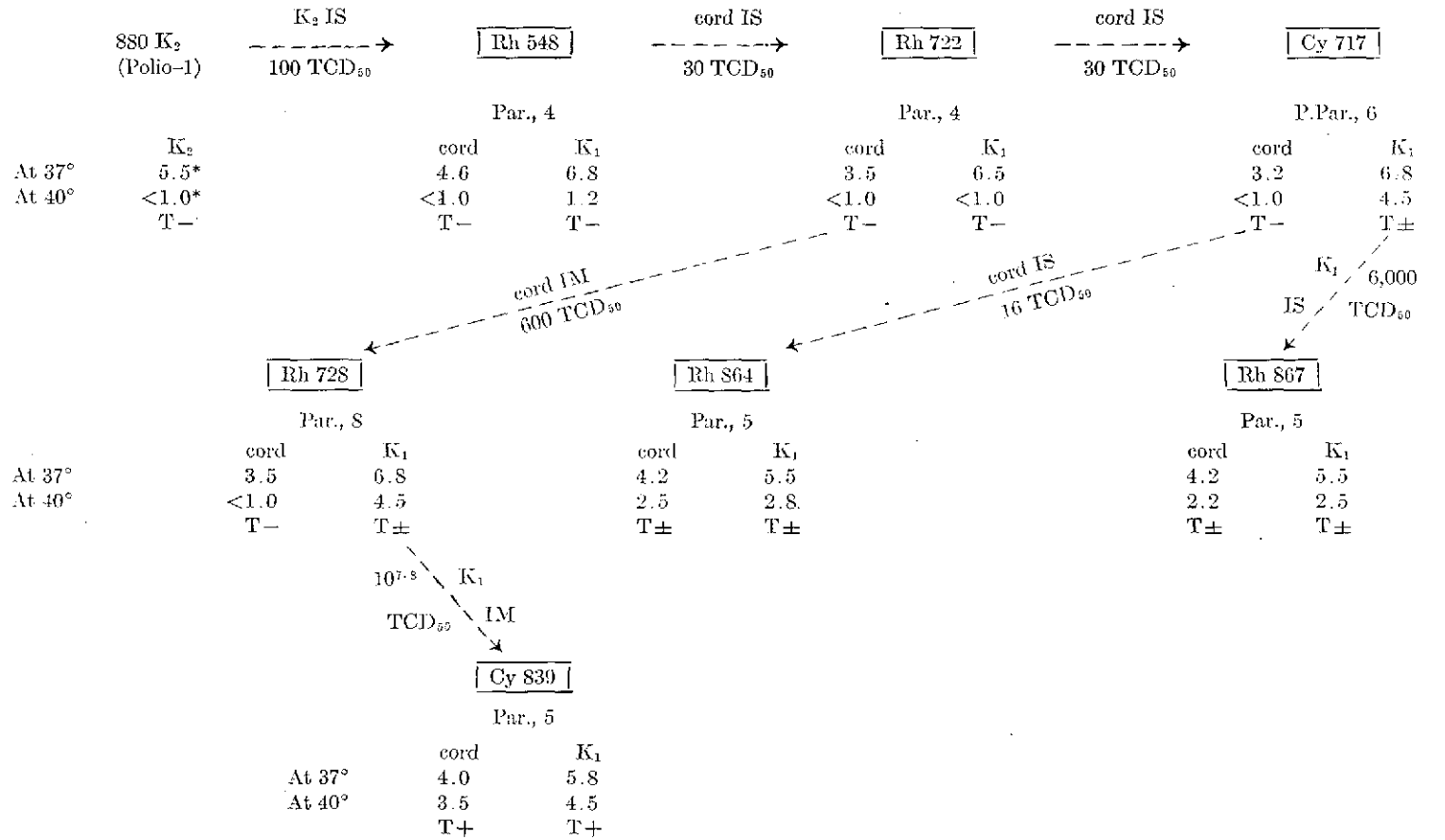
* Numbers in this column refer to the level or levels where the needle tract lesion was found; for this purpose the lumbar cord was arbitrarily divided into 10 consecutive blocks.

TABLE 7. EFFECT OF SERIAL, CORD TO CORD, MONKEY PASSAGES ON THE NEUROVIRULENCE AND *T* CHARACTER OF *T*— TYPE 2 POLIOVIRUS ISOLATED FROM A VACCINATED CHILD

	MEXICO ISOLATE #3485 R.Sw. (Polio–2)		R.Sw. IS → 60 TCD_{50}	Cy 502 CPBL, 5 C 25 (0/++) L 65 (++/++++)		cord IS → 10 TCD_{50}	Rh 726 CPRL, 6 C 27 (0/++) L 52 (++/++++)		cord IS → 30 TCD_{50}	Cy 706 CPBL, 5 C 25 (0/++) L 70 (++/++++)	
	R.Sw.	K_1		cord	K_1		cord	K_1		cord	K_1
At 37°	1.8*	6.0		2.9	7.5		2.5	6.2		2.8	5.8
At 40°	<1.0*	<1.0		<1.0	<1.0		<1.0	<1.0		<1.0	<1.0
	?	T–		T–	T–		T–	T–		T–	T–

* Log_{10} TCD_{50} per 0.1 ml.

TABLE 8. EFFECT OF SERIAL, CORD-TO-CORD, MONKEY PASSAGES ON THE NEUROVIRULENCE AND *T* CHARACTER OF *T*— TYPE 1 POLIOVIRUS ISOLATED FROM A VACCINATED CHILD



* Log_{10} TCD_{50} per 0.1 ml.

stable for a certain number of passages in the CNS but under certain conditions may change to the $T+$ state, that is, the virus now isolated has the property of multiplying at 40°, as do most wild viruses recovered from patients paralyzed in nature.

It is evident from these data that viruses neurovirulent for monkeys, may either retain their $T-$ character as they are passed serially in monkeys, or they may after a number of passages change to the $T+$ state. The data also show that a virus after multiplication in man may possess a marked increase in monkey neurovirulence without an alteration of its $T-$ character. Furthermore, we have performed T tests with wild polioviruses isolated in Houston from 54 paralytic and 10 aseptic meningitis patients. As seen in Table 9, of the 43 Type 1 strains isolated from the paralytic cases, 33 were $T+$, 4 $T\pm$ and 6 $T-$; on the other hand, of the 10 Type 1 strains isolated from the aseptic meningitis cases, 9 were $T+$ and only one was $T-$, thus showing again that the $T-$ marker is not necessarily linked with lack of neurovirulence. Similar results were recently presented by Sabin.[10]

Monkey Neurovirulence and Viremia after Intramuscular Inoculation. We have recently carried out studies on another aspect of neurovirulence. By inoculation of virus into the muscle, we avoid any needle trauma to the brain or spinal cord, so that the total clinical response in the monkey can be attributed only to virus multiplication.

Furthermore, these studies measure two other properties of poliovirus: first, its capacity to multiply outside the central nervous system and produce viremia; and second, the capacity of the virus to gain entrance into the susceptible spinal cord from the peripherally-located muscle.

Table 10 shows a comparison of 4 virulent strains from nature; 3 attenuated strains (these being the Sabin strains used in the live polio-vaccine); and 24 strains designated "isolates." The latter are strains isolated from children who had received the Sabin material; they include 3 Type 1, 2 Type 2, and 19 Type 3 strains. The d and T markers are indicated in the second column, the virulent strains being $d+T+$, and the attenuated strains being $d-T-$. The isolates selected for tests in monkeys were those which had changed after multiplication in children, from the $d-$ state of the vaccine to the $d+$ state. Also, 21 of the 24 isolates had changed in the T marker, from negative to positive. The next column on the table indicates the dosage which was used. All the attenuated viruses and the isolates were tested at a dosage of at least seven logs of virus; the virulent strains were tested in two dose ranges as indicated in the table.

With the virulent strains, of 36 monkeys inoculated with undiluted tissue-culture material, 35 showed clinical responses (34 with paralysis, and 1 with only weakness); all 35 developed polio lesions. With the lower dose of virulent virus, 11 of the 16 animals developed disease, and this was also associated with lesions. As

TABLE 9. RESULTS OF T TESTS WITH POLIOVIRUS TYPE 1 AND 3 STRAINS FROM PARALYTIC AND ASEPTIC MENINGITIS PATIENTS IN HOUSTON IN 1958-59

DISEASE	NUMBER OF STRAINS TESTED	VIRUS TYPE	IN VITRO CHARACTER			TOTAL
			T+	T±	T−	
Paralytic	54	P-1	33	4	6	43
		P-3	9	1	1	11
Aseptic Meningitis	10	P-1	9	0	1	10
Total			51	5	8	64

TABLE 10. NEUROVIRULENCE AND VIREMIA AFTER INTRAMUSCULAR INOCULATION OF MONKEYS WITH VIRULENT AND ATTENUATED POLIOVIRUSES AND POLIOVIRUS ISOLATES FROM VACCINEES AND CONTACTS

STRAINS AND NO. TESTED	d, T MARKERS	DOSE Log TCD_{50}	INTRAMUSCULAR NEUROVIRULENCE				VIREMIA		
			NO. OF MONKEYS	NO. WITH DISEASE Par.*	Wk.*	NO. WITH CNS LESIONS	NO. POS/TOTAL AT DAYS 2	3	4
Virulent									
4	d+T+	7.8–8.8	36	34	1	35 (1 mild)	20/22	18/24	
		3.8–6.5	16	10	1	11	10/12	12/16	9/16
Attenuated									
3	d−T−	7.8–8.5	64	0	0	5 (all mild)	12/33†	0/8	0/12
Isolates									
3‡	d+T−	7.4–8.5	6	2	3	6 (4 mild)	0/2		0/2
21**	d+T+	7.0–8.5	52	17	5	26 (2 mild)	33/50		7/46

* Par. = paralysis.
Wk. = weakness.
† All 12 monkeys with viremia received Type 2 P_{712} vaccine virus.
‡ 3 Polio-1.
** 2 Polio-2 and 19 Polio-3.

shown in the last three columns of the table, viremia was present on days 2, 3, and 4, being a relatively common finding when a virulent strain was inoculated intramuscularly into monkeys.

The attenuated strains yielded an entirely different picture. Of 64 monkeys in these tests, none developed disease, and only 5 showed lesions, all of these being mild. The pattern of viremia was also different. Of 36 animals studied on the second day, 12 were positive; all 12 positives were Type 2. The chief importance of these data reside in the bottom two rows of the table—the data pertaining to the isolates. For the three $d+T-$ isolates, 5 of 6 monkeys developed disease, and all 6 animals showed lesions in the spinal cord. For the 21 $d+T+$ strains, 52 monkeys were used. Seventeen developed flagrant paralysis, 5 developed weakness, and 26 of the animals developed lesions—an attack rate of 50 per cent. As regards viremia, this was a common finding on the second day, when 33 of 50 animals were positive, but tapered off on the fourth day, when only 7 out of 46 animals were positive.

The data on the isolates are shown in greater detail in Table 11. In general, the pattern was the same for the different areas—for children fed during the winter in Houston and Cincinnati, or from children fed in warmer climates in Mexico. Paralysis occurred in many of the monkeys receiving strains with changed markers. However, a number of $d+T+$ Type 3 isolates failed to paralyze the injected monkeys, although they did produce viremia and in this way differed from the $d-T-$ Type 3 vaccine.

Thus, genetic change as measured by reversion in the $d-T-$ markers to $d+T+$ is not always associated with full intramuscular neurovirulence. Other factors, as yet unknown, are apparently also involved. This is further illustrated in the data in Table 12, which represents two families fed Type 3 vaccine virus in the Fox-Gelfand study in New Orleans. The vaccinated children in two families excreted a $T-$ virus on the first day and a $T\pm$ virus on the second day after the feeding, but later samples were $d+T+$. The $d+T+$ specimens from the vaccinated children failed to produce lesions or disease in four monkeys although all four animals developed viremia on day 2, and three on day 4. In contrast, $d+T+$ specimens from three

TABLE 11. NEUROVIRULENCE AND VIREMIA AFTER INTRAMUSCULAR INOCULATION OF ISOLATES FROM VACCINEES AND CONTACTS

STRAINS AND NUMBER TESTED	d, T MARKERS	DOSE (log TCD_{50})	INTRAMUSCULAR NEUROVIRULENCE				VIREMIA	
			NUMBER OF MONKEYS				NUMBER POSITIVE/TOTAL AT DAYS:	
			TOTAL TESTED	WITH DISEASE PARALYSIS	WEAKNESS	WITH CNS LESIONS	2	4
Cincinnati vaccinees:								
5 Polio–3	d+T+	7.0	20	5	3	10	11/20	1/20
Houston vaccinees:								
6 Polio–3	d+T+	7.4	12	0	1	2 (mild)	6/12	2/8
New Orleans vaccinees:								
2 Polio–3	d+T+	7.4	4	0	0	0	4/4	3/4
contacts:								
2 Polio–2	d+T+	7.4	4	3	0	4	4/4	0/4
5 Polio–3	d+T+	7.4	10	7	1	8	8/10	1/10
1 Polio–1	d+T−	7.4	2	1	0	2 (1 mild)	0/2	0/2
Mexico vaccinees:								
2 Polio–1	d+T−	8.5	4	1	3	4 (3 mild)		
1 Polio–3	d+T+	8.5	2	2	0	2		

family contacts were virulent for the six monkeys used in the tests. Thus here we have an example of a virus which had an initial change in its *dT* character in the vaccinated child, but which underwent a further increase in neurovirulence upon serial human passage of the virus.

Summary. The material presented above has been collected in an attempt to elucidate the following problems:

1. *The correlation of the d and T characters of polioviruses and their neurovirulence for monkeys and man.* It is evident from the data presented, that we do not possess as yet a clear *in vitro* method for discriminating among strains according to their degree of attenuation for monkeys. The relationship between the *d*+ character and increased neurovirulence appears suggestive. As regards the significance of the *T* character the interpretation of the data is difficult. Many strains isolated from vaccinated children were *T*−, but being *d*+, they still produced extensive damage in the CNS of monkeys. In addition, some *T*− strains isolated from paralytic patients were also found to be paralytogenic for monkeys.

It is obvious that there is a need to search for additional genetic markers of polioviruses. With

TABLE 12. CHANGES IN *d* AND *T* CHARACTER AND INTRAMUSCULAR MONKEY NEUROVIRULENCE OF TYPE 3 VACCINE VIRUS AFTER MULTIPLICATION IN VACCINEES AND CONTACTS

NEW ORLEANS FAMILY NUMBER	STATUS	DAY AFTER FEEDING	d and T CHARACTER	INTRAMUSCULAR MONKEY NEUROVIRULENCE: NUMBER OF MONKEYS INOCULATED	NUMBER OF MONKEYS WITH PARALYSIS	NUMBER OF MONKEYS WITH LESIONS
147	Vaccinated	1	T−			
		18	T+			
		75	T+d+	2	0	0
	Contact A	18	T+			
		54	T+d+	2	2	2
121A	Vaccinated	2	T±			
		26	T+			
		52	T+d+	2	0	0
	Contact A	29	T+d+	2	2	2
	Contact B	29	T+d+	2	2	2

a better understanding of virus markers and their mode of linkage in different strains, the task of discriminating between virulent and attenuated poliovirus strains may become simplified.

Nevertheless, the use of only two markers in the present study has proved of value in screening viruses isolated from vaccinated children, so that we were able to conserve our monkeys for neurovirulence tests only on altered strains, and not on strains selected at random.

2. *The genetic stability of attenuated polioviruses after multiplication in man.* A significant proportion of vaccinated children were found to excrete vaccine viral progeny possessing increased neurovirulence for monkeys inoculated by the intraspinal and intracerebral routes. Data obtained by tests using the intraspinal route have been severely criticized on the basis of associated traumatic damage in the cords, which has been said to explain the high rate of paralysis observed in our intraspinally inoculated monkeys. The new intramuscular data indicate clearly that viruses which are recovered from vaccinated children and which show changes in certain of their genetic characters show concomitant increases in their neurovirulence for the monkey, even when inoculated by a peripheral route which deposits the virus far from the site of the lesion in the central nervous system. Thus the vaccines used cannot be said to be genetically stable.

There is an important question which still remains to be answered: Do these changes in the *in vitro* markers and the associated increase in monkey neurovirulence have any significance as regards the use of these vaccines in man? This question is probably of greater importance for the contacts than for the vaccinees. Under certain epidemiologic settings, will the altered strains excreted by the vaccinees undergo progressive changes after several passages in the community and eventually reach a degree of virulence comparable to that of wild epidemic polioviruses?

REFERENCES

1. Melnick, J. L., and Brennan, J. C.: Monkey neurovirulence of attenuated poliovirus vaccines being used in field trials. In: *Live Poliovirus Vaccines, First International Conference.* Scient. Pub. no. 44 of the Pan

American Health Organization, pp. 65-101, 1959.

2. Murray, R., Kirschstein, R., Van Hoosier, G., Jr., and Baron, S.: Comparative virulence for Rhesus monkeys of poliovirus strains used for oral administration. In: *Live Poliovirus Vaccines, First International Conference.* Scient. Pub. no. 44 of the Pan American Health Organization, pp. 39-64, 1959.
3. Vogt, M., Dulbecco, R., and Wenner, H. A.: Mutants of poliomyelitis viruses with reduced efficiency of plating in acid medium and reduced neuropathogenicity. *Virology* **4:** 141-155, 1957.
4. Hsiung, G. D., and Melnick, J. L.: Effect of sodium bicarbonate concentration on plaque formation of virulent and attenuated polioviruses. *J. Immun.* **80:** 282-293, 1958.
5. Kanda, Y., and Melnick, J. L.: *In vitro* differentiation of virulent and attenuated polioviruses by their growth characteristics on MS cells. *J. Exp. M.* **109:** 9-24, 1959.
6. Lwoff, A.: Factors influencing the evolution of viral diseases at the cellular level and in the organism. *Bact. Rev.* **23:** 109-124, 1959.
7. Hodes, H. L., Zepp, H. D., and Ainbender, E.: A physical property as a virus marker. Difference in avidity of cellulose resin for virulent (Mahoney) and attenuated (LSc, 2ab) strain of Type 1 poliovirus. *Virology,* 1960. In press.
8. Hollinshead, A. C.: Differences in chromatographic behavior on cellulose columns of virulent Mahoney and attenuated LSc strains of poliovirus and similarities of base ratios of their nucleic acids. *Medicina Experimentalis,* 1960. In press.
9. McBride, W. D.: Antigenic analysis of polioviruses by kinetic studies of serum neutralization. *Virology* **7:** 45-58, 1959.
10. Sabin, A. B.: Paper presented at the *Gustav Stern Symposium-Perspectives in Virology,* New York City, January, 1960.

DISCUSSION

CHAIRMAN ANDERSON: There are two more papers this morning dealing with markers, and one the first thing this afternoon, so I am certain there will be discussion that revolves around all four of these papers.

Are there any comments at this time? This does not preclude coming back later for further discussion, after the other papers have also been presented.

DR. SABIN: The data that Dr. Melnick has presented are not new data. We have known for several years—and many people have demonstrated it before—of the changes in the viral population that occur after multiplication in the intestinal tract.

As regards these changes with the strains that I developed, it has previously been reported by myself and others that they are greater for Type 3 than they are for Type 1 and Type 2. This Dr. Melnick has again very nicely shown by indicating, for example, that he had in his studies no *rct*/40+ cultures of excreted virus after administration of Type 1 and Type 2, but a considerable number of such cultures of Type 3.

I have previously reported similar findings, although I did find, in testing a larger number of specimens, that occasionally also in the case of Type 2, after a long period of multiplication, one may get a culture that has increased capacity to multiply at 40° C.

The most important thing that has come out in our studies on Type 3 is that this change in the capacity to multiply at 40° C. seems to be acquired rather rapidly after multiplication in the intestinal tract, within a few days.

Furthermore, this property may not be present at all in the original stool, but may come out after a single propagation at 36° C. in monkey-kidney tissue culture.

I think that, in speaking of changes in viral progeny, it is important to keep in mind not only the changes that one finds in the culture, but also quantitative aspects. I believe that anyone who thinks in terms of viral genetics must think in terms of quantitative aspects of the property in the population of virus particles.

It is evident that the whole population of virus particles does not undergo a change. Therefore one must pay attention to the proportion of viral progeny that changes, which may then be selected either in the tissue culture or the monkey.

When studies are carried out on the original stool specimens, one can show that only 1 per cent or less of the viral particles may be involved in a given change. Therefore, even when one inoculates 60 tissue-culture doses intraspinally, a very sensitive place, it is potentially possible to give advantage to those few viral particles that have a greater capacity for multiplication in these neurons.

The new data that Dr. Melnick has presented on intramuscular inoculations, I think, again are not a reflection of anything more than has been observed before.

When you have a viral culture that has an increased capacity for producing paralysis by spinal inoculation, and one inoculates, as he has done, 10 million to 100 million tissue-culture doses intramuscularly, I know from previous experience that that will lead to greater invasion of the axis cylinders of the neurons which are present in the muscles. The muscles contain the processes of the anterior horns in the spinal cord.

No one has ever criticized the results presented by Dr. Melnick last year on the basis of the traumatic changes produced in the spinal cord. It was merely pointed out that if the virus inoculum is artificially spread out over a larger portion of the spinal cord, the incidence of paralysis can be greater.

Now, with regard to the data on contacts of children fed the Type 3 vaccine that Dr. Melnick has presented, I have also tested those same specimens, and the results may be seen in the proceedings of last year's Conference. Dr. Melnick has presented results only with one large dose of virus, and I presented data with several doses.

When the thing is done quantitatively, one can show that the virus is still attenuated, because with 100,000 TCD_{50} you get no effect.

There was a time when Dr. Melnick worked

with 100,000 TCD_{50}. Now he is doing all his tests with larger doses intracerebrally and disregards the quantitative aspects. The significance of the observed changes which were first reported by myself and others—and only being mentioned again by Melnick—must be based upon the epidemiologic observations that have been made in the field, when these particular viruses were used, and the abundant epidemiologic observations now available indicate that these changes which have been shown in monkeys and in tissue culture are not associated with harmful effects in human beings.

DR. ZHDANOV *(through an interpreter)*: At this moment we are dealing with an important matter, the stability or nonstability of the strains. This is the point being discussed.

When we use the vaccine of the active virus, I think we could have understood apropos that the virus progeny cannot be stable. I will repeat what Dr. Koprowski said, that the virus not only is not stable, but develops in several directions, including the partial return to the previous virulence.

Of course, this is a very important fact, but it seems to me that however interesting it may be from the point of view of the genesis of viruses, and the question of the safety of live antipolio vaccine, we must take into consideration ecological considerations. I replace epidemiology by ecology, because I think epidemiology is better defined by the ecology.

From this point of view it will of course be exceedingly interesting, in connection with the fact that many countries at present are conducting serious campaigns against poliomyelitis with live vaccines, to follow the fate of these viruses. But if one takes the ecological approach—which, of course, is perhaps the more difficult one, but which should be taken into consideration—it seems to me that the live virus of poliomyclitis, attenuated and taken as a vaccine, does not have any chances, not any chances whatever, to compete with wild viruses.

That is why, with all the interest in the importance of this research, I do not think that this concerns the question of the safety of applying vaccine as a mass vaccination.

I will bring you an example from another field, which shows how difficult it is to circulate a virus among the population I am speaking here of the influenza virus. You know that it is sufficient for a new antigenic variety of influenza to appear, for its spread to begin among the population. And at the same time the old viruses, the very energetic wild viruses that live very well in the human organism, do not survive any competition with this new virus. They very quickly decrease and completely disappear and remain only in laboratories or in animals.

It is not so easy for a new virus to take its place among the population. Therefore, it seems to me that from this point of view, taking the question from the viewpoint of the safety of live vaccine, we must take into account this ecological consideration; the 10-year experience of which Dr. Koprowski spoke here confirms the thought that, even in the most massive immunization, live attenuated viruses have no chance to compete with wild viruses.

There is a condition of immunity which kills both types of viruses. These are the remarks I wish to add to what has been advanced here.

DR. MELNICK: I would like to say that I am glad to see first Dr. Koprowski in his talk, and Dr. Sabin later, emphasizing the fact that the viruses in the vaccines do change after multiplication in man. Because this emphasis was not clearly expressed by them a year ago, I feel that it is all to the good to have agreement on this point. The question on which we now agree is: Just how much change in the virus can be tolerated by a child who has received the oral polio vaccines, or their progeny? Last year we argued about whether the virus changes or not in the course of multiplication in man. I am glad to see we are all agreed now.

I would also like to point out that the change observed is considerable. Dr. Sabin indicated that when we inoculated rectal swabs, some of them contained 60 tissue-culture doses, but many of these contained only 10 tissue-culture doses. This is as small a dose as we can give to a monkey with virulent virus and expect a take. When 10 tissue-culture doses are able to produce paralysis in the monkey, whereas it takes 10,000 doses of the original vaccine, this fact, to me, represents a considerable change in the virus and does not represent a selection of virulent virus by the central nervous system of the monkey.

CHAIRMAN ANDERSON: The next paper will be presented by Dr. Cabasso of the Lederle Laboratories, Pearl River, New York, on "Assessment of Correlation between Certain *in vitro* Poliovirus Markers and Monkey Neurovirulence."

2. ASSESSMENT OF CORRELATION BETWEEN CERTAIN *IN VITRO* POLIOVIRUS MARKERS AND MONKEY NEUROVIRULENCE

V. J. CABASSO, E. L. JUNGHERR, S. LEVINE, A. W. MOYER, M. ROCA-GARCÍA, AND H. R. COX

Viral and Rickettsial Research, Lederle Laboratories, American Cyanamid Co., Pearl River, N. Y.

DR. CABASSO (*presenting the paper*): The availability of proved and stable laboratory assays other than the monkey test for determining the degree of virulence of polioviruses would indeed be of great value. Search for such assays has uncovered in recent years certain *in vitro* characteristics, the so-called *d*, *t* and *MS* "markers," which have been proposed as criteria of acceptability of poliovirus strains intended as live oral vaccines.[1] But already evidence suggests that at least the *d* marker is not as well correlated with monkey neurovirulence as was originally thought and information accumulated about the *MS* character is as yet insufficient. Consequently, until the value of these markers is definitely established, the residual neurovirulence of a poliovirus strain for monkeys remains its most reliable marker and the most acceptable measure of its attenuation.

It is the purpose of this communication to present results of monkey tests on recent lots of Lederle oral poliovirus vaccine, to examine the degree of correlation between the findings and the marker pattern of the vaccine strains and to discuss the relative validity of these *in vitro* characters.

Results of recent monkey tests on Lederle oral poliovirus vaccine. As all known poliovirus vaccine strains have some degree of neuroactivity in monkeys when inoculated intraspinally, the present discussion of Lederle virus strains will be limited to results of intracerebral inoculation. Detailed monkey test results have been reported[2] on 8 Type 1, 11 Type 2, and 11 Type 3 vaccine lots. The histological examination, however, was limited in these tests to the cervical and lumbar enlargements of the cord, as brains were not removed from inoculated animals.

With recent vaccine lots, the animals were submitted to a more complete histological scrutiny. The brain stem was dissected into three portions at approximate levels of the red nucleus, and the abducens and hypoglossal nerves, and the thalamus was examined for inoculation trauma. Only animals with a definite track in the thalamus were considered valid. The three portions of the brain stem, the thalamus and pre- and post-central gyri, as well as the upper, middle, and lower segments of the cervical and lumbar enlargements, were embedded in paraffin, orientation being maintained by ink marks.

A total of 41 sections per monkey were stained by the gallocyanin method and examined. Lesions were estimated according to Melnick's system[3] on a scale of + to ++++. Although this system permitted immediate appraisal of lesions as to location, distribution, severity, and relation to needle track, a formula was still required to evaluate relative neurovirulence among vaccine lots. Such a formula would allow detection of any undue changes when successive lots of vaccine are produced from the same or different seed virus. Accordingly, criteria were established which were based primarily upon spread or extension of lesions from site of inoculation. These were expressed in grades 1 to 4 as shown in Table 1. Grade 0 was assigned to no lesions or to track lesions with local inflammatory reactions; one to significant inflammatory and neuronal changes homolateral to the track; two to lesions in brain stem; three to spread to cervical or lumbar cord; and grade four to spread to all portions of the CNS.

In the final evaluation of results, grades 1 and 2, which symbolized little or no spread from the site of inoculation, were considered not significant, and the relative neurovirulence of a vaccine lot was expressed as the sum of per cent of animals showing grades 3 and 4. By this grading system it was found that, in consecutive lots involving the examination of a total of 574 monkeys, the average incidence of combined

TABLE 1. FORMULA FOR EVALUATION OF RELATIVE MONKEY NEUROVIRULENCE, OR DEGREES THEREOF, AFTER INTRACEREBRAL INOCULATION

GRADE	CRITERIA
0	NO LESIONS OR TRACK LESION W/LOCAL INFLAMMATORY REACTION
1	SIGNIFICANT INFLAMMATORY & NEURONAL CHANGES HOMOLATERAL TO TRACK
2	LESIONS IN BRAIN STEM
3	LESIONS IN BRAIN STEM & SPREAD TO CERVICAL OR LUMBAR CORD
4	SPREAD TO ALL PORTIONS OF THE CNS

grades 3 and 4 was 21 per cent for Type 1, 57 percent for Type 2, and 16 per cent for Type 3. Thus, a base line of normally expected neurovirulence for each type of Lederle polioviruses was established as a reference for recognizing a vaccine lot with increased activity in monkeys.

As summarized in Table 2, 4 of 12 tests performed on 10 Type 1 lots of vaccines, 3 of 7 tests on 6 Type 2 lots, and 5 of 10 tests on 5 Type 3 lots were above average. It must be emphasized, however, that the animals were inoculated with very large doses of virus (from over 10^5 to over 10^7 TCD_{50}); that most reactions occurred at 10^7 and 10^6 levels except for Type 2 virus; and that the paralyses or weaknesses observed were non-progressive and non-fatal. In contrast, with much smaller doses of virulent polioviruses, sometimes only a few TCD_{50}, close to 100 per cent of animals showed not only severe histo-pathological changes but also developed progressive paralysis and died of the disease 5 to 10 days following inoculation.

Some variation was observed between two consecutive tests with the same lot of vaccine (Table 2). For example, Type 1 tests Nos. 2 and 3 were obtained at two different times on the same lot

TABLE 2. PARALYTIC RATES AND DEGREES OF HISTOLOGIC LESIONS OF LEDERLE STRAINS OF POLIOVIRUS VACCINE IN SUCCESSIVE LOTS

	TEST NO.												
	1	2	3	4	5	6	7	8	9	10	11	12	Av.
TYPE I													
PARALYTIC RATE (%)	13	[0	13]	0	0	0	[44	0]	0	0	0	15	8
HISTOL. LESIONS (%)	32	[5	21]	0	10	10	[50	10]	6	13	26	50	21
TYPE II													
PARALYTIC RATE (%)	0	0	0	0	[0	5]	13						2
HISTOL. LESIONS (%)	45	54	30	70	[53	85]	65						57
TYPE III													
PARALYTIC RATE (%)	[0	0]	0	0	27	0	10	0	29	0			6
HISTOL. LESIONS (%)	[5	50]	0	0	36	16	25	20	20	0			16

Underlined figures denote pools showing above average neurovirulence.
Brackets indicate results of duplicate tests on the same vaccine lot.

of virus, as were tests Nos. 7 and 8 on another lot. Similarly, Type 2, Nos. 5 and 6, and Type 3, Nos. 1 and 2, results were also obtained in duplicate tests on the same materials. No ready explanation can be advanced for these seeming discrepancies except that the physical condition of the monkeys in the different tests varied substantially.

The Type 2 Lederle vaccine strain has always shown greater intracerebral activity in monkeys than the other two types. It must be emphasized again, however, that this activity manifests itself only in histo-pathologic changes and not in paralysis or other clinical signs in the animals. It may well be that this particular Type 2 strain owes its neuronophilic characteristic to its long intracerebral passage history in mice and suckling hamsters [4-7], and that, in this respect, it may be compared to the 17D yellow fever vaccine strain.[8]

Paradoxically, however, this same Type 2 virus seems to possess most of the other laboratory characteristics currently attributed to avirulent polioviruses as well as the lowest infectivity for the human intestinal tract. Of the three Lederle vaccine strains, it is, as will be indicated later, the one with the most favorable "marker" picture. It is also the one which results in the lowest conversion rates from seronegative to seropositive (65 to 75 per cent in most instances as compared to 85 to 95 per cent for Types 1 and 3). Furthermore, it has consistently shown the least spread from vaccinees to either contact siblings or the community and, unlike Lederle Types 1 and 3 strains, Type 2 virus recovered from either vaccinees or their contacts has caused no paralysis in monkeys after intracerebral inoculation of stool suspensions containing up to $10^{5.3}$ TCD_{50}/ml.

In vitro Marker Characterization of Lederle Poliovirus Vaccine Strains. The *d*, *t* and *MS* characters of Lederle poliovirus strains in relation to their residual monkey neurovirulence are shown in Table 3. The *MS* character was obtained by the tube titration method rather than by efficiency of plating, and ratings of the three markers were made by criteria described by Melnick and co-workers [9] and Kanda and Melnick.[10] All three viruses possessed the *t*—character, but they differed in their *d* and *MS* markers; these were *d*+ *MS*± for Type 1, *d*± *MS*— for Type 2 and *d*+ *MS*+ for Type 3. Type 2 virus, which has consistently produced the most histologic change in monkeys, has a marker pattern most like that suggested for avirulent polioviruses.

Pertinent questions at this time are how truly representative of avirulence these markers are, and how stable they may be in relation to monkey neurovirulence. Correlation between these characters and monkey neurovirulence was initially established by the use of known attenuated and virulent poliovirus strains, but whether these characteristics were inevitable accessories of avirulence in all polioviruses, or only characteristic of the particular attenuated strains studied, with incidental or no relation to avirulence, remains to be determined. Obviously this aspect of markers can be evaluated only through extensive investigation of a whole range of natural and laboratory induced poliovirus variants.

A discrepancy connected with the *d* marker was already apparent in the original published report on this character [11]; Type 1 SM 90 strain, which was judged attenuated by the neuro-

TABLE 3. "MARKER" CHARACTERIZATION OF LEDERLE POLIOVIRUS VACCINE STRAINS IN RELATION TO THEIR RESIDUAL MONKEY NEUROVIRULENCE

VIRUS TYPE	MARKER			DEGREE OF INTRACEREBRAL NEUROVIRULENCE (%)
	d	t	MS*	
I	+	–	±	21
II	±	–	–	57
III	+	–	+	16

* MS values were obtained by tube titration method rather than efficiency of plating.

virulence test, possessed the $d+$ character associated with the more virulent strains used in the same study. Similarly, Hsiung and Melnick [12], working with natural poliovirus isolates, or with viruses recovered from persons fed avirulent virus, found several $d+$ strains which were classified as attenuated by the monkey test. Furthermore, Sabin has reported [13] that a laboratory induced $d+$ variant of his Type 1 vaccine strain remained as avirulent for monkeys as was the original d variant. More recently Yoshioka and co-workers,[14] in a study of polioviruses excreted by children fed Sabin's Type 1 vaccine strain, or those isolated from flies trapped in the trial area, found that the d marker was not always in agreement with results of the monkey virulence tests.

A systematic study of the effect that induced variation of the d and t characters may have on the monkey virulence of an attenuated $d-t-$ poliovirus was undertaken in our laboratory with the original LSc Type 1 virus, for which we are indebted to Dr. Morris Schaeffer. The history of the virus sample received is summarized in Table 4.

In Dr. Schaeffer's laboratory the virulent Mahoney strain was successively passed 14 times intracerebrally in monkey, twice in monkey testis tissue culture, six times in monkey skin graft in chicken embryos, 34 times in monkey testis, 11 times in monkey skin and, finally, 13 times in monkey kidney-tissue culture. Upon receipt in our laboratory, the 13th monkey kidney-tissue culture passage was plaqued four consecutive times on monkey kidney monolayers by the Dulbecco method [15]; pool 410 LSc, used in this study, represented the progeny of the fourth plaquing. This pool clearly possessed the $d-t-$ characters. Starting with pool 410, variants of various marker combinations were induced as indicated in Fig. 1. A $d+t-$ pool was obtained after three plaquings in monkey-kidney tissue culture under low bicarbonate overlay. Three terminal dilution passages in monkey-kidney tube cultures at 39° C., followed by three similar passages at 40° C., yielded a $d+t+$ variant from the $d+t-$ pool. Finally, the $d-t+$ variant came directly from the $d-t-$ pool, after successive passages, twice at 39° C. and twice more at 40° C. by the terminal dilution method.

Table 5 summarizes the results obtained by virulence tests in monkeys with the four pools of virus as well as the effect which variation of the d and t markers had on the *MS* marker. It can be observed that as long as the $t-$ marker remained unchanged, varying $d-$ to $d+$ did not induce any change in the virulence of the virus for monkeys; no paralysis followed intramuscular or intracerebral inoculation of either $d-t-$ *or* $d+t-$ variants, and histologic lesions were only of grades 1 and 2 as defined earlier.

In contrast, changing the $t-$ character to $t+$ was accompanied by reappearance of monkey virulence by both intramuscular and intracerebral routes. Similarly histologic lesions were more intense.

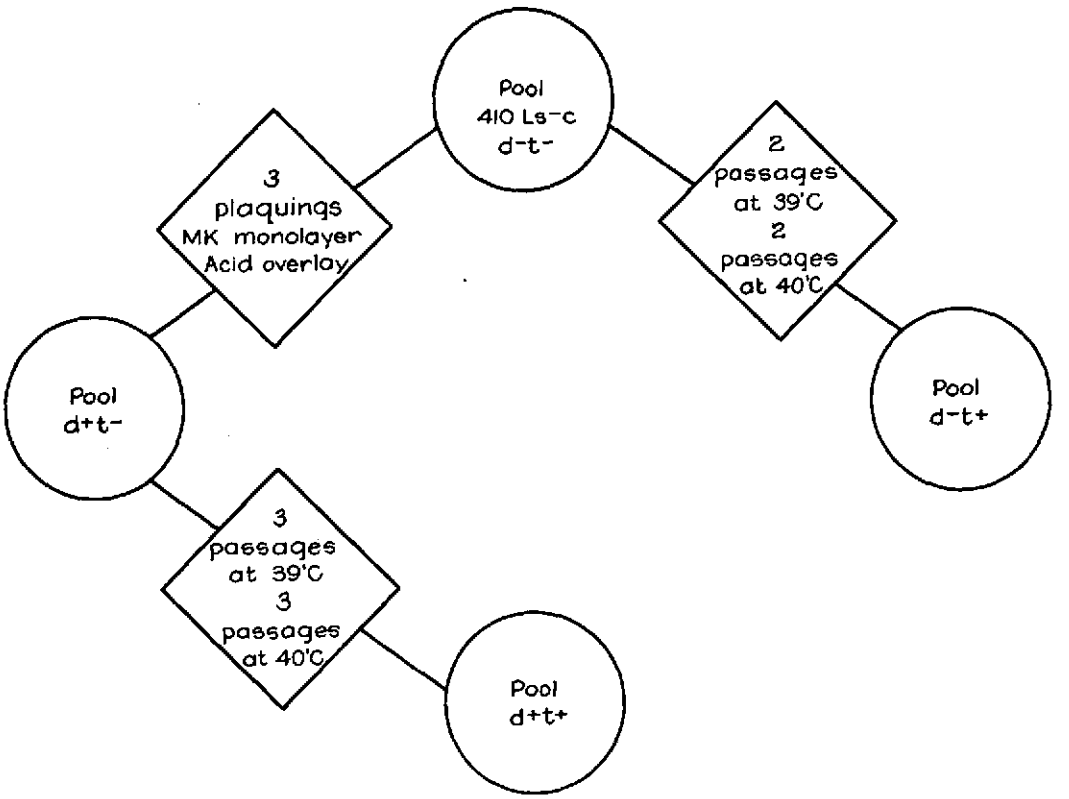
Variation of either the d or t characters had no effect on the *MS* marker, which remained unchanged in all three induced variants.

Thus, in confirmation of the findings of others, it appears that presence of a $d+$ marker in a

TABLE 4. HISTORY OF TYPE 1 LSc VIRUS OBTAINED FROM DR. SCHAEFFER

SCHAEFFER'S LAB	MAHONEY STRAIN (VIR) ⟶ 14 MONKEY PASSAGES (IC) ↓ 2 MONKEY TESTIS PASSAGES ↓ 6 MONKEY SKIN GRAFT IN CHICK EMBRYOS ↓ 34 MONKEY TESTIS PASSAGES ⟶ 11 MONKEY SKIN PASSAGES ↓ 13 MONKEY KIDNEY PASSAGES
LEDERLE LAB	↓ 4 CONSECUTIVE PLAQUINGS IN MONKEY KIDNEY MONOLAYERS ↓ POOL 410 Ls-c PROGENY OF 4th PLAQUING USED IN PRESENT STUDY (d-t-)

FIG. 1. Induction of variants of various marker combinations from d—t— Schaeffer's LSc Type 1 poliovirus.



poliovirus strain is not necessarily synonymous with virulence for monkeys.

As regards the *t* marker, however, the finding by Lwoff and co-workers [16] that selection of *t*+ variants from an originally *t*— strain was accompanied by reappearance of monkey virulence implied a closer correlation between the *t* marker and neurovirulence than is the case for the *d* character. Our results support this implication. It may be, as intimated by Lwoff, that this correlation is due to the relative multiplication rates of "cold" or "hot" virus variants within the central nervous system of the monkey whose normal body temperature varies between 39° C. and 40° C. In this regard, it may be interesting to speculate on what the results might be if Pasteur's classic experimental method with anthrax in chickens were applied to an avirulent *t*— poliovirus strain in monkeys. Would the behavior of the virus remain the same in an animal whose body temperature was lowered to that optimal for virus multiplication? Be that as it may, as yet insufficient investigation of field isolates has been reported to be sure that a *t*+ character is invariably associated with neurovirulence, and some exceptions have already been pointed out. For example, a mouse-selected derivative of Type 2 MEF_1 virus which lost its neurovirulence for monkeys was shown by Sabin to be still capable of growing readily at 39° and 40° C., perhaps even better than a fully virulent Type 1 Mahoney strain.[13] Also, in at least one instance a *d*+*t*+ Type 1 isolate from children fed Sabin's LSc strain was found by Yoshioka and others [14] to be still avirulent for monkeys by the intracerebral route. On the other hand, Melnick *et al.*,[17] studying viruses excreted by children vaccinated with Sabin's strain of virus, obtained evidence

TABLE 5. MONKEY TEST RESULTS AND *MS* CHARACTER OF VARIOUS *d* AND *t* VARIANTS OF SCHAEFFER'S *d*—*t*—LSc TYPE 1 POLIOVIRUS

	VARIANT			
	d-t-	d+t-	d-t+	d+t+
TCD_{50}/ML	7.5	7.5	6.5	7.5
MS CHARACTER	MS-	MS-	MS-	MS-
PARALYTIC RATIO:				
I.M., UND.	0/4	0/4	3/4	5/5
I.C., UND.	0/4	0/4	2/4	3/3
I.C. 10^{-1}	0/4	0/4	2/4	4/4
HISTOL. RATIO:				
I.M., UND.	3/4*	1/4*	3/4	5/5
I.C., UND.	0/4	2/4*	4/4	3/3
I.C. 10^{-1}	0/4	3/4*	4/4	4/4

* LUMBAR LESIONS ONLY

that excreted virus which became of increased neurovirulence for monkeys could still retain the *t*— character.

Information available on the *MS* marker is based primarily upon laboratory studies with a limited number of strains and little can be said at this time about its correlation with monkey virulence. However, it should be pointed out that in the original study by Kanda and Melnick,[10] the correlation did not hold for a virulent Type 3 strain (Saukett) which possessed an *MS*— instead of an *MS*+ character.

It would be of interest to study induced *MS*+ variants from known attenuated *MS*— strains to determine whether associated changes occur in their monkey virulence or other markers. Such an attempt is now in progress.

DISCUSSION

No one questions the necessity for thorough laboratory testing of poliovirus strains intended for use in human immunization, by methods which genuinely portray the safety of these strains. But it should be stressed at this point that, in considering the acceptance of a vaccine strain, the laboratory criteria employed must be consonant with the behavior of the strain in the ultimate host, and that laboratory and field findings supplement one another in the characterization of the agent. For example, 17D yellow fever virus has a long history of proved safety in man although still capable of paralyzing and killing up to 20 per cent of monkeys inoculated intracerebrally, and of provoking central nervous system changes in the rest.

No doubt the *d*, *t* and *MS* markers do represent useful investigational tools of polioviruses. But it is important to recognize our ignorance concerning the basic mechanisms involved in neurovirulence and the degree to which these various markers reflect this mechanism. The correlation of these markers with neuroactivity is imperfect, indicating that other and unknown factors also play a role in neurovirulence. This is in all probability to be expected, since the growth of a virus within a cell is dependent not only upon the environmental conditions outside the cell, as pH and temperature, but also upon the presence of proper receptors on the cell surface and perhaps an entire battery of as yet unknown enzymes necessary for initiating and carrying out the complex operation of virus synthesis and release. It seems therefore, that at this stage of knowledge one is hardly justified in considering as indicators of avirulence unstable secondary characteristics of as yet unproved significance, when

the direct method of assay in monkeys is readily available and accords well with the field behavior of the strains in the human host.

SUMMARY

Base lines of normally expected neuroactivity for each type of Lederle attenuated polioviruses in consecutive lots of vaccine were established by grading on a scale of 1 to 4 the spread of lesions from the site of inoculation in 574 monkeys inoculated intracerebrally with consecutive lots of vaccine. Relative neuroactivity of a vaccine lot was expressed as the sum of per cent of animals showing grades 3 and 4.

The average incidence of combined grades 3 and 4 was 21 per cent for Type 1, 57 per cent for Type 2, and 16 per cent for Type 3 virus. The greater intracerebral activity of Type 2 virus, which expresses itself in histopathologic lesions and not in paralysis, is in marked contrast to its clinical behavior in man, where it shows the least "aggressiveness" of the three poliovirus strains, and to its marker pattern which conforms most closely to the one proposed for avirulence.

All three Lederle poliovirus strains possess the *t*— character, which appears to have the best correlation with monkey avirulence, but differ in their *d* and *MS* markers; Type 1 was found to be *d*+ *MS*±; Type 2, d± *MS*—; and Type 3, *d*+ *MS*+. Some of the discrepancies in the correlation between markers and monkey virulence are discussed.

Since the value of *d*, *t*, and *MS* markers as criteria of attenuation for polio vaccine viruses has not been definitely established, residual neurovirulence for monkeys remains the most acceptable measure of strain attenuation.

REFERENCES

1. Advisory Committee to the Surgeon General. Public Health Service. 2nd Report of the Committee on Live Poliovirus Vaccine. 1959.
2. Cabasso, V. J., Jervis, G. A., Moyer, A. W., Roca-García, M., Orsi, E. V., and Cox, H. R.: Cumulative testing experience with consecutive lots of oral poliomyelitis vaccine. *Brit. M. J.* I (5170): 373-387, (Feb. 6) 1960.
3. Melnick, J. L. and Brennan, J. C.: Monkey neurovirulence of attenuated poliovirus vaccines being used in field trials. In: *Live Poliovirus Vaccines, First International Conference.* Scient. Pub. no. 44 of the Pan American Health Organization, pp. 65-101, 1959.
4. Moyer, A. W., Accorti, C., and Cox, H. R.: Poliomyelitis. I. Propagation of the MEF_1 strain of poliomyelitis virus in the suckling hamster. *Proc. Soc. Exp. Biol.* **81**(2): 513-518, 1952.
5. Roca-García, M., Moyer, A. W., and Cox, H. R.: Poliomyelitis. II. Propagation of the MEF_1 strain of poliomyelitis virus in developing chick embryo by yolk sac inoculation. *Proc. Soc. Exp. Biol.* **81**(2): 519-525, 1952.
6. Cabasso, V. J., Stebbins, M. R., Dutcher, R. M., Moyer, A. W., and Cox, H. R.: Poliomyelitis. III. Propagation of MEF_1 strain of poliomyelitis virus in developing chick embryo by allantoic cavity inoculation. *Proc. Soc. Exp. Biol.* **81**(2): 525-529, 1952.
7. Cabasso, V. J., Stebbins, M. R., and Cox, H. R.: Poliomyelitis. IV. Some cultural and other characteristics of chick embryo-adapted Type 2 strain of poliomyelitis virus. *Proc. Soc. Exp. Biol.* **85**(1): 167-171, 1954.
8. Strode, George K.: *Yellow Fever.* New York. McGraw-Hill Book Co., 1951, 710 pp.
9. Melnick, J. L., Benyesh-Melnick, M., and Brennan, J. C.: Studies on live poliovirus vaccine. Its neurotropic activity in monkeys and its increased neurovirulence after multiplication in vaccinated children. *J. Am. M. Assn.*, **171**(9): 1165-1172 (Oct. 31) 1959.
10. Kanda, Y., and Melnick, J. L.: *In vitro* differentiation of virulent and attenuated polioviruses by their growth characteristics on *MS* cells. *J. Exp. M.* **109**(1): 9-24, 1959.
11. Vogt, M., Dulbecco, R. and Wenner, H. A.: Mutants of poliomyelitis viruses with reduced efficiency of plating in acid medium and reduced neuropathogenicity. *Virology* **4**(1): 141-155, 1957.
12. Hsiung, G. D., and Melnick, J. L.: Effect of sodium bicarbonate concentration on plaque formation of virulent and attenuated polioviruses. *J. Immun.* **80**(4): 282-293, 1958.

13. Sabin, A. B.: Recent studies and field tests with a live attenuated poliovirus vaccine. In: *Live Poliovirus Vaccines, First International Conference.* Scient. Pub. no. 44 of the Pan American Health Organization, pp. 14-33, 1959.

14. Yoshioka, I., Riordan, J. T., and Horstmann, D. M.: Thermal sensitivity of polioviruses isolated during oral vaccine field trial: comparison with monkey neurovirulence. *Proc. Soc. Exp. Biol.* **102**(2): 342-347, 1959.

15. Dulbecco, R. and Vogt, M.: Plaque formation and isolation of pure lines with poliomyelitis viruses. *J. Exp. M.* **99**(2): 167-182, 1954.

16. Lwoff, A. and Lwoff, M.: Remarques sur les facteurs aspécifiques, gouvernant l'évolution des infections virales: La notion d'état critique. *Compt. rend. Acad. sc.* **248:** 154-156, 1959.

17. Melnick, J. L. and Benyesh-Melnick, M.: Correlation of *in vitro* markers of poliovirus strains and monkey neurovirulence. 2. Intramuscular inoculation of vaccine progeny strains and serial spinal cord passage of t— strains. *Fed. Proc.* **19**(1): 407 (Abst. #13), 1960.

DISCUSSION

CHAIRMAN ANDERSON: These papers are now open for discussion.

DR. ARMSTRONG: The virus of which I speak is not a poliovirus, but is a neurotropic virus. The mouse is its natural host, and these observations, I think, may have some bearing on some of the unknown factors which play a part in susceptibility following feeding.

I had an isolate of lymphocytic choriomeningitis from 1933. It had been carried through about 200 mouse-brain-to-mouse-brain passages, and had been stored in the deep freezer. So I took that out and thought I would try feeding it, as we have had so little experience with vaccines administered by feeding.

I found that this virus, when fed to mice, or given by stomach tube, failed to give any symptoms whatever. But when I tried it intracerebrally it was lethal in about three hundredths of a cc. of one to a million dilution. When fed, it produced high immunity. Here, then, was a virus which was harmless when fed, but which was lethal in one to one million of a cc. dose when given intracerebrally.

Now, I think it would mean more to me if these viruses which were tested and found to change their virulent markers, had been found outside the intestinal tract—if it is more virulent, it should invade the intestinal tract. But these viruses were isolated from the intestinal tract. One cannot be sure it was the same virus.

I think that search should be made for these viruses of changed character, not in the intestine, but after invasion from the intestine.

What enables it to penetrate the intestine may be an entirely different thing than what enables it to affect the brain.

Now, when we give a virus into the brain, we do several things. We break the meninges, injure nerve tissue, rupture capillaries and blood vessels, and put the virus on high concentration, under pressure, in contact with injured cells which have had no experience at all in dealing with infection by this route.

In the intestinal tract, however, we are dealing with a tissue that has been for ages the first line of defense against the infection invasion from that route.

DR. SABIN: I would like to ask Dr. Armstrong to tell us whether or not the LCM virus he fed to mice showed any evidence of multiplication in the intestinal tract, and then I shall be able to digest his interesting data a little better.

DR. ARMSTRONG: I cannot absolutely say as to multiplication, but I was able to recover it from the intestinal tract, after considerable time.

DR. COX: I wish to ask whether they become immune.

DR. ARMSTRONG: In seven or eight days they become immune, completely immune.

CHAIRMAN ANDERSON: He said was there an immunity in the mice.

Are there any further comments? Dr. Voroshilova.

DR. VOROSHILOVA (*through an interpreter*): The experiences and observations regarding these questions concern the immunizations which were conducted in Moscow.

We had to choose children of more advanced age, because the virus was not influenced by preceding immunity. Here we took eight strains which had been excreted by four children, triple-positive, from Karaganda 3–10 days after their feeding, and after four and five weeks, respectively.

In another research, which we conducted in the Estonian Republic, the strains of Type 3 were excreted by small children who had no antibodies.

In the third research, we had strains $T\pm$ from children up to three years of age who had been in contact with immunized children.

Among these strains we noted multiple variations; the strains which were excreted in Karaganda from the older children, in general, had $T-$ marker.

Of the 22 monkeys infected intraspinally there was paralysis only in one on the 28th day.

Perhaps these results are interesting in the sense that they were conducted during field research work, on a large scale, and showed that no changes had occurred in children. All those who had been vaccinated were absolutely healthy. Therefore, the differences of strains practically showed no influence in the final results of the vaccination.

3. A PHYSICAL PROPERTY AS A VIRUS MARKER: DIFFERENCE IN AVIDITY OF CELLULOSE RESIN FOR VIRULENT (MAHONEY) AND ATTENUATED (LSc, 2ab) STRAIN OF TYPE 1 POLIOVIRUS

HORACE L. HODES, HELEN D. ZEPP, AND EUGENE A. AINBENDER
The Mount Sinai Hospital, New York, New York

DR. HODES (*presenting the paper*): The work which I am about to describe came about as a result of studies we were making with radioactive poliovirus. We had grown polioviruses in the presence of radioactive phosphorus. In attempting to separate the virus from other materials, we made use of cellulose resin columns. The one we finally came to use was the diethylaminoethyl cellulose resin ion-exchange column which had been used by Hoyer and his associates.

In working with the viruses it became clear that the manner in which the viruses were eluted from this column was quite regular and quite reproducible.

This gave us the idea that perhaps the pattern of elution of a virus strain from the DEAE column was a constant characteristic, and that if this were so, we might have a physical marker which could conceivably be correlated with neurovirulence or lack of it.

We showed very soon that the Type 1 Mahoney strain came off the column in a very regular and reproducible way. We obtained from Dr. Sabin a number of attenuated strains.

The first one we worked with was the LSc,

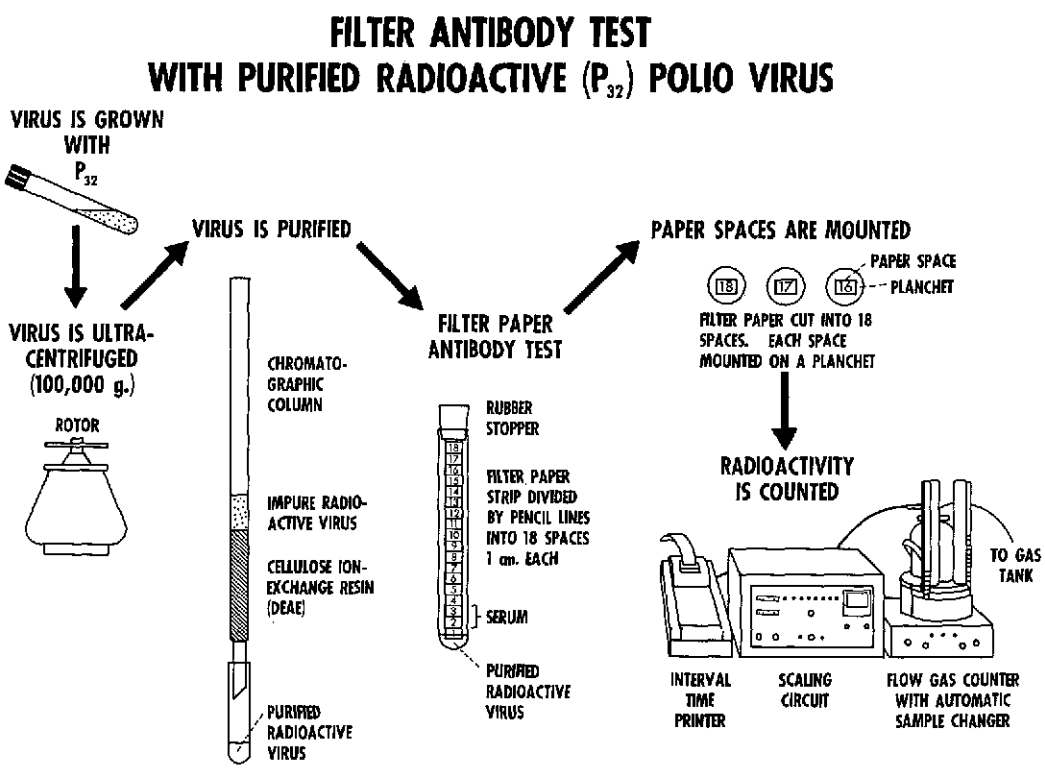


FIG. 1

2ab strain, which had not been through human beings. The elution pattern of this virus of strain was quite different from that of the Mahoney strain.

Dr. Sabin then sent us two additional strains, obtained from a child which he had fed the LSc, 2ab strain. He recovered the first strain seven days after the child had been fed the vaccine virus, and the second strain he sent us had been recovered 19 days after feeding of the strain of the vaccine material.

The seven-day strain proved to have exactly the same elution pattern as that of the vaccine strain, but the 19-day pattern had an intermediate sort of pattern.

At this point, I would like to explain the method we used in more detail.

Your attention is called to the left-hand part of Figure 1. The method we follow is to grow virus in tissue culture. With this work the monkey-kidney tissue cultures are used, 30 cc. of virus are obtained, and centrifuged at 100,000 G. This centrifugate is made up in 2 ml. of 0.02 M phosphate buffer. This material is loaded onto a cellulose ion-exchange column, the column being 8 centimeters long and 2 centimeters in diameter.

Before the virus is put on, this column has been equilibrated with the 0.02 M phosphate buffer. In other words, the virus pellet is suspended in 2 ml. of the buffer, then put on the column and eluted off with 2 cc. amounts of the phosphate buffer, and the corresponding eluates are collected.

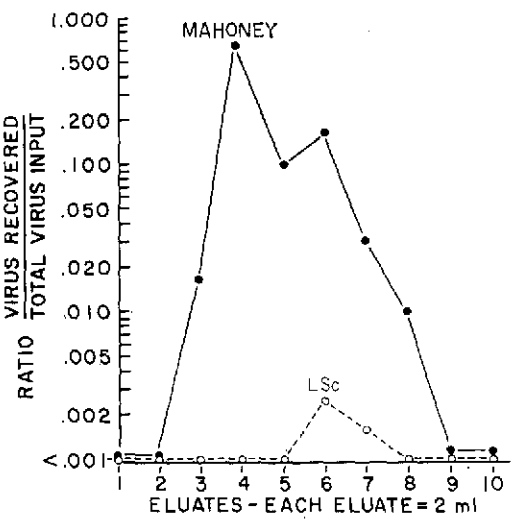


FIG. 2

Figure 2 shows the results obtained with Mahoney virus and LSc, 2ab strain. Along the x axis are plotted the various eluates, each eluate being 2 ml. Along the y axis is plotted a fraction which represents the ratio of the virus recovered to the total virus input. In the fifth and sixth eluates, almost all the Mahoney virus put on the column is recovered. The LSc, 2ab strain gives a recovery, in this instance, of under half of 1 per cent.

In all the tests we have run with the LSc, 2ab, less than 3 per cent of virus put on the column is recovered. The maximum we have recovered is under 3 per cent.

From these data it may seem that the column actually inactivates the L.Sc. But this is not the case, because if one at this point adds to the column sodium chloride in a concentration of .85 per cent, the LSc strain comes off the column and is recovered in the eluates. It then can be shown by tissue culture that its cytopathogenicity is still present.

Therefore, this is not an inactivation. What has occurred here is that the DEAE column permits the passage of the Mahoney strain and holds on very avidly to the LSc, 2ab strain.

The seven-day excreted material which Dr. Sabin sent us behaved like the LSc, 2ab strain in the Sabin vaccine.

The 19-day material was eluted in about 10 per cent of the amount which we put in. That is, it is somewhat less avidly held by the DEAE column than is the original LSc, 2ab strain. But it is much more strongly held than is the Mahoney strain.

We found, by checking with Dr. Sabin, that this particular strain had shown a partial reversion to neurovirulence in the monkey. As I recall, two out of five monkeys inoculated showed clinical evidence of disease.

Dr. Koprowski's Type I vaccine was also tested, and about 10 per cent of this strain comes off the column.

Some other interesting data have come out of this, and I shall mention them very briefly. Altogether, we have tested 24 Type 1 strains, obtained from various sources, of which 14 have been virulent.

For instance, from five children who had

paralytic poliomyelitis due to Type 1 poliovirus, the isolates obtained from Dr. Paul's laboratory all behaved in the way Mahoney does when tested by the DEAE column. We also have from Dr. Paul's laboratory five other strains obtained from the experiment which was referred to earlier, I believe, by Dr. Melnick. These behaved as follows: Four of the five were eluted in exactly the same way as is the Mahoney strain, and published data from Dr. Paul's laboratory indicate that the results of monkey inoculation go along with this.

The fifth strain is again an intermediate strain. It was one obtained from a child fed LSc, 2ab strain some days earlier, and it did not produce clinical disease in either of the two monkeys inoculated, although it did produce lesions in one of the two.

One other point might be mentioned: If one takes the Mahoney eluate and puts it into a capillary tube with hyper-immune rabbit serum, a flocculation occurs either after an hour in the incubator or overnight. This does not happen when the LSc, 2ab eluate is tested. It may be merely a difference in concentration of virus.

Another point of difference I should mention is this: If we take the LSc, 2ab virus which does come off the column, or the LSc, 2ab which we can elute off the column by adding saline, grow them in tissue culture, and then put them through the column, they behave again as does the original LSc, 2ab strain. The difficulty of elution, therefore, appears to be a characteristic of the strain and does not indicate that there is a mixture. It seems to be a characteristic which, through at least several tissue-culture passages, is a constant one.

We have done a little work with the Type 2 strains, using the strain which Dr. Sabin has in his vaccine. Using the buffer system, which I have described, one cannot detect a difference between the virulent and nonvirulent. The two strains come out very much in the manner as the Mahoney shown here.

However, if we use a different buffer system with a low concentration sodium chloride, .05 molar in this buffer, then the same result occurs; the virulent strain comes off the way the Mahoney does. The vaccine strain behaves like the LSc, 2ab strain in that it is held avidly by the column.

To summarize, this marker we call the *E*, or elution marker, appears to be a characteristic of a particular strain. In other words, it has been correlated with virulence or lack of it, as judged by monkey inoculations.

DISCUSSION

CHAIRMAN ANDERSON: Thank you very much, Dr. Hodes. The papers which have been presented are now open for discussion.

Dr. Lépine.

DR. LÉPINE: I was very much interested in Dr. Hodes' paper because in Paris we have been carrying out a similar piece of work, the results of which are about to be published.

More than one year ago, in the purification of poliovirus, we started using cellulose columns of the DEAE type, on which the virus was passed and then eluted with solutions of NaCl of increasing molarity. Fractions were collected by an automatic collector. We started by using the virulent Mahoney strain and we obtained a pattern in which most of the virus, more than 95 per cent, was eluted in one of the first fractions.

Next, we tried an attenuated strain, the strain 1342 which is included in the French inactivated vaccine, and to our great surprise we obtained quite a different pattern of elution of the strain. Most of it was eluted in the fraction which we call fraction 50; then we wondered whether there was a correlation between the different patterns with attenuation, or whether it was only a characteristic of a given strain.

It so happened that we had in store the 1342 strain, which, since its isolation from samples, was kept in the deep freeze at different stages of attenuation.

Starting from the non-attenuated original strain, we found that it gave a pattern which is almost similar to the Mahoney strain. Gradually, as the different degrees of attenuation were used, we obtained a shift to the right, that is, a shift from fraction 1 to fraction 50.

The same was done with the Type 3 strain which we have and we obtained an almost similar evolution of the pattern.

In connection with the Type 2 strain, the work is at present in progress.

Surprising as it may be, we were expecting to obtain fractions which would give a good complement fixation, but so far we have failed. It seems that the complement-fixing fraction is lost on the column, or is not eluted; in our tests, the virulence was evaluated by inoculation into the monkey and the titrations were made using the tissue culture and CPE technique.

Thus we can characterize a strain by its specific pattern of elution from the cellulose column, and there seems to be a great degree of correlation between the shape of the elution pattern and the degree of attenuation of the strain.

In the present stage of our research, we think that such elution patterns are significant markers which could be used for the selection or the characterization of strains. As an example of selection, we have grown in tissue culture the fraction number 50, which is present only in small amounts in virulent strains; then we collected the tissue-culture fluid and passed it again on the cellulose column. Repeating this three times, after three passages, we were able to obtain a substrain which had the same elution pattern as the attenuated strain obtained after 40 to 50 passages in tissue culture with our maximal dilution technique. We therefore believe that the elution pattern technique may be a help in selecting the attenuated component of the strain, as well as in characterizing the strain itself.

PROFESSOR CHUMAKOV (*through an interpreter*): My remarks with reference to the problem of markers, under discussion, concern only the methodological aspects. They are related to a recent announcement by our colleagues at the Institute of Poliomyelitis in Moscow, Drs. V. I. Agol and M. Ya. Chumakova, on the mechanisms of reaction of the so-called *d* marker.

Until now it has been generally accepted that the ability of cell cultures to fully differentiate attenuated strains from the virulent strains of poliovirus is due to the differences in multiplication of viruses in culture media with variable concentration of bicarbonate (Na_2HCO_8). Therefore, not infrequently the *d* marker is also referred to as the *bicarbonate* marker.

However, in 1960, Agol and Chumakova, while studying fractions of infectious RNA derived from attenuated and virulent strains of polioviruses, unexpectedly discovered that the *d*—marker can be completely reduplicated if, in-

stead of variable bicarbonate concentration, variations in the concentration of sodium chloride in the medium are substituted. Agol and Chumakova concluded that the active factor was *not* the bicarbonate (Na_2HCO_3), but merely the Na+ cation. An appropriate communication giving the experimental evidence will appear in a forthcoming issue of the Russian journal *Problems of Virology*. Of course, one cannot completely rule out the possibility of a coincidence of the *d* marker and the *bicarbonate* marker, but such a coincidence would be so remarkable that one has to consider first a modification of our views regarding the mechanism of the *d* marker, used so widely in the studies on poliovirus genetics.

DR. ROBBINS: In the course of our studies of newborn infants, we thought that perhaps the high acidity of their stomachs might have something to do with the lower rate of intestinal infection in these children. Therefore, in conversation with Dr. Sabin, it occurred to us that perhaps we could adsorb the virus to aluminum hydroxide, a technique he described many years ago, along with other people.

You will recall that the virus adsorbs at an acid pH and elutes at an alkaline pH. This seemed made to order for our purpose, since the virus presumably would remain adsorbed in the stomach and elute in the intestine.

However, in testing this *in vitro*, we found that the Type 1, LSc_1ab vaccine strain behaved quite differently from the Mahoney strain. The pattern of behavior is quite similar to that described by Dr. Hodes in that the more attenuated virus fails to elute, whereas the Mahoney virus elutes very readily, as expected.

In Table 1, data from a series of experiments with Mahoney virus are presented. These experiments were done very simply by suspending virus with the aluminum gel at pH 5.5 to 6. The suspension is centrifuged and the adsorption determined by titration of the supernate. The gel is then washed with the acid buffer and resuspended in a buffer of pH 7.5. The suspension is centrifuged again and the eluate tested for viral content. The recovery of the Mahoney virus is almost quantitative, whereas with the Sabin LSc strain a very small proportion of the virus was eluted.

We have tested one Type 1 virus isolated from

ADSORPTION AND ELUTION OF TYPE 1 POLIOVIRUSES TO $AL(OH)_3$ GEL SUSPENSIONS

VIRUS	TITER (LOG $TCID_{50}$/0.1 ML.) ORIGINAL T.C.F.	AFTER ADSORPTION	ELUATE
MAHONEY	7.5	3.8	7.4
	7.6	3.8	7.6
	4.5	0.5	4.0
SABIN (LSC)	7.7	4.0	2.0
	7.0	4.5	4.0
	4.5	0	< 2.0
TYPE 1 (FROM PARALYTIC CASE)	4.0	0	3.0
SABIN (LSC)			
2 DAYS POST-FEEDING	7.0	5.0	< 1.0
32 DAYS POST-FEEDING	7.0	4.2	2.5

TABLE 1

a paralytic case. This behaves like the Mahoney virus.

Two isolates from a child fed the Type I vaccine virus were examined. These were isolated two days and 32 days after feeding. Both behaved in a similar manner and resembled the virus fed.

Figure 1 illustrates the behavior of the Mahoney and LSc_1ab viruses when eluted from columns of aluminum hydroxide. The pH of the buffer added is recorded along the bottom and the pH of the eluted fractions is illustrated by the curve with open circles. This is a rather smooth curve. The fractions collected were approximately 10 cc. in amount.

The Mahoney virus, as shown in Fig. 1, is eluted sharply and almost completely when the pH reaches 6.5, whereas very small amounts of the LSc_1ab virus are found in any of the fractions.

We have not yet done any experiments with the other types of vaccine virus.

DR. SMORODINTSEV (*through an interpreter*): I would like to comment on the evaluation of different markers for the estimation of vaccine safety. Among these markers, the pathogenic behavior in monkeys are the most interesting.

A year ago I reported the results obtained on prolonged passages of Dr. Sabin's attenuated strains through children.

We have now finished 12 consecutive passages for all three strains of viruses. We have noted the periodic increase in the neurovirulence of these viruses with cerebral, or with spinal introduction in monkeys.

Neurovirulence in monkeys sometimes reached quite a high level corresponding to two and a half logs of virus concentration for the minimal paralytic intraspinal dose for monkeys, but in subsequent passages these strains gave normal non-pathogenic viruses again.

We have now returned to the strains which were kept in a deep freeze and have confirmed the fact that after a six-month or year-and-a-half observation, they bring about the same results in monkeys. We have studied the very important question of whether these viruses are capa-

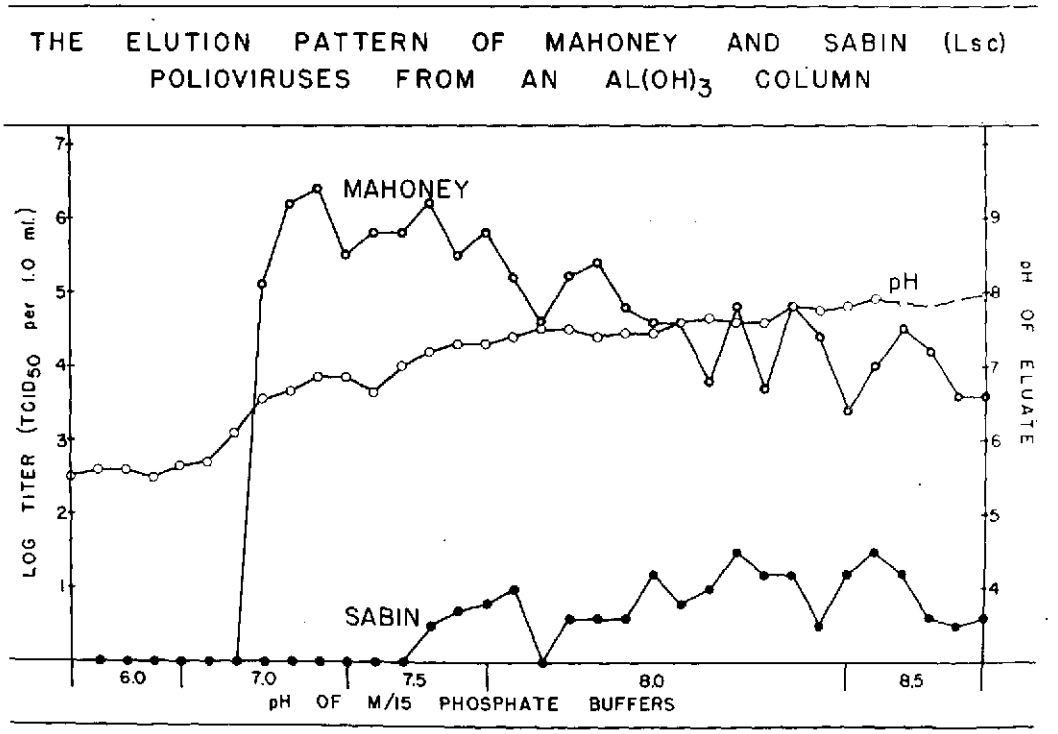


FIG. 1

ble, by introduction into children, of producing viremia which is not usually observed with original strains.

We therefore took monolayer cultures and conducted blood tests on children at various periods after the introduction of such strains to test the possible appearance of viremia and the increase of neurovirulence.

The results are now in progress, but we have preliminary data with Type 3 strain which show that this more neurovirulent strain does not cause viremia in children. I believe that these results are more important for the evaluation of safety of these viruses than are the various laboratory markers, which are so interesting in theoretical investigations of different variants. Viremia, after all, is the first stage which can be a menace to the generalization of such viruses into the central nervous system and its evaluation, among other markers, seems to be an especially important task.

DR. DULBECCO: The question of relationship between various markers and neurovirulence is actually a part of the general problem of correlation in variation of markers of any kind that occurs in poliovirus.

In the work carried out in our laboratory by Dr. McBride, Dr. Vogt, and myself, we have obtained the information which is summarized in Table 1. We have markers which show extreme cystine requirement, the inhibition by cystine, the temperature resistance for inactivation, the *d* character, the 30 and 40 centigrade growth, the *MS* marker, the resistance to inhibitors, and neurovirulence.

Now, there are many pluses and many minuses. The plus indicates, in a qualitative way, that the coexistence of the markers in the same virus is possible and frequent. The minus indicates that very frequently we have exclusion between these markers.

Now there are exceptions as we very well know. You will note that there are quite a number of minuses, meaning that if mutation occurs, let us say, from the *d* plus to the *d*, then it is very likely that at the same time the temperature resistance marker will also change simultaneously.

A close look at the minuses shown in Table 1 will reveal that these concern essentially a group of markers which include the temperature resistance, the 30 and 40 degree growth, the *MS* character, the *d* character, cystine requirement, and neurovirulence.

The bovine and equine inhibitor markers are completely out of the group. This means that whenever a marker in this group changes, the bovine or equine inhibitor markers are not affected, and vice versa. Therefore, all the markers constitute two groups. In one there is an extensive degree of covariation, meaning that a variation of one marker may involve a variation of another one, or variation of one marker may cause exclusion of another marker.

I believe that, in general, the use of markers serves a dual purpose in the study of vaccines. One of these would be, as emphasized very frequently at this session, to have markers that can be used as *in vitro* markers for neurovirulence. The other would be to have a marker which can be used to trace a certain strain introduced in the population, in order to determine, for instance, whether the strain recovered is identical to the strain which was introduced.

Markers of the first group may be useful for the first purpose. For the second purpose, it is obvious that none of the markers in this group is useful because if a mutation arises, it may involve the marker employed. Only the bovine and equine markers are suitable for the second purpose. Perhaps the marker of antigenicity referred to earlier could be considered.

As for the covariation of markers of the first group, many exceptions have already been pointed out. None of these markers truly reflects the behavior of neurovirulence.

This also raises further questions: the direction that research will have to take in this field and the significance of all these findings.

I should like to call attention to a theoretical point, namely, the fact that we have so many different markers for poliovirus does not imply that we have many genes in the poliovirus.

The hypothesis of many genes, which may seem to be the simplest explanation, does not take into account two facts: one, the construction of the virus; the other, the extent of covariation. If there were different genes, one would not expect this extensive covariation.

The structure of the virus is against the hypothesis of many genes because we know that the RNA of poliovirus, the genetic material, is very small, of a size equivalent to that considered as

TABLE 1. COVARIATION BETWEEN POLIOVIRUS MARKERS

	Cy^r	Cy^+ Cy^i	t^s t^r	d^+ d^-	30° 40°	MS^+ MS^-	bo^+ bo^-	ho^+ ho^-	nv^+ nv^-
Cy^r			+ −	+ +	(+) −	− +	+ +	+ +	− +
Cy^+			+ −	+ +	+ +	− +	+ +	+ +	+ +
Cy^i			− +	+ −	+ +	+ −	+ +	+ +	+ −
t^s				+ +	+ +	+ +	+ +	+ +	+ +
t^r				+ −	+ +	+ −	+ +	+ +	+ −
d^+					+ +	+ +	+ +	+ +	+ (±)
d^-					+ −	− +	+ +	+ +	− +
30°						− +	+ +	+ +	− +
40°						+ −	+ +	+ +	+ −
MS^+							+ +	+ +	+ −
MS^-							+ +	+ +	− +
bo^+								+ +	+ +
bo^-								+ +	+ +
ho^+									+ +
ho^-									+ +

+ = The two markers frequently occur in the same virus particle.
− = The two markers tend to exclude each other.

belonging to one gene, or approximately so.

We also know of only one protein and it is not likely that there are many more, owing to the structure and the compactness of the virus.

In work done recently at our Institute in Pasadena, it was found that in bacterial viruses covariation of markers occurs only when the genetic markers are very closely situated in the gene. Markers that are a few tenths of 1 per cent apart can show an extensive degree of covariation in the form of suppression.

Therefore, if we can extend these results, it is likely that the markers of the first group, the temperature, the *d*, the growth requirement, the *MS* and neurovirulence, and probably also the adsorption marker, discussed earlier, belong to a very small fragment of the genetic material of the virus.

If so, one would explain covariation as follows: If we have a protein containing a certain number of active groups, each one of these markers could reflect the function of one independent group. When there is a mutation in one of these groups, the functionality of the neighboring group would be affected, as we know from protein chemistry, and covariation would be produced.

Thus, lots of covariations we see are not due to the fact that markers are mixed, i.e., have a common identical genetical determinant. The covariation of many characters with neurovirulence can be explained as follows. These markers would correspond to functional groups near the group responsible for neurovirulence. One would be inclined to predict that there does exist a special group responsible for neurovirulence. To proceed in this kind of work, one should try to find possible markers reflecting the function of this group, which can be tested *in vitro*.

From what I have heard this morning, I must say that the results mentioned by Dr. Hodes, Dr. Robbins, and Dr. Lépine give the impression that perhaps their marker has to do more directly with the neurovirulence than any other character. Because it is very possible that the neurovirulence is essentially an expression of a surface property of the virus, related to the attachment of the virus to special receptors or special types of cell, namely, the neurons, or maybe another cell, which could be from what Dr. Smorodintsev has just stated, perhaps a cell involved in allowing viremia.

CHAIRMAN ANDERSON: Are there any comments on Dr. Dulbecco's statements? Dr. Sabin.

DR. SABIN: I believe that when we have spoken of correlations of these findings, we have neglected to mention one property which I described several years ago, namely, the property of combining with the neuron receptor substance.

I showed that virulent Type 1 poliovirus combined readily with suspensions of gray matter from monkey, chimpanzee, and human spinal cord, but did not combine with that derived from rabbit or dog spinal cords. At that time, I also showed that the Type 1 vaccine strain combined very poorly with the receptor substance of monkey, chimpanzee, and human material.

Now the question is whether the specific receptor substance derived from susceptible nerve cells possesses physical properties which are the reverse of the colloids described this morning, which have greater avidity for the attenuated than the virulent virus.

There is one other point I would like to add to what Dr. Hodes has presented. When I am asked for certain viruses, I am given a prescription and I try to fill that prescription. When I sent him the two strains of virus excreted by a child that had been fed Type 1 vaccine, I selected the one child that showed the maximum change in the monkey tests observed with Type 1 excreted virus. That maximum change was observed only after inoculation of 10 million tissue-culture infective doses intracerebrally. With smaller doses there was no effect, so we were still dealing with a highly attenuated virus.

DR. BODIAN: I would like to ask Professor Smorodintsev whether he has continued to subculture his fecal isolates in his consecutive human passages before monkey inoculation, and whether he feels that the use of tissue-culture passage fluids instead of fecal suspensions may influence the picture of reversion of neurovirulence.

DR. SMORODINTSEV (*through an interpreter*): I should like to say that, as we have already published in our works, we have observed a rather well-defined process of periodical increase of neurovirulence, particularly with strains of Type 3; we did not use additional *in vitro* pas-

sages of neurovirulent strains which we got from the intestinal tract of children.

Even if we observed an increase of neurovirulence for monkeys, we continued to feed this material to triple-negative children so as to follow the fate of this neurovirulence.

As I mentioned previously, as a rule, this neurovirulence of virus disappeared in the following passages, so that the intestinal tract of other children did not continue to support these neurovirulent viruses. There is no indication of a progressive increase of neurovirulence. This quality appears and then disappears.

We did not conduct lengthy sub-passages of this strain on cell cultures because our aim was to get quantitative information on the changes in neurovirulence, to which I am referring at the moment.

At the present time, we are trying to establish the correlation between these neurovirulent viruses and their pathogenic meaning. Can they be more invasive through the intestinal tract and produce more pronounced viremia? Viremia, so common in naturally infected children, is not found in vaccinated children.

DR. DULBECCO: I should like to make a comment on what Dr. Smorodintsev has just said, namely, the failure of passing further the pathogenic, neuropathogenic characters for monkeys with the virus obtained from those children in which the passage of the vaccine virus was shown to be increased in neurovirulence. I think that this failure of continuing the passage of neurovirulence may be interpreted in a different way: it could be due simply to a statistical sampling problem.

In fact, 10^5 tissue-culture doses are an adequate dose to cause infection with the vaccine, but if much less than that is inoculated one will not have a positive result all the time. This means that 10^5 tissue-culture doses are essentially equivalent to probably a few effective doses, which, by the oral route, can give rise to extensive intestinal multiplication and cause antibody production.

We may therefore consider that 10^5 tissue-culture doses probably correspond to a few infectious doses for the child.

Now, the virus which comes from the feces contains a small proportion of neurovirulent virus; of this you inject a large dose intracerebrally in monkeys, where the minority virulent type can be thus detected; but in testing the child you inject a few infectious doses (for the child) and therefore you discriminate against any minority type. The failure of passing further the character of neurovirulence for monkeys could simply be due to this sampling process and may not imply that neurovirulence is actually lost in subsequent passages.

DR. SMORODINTSEV (*through an interpreter*): In connection with the comments just made, I should like to say that we introduced not 100,000 doses but rather one and a half logs more. The average concentration fed to children was 6.5 logs of 10. These were studied quantitatively. Only those children who gave a high multiplication of virus introduced into the intestinal tract were considered by us to be interesting and we conducted observations on monkeys to titrate the level of neurovirulence.

Of course, here there can be significant variation in results for different children, but this was usual during 12 passages of each of the three attenuated strains: neurovirulence either appears or, what occurs more frequently, completely disappears during subsequent passages.

The same results are observed during the natural passage of neurovirulent pathogenic strains which do not select out strains of the greatest virulence. Rather, there is a distinct predominance of strains of moderate or low neurovirulence.

CHAIRMAN ANDERSON: The presentation of papers under Topic II will be continued during the second session.

SECOND SESSION

MONDAY, 6 JUNE 1960, 2:00 p.m.

Chairman

SIR F. MACFARLANE BURNET
Director, Walter and Eliza Hall
Institute of Medical Research
Melbourne, Australia

TOPIC II. SAFETY. (A) LABORATORY EVIDENCE OF ATTENUATION AND SAFETY (*continuation*)

Presentation of Papers by:

Dr. Hilary Koprowski

(DISCUSSION)

Dr. H. Pette

Dr. Maurice R. Hilleman

(DISCUSSION)

Dr. Ruth L. Kirschstein

(DISCUSSION)

Dr. Albert B. Sabin

(DISCUSSION)

TOPIC II. SAFETY. (A) LABORATORY EVIDENCE OF ATTENUATION AND SAFETY *(continuation)*

4. THE APPLICATION OF GENETIC MARKERS TO THE DEVELOPMENT AND CONTROL OF LIVE POLIOVIRUS VACCINE

HILARY KOPROWSKI, M.D., RICHARD CARP, M.D.,
T. W. NORTON, M.D., BARBARA COHEN, M.D.,
AND STANLEY PLOTKIN, M.D.

DR. KOPROWSKI (*presenting the paper*): Today, when over 60 million people in many parts of the world have been vaccinated against poliomyelitis with various attenuated viruses, the study of markers of poliovirus strains assumes an enormously important role. In addition to thorough clinical surveillance, the protagonist of live virus vaccination must maintain a close check on the fate of the vaccine strains fed to the population, if he is to stave off antagonists who see the somewhat imaginary instability of live viruses as a menace to the population of this globe, rather than as its salvation.

The high cost of monkeys and the relative crudity of the so-called monkey virulence tests once presented serious obstacles to the development of live attenuated virus vaccines since no other markers were available. Today, however, the laboratory can use several types of genetic markers to characterize a given strain of poliovirus, and particularly striking differences are apparent as between virulent and attenuated strains. In addition, McBride,[1] Wenner,[2, 3] Gard,[4] and Wecker [5] have provided laboratory workers with a tool which permits serologic differentiation between strains of the same type.

Serologic Differences of Poliovirus Strains within the Same Type: Intratypic Serodifferentiation Test (IST). Although several methods have been provided for this type of test, the procedures used in our laboratory are based chiefly on techniques developed by Gard and Wecker. A brief description of the procedures is as follows: Not less than 10 guinea pigs are immunized against the respective strains of poliovirus through parenteral administration of at least two and not more than three injections of undiluted tissue-culture fluid infected with a cloned progeny of the virus to be tested. The animals are bled individually, one week after the last injection, and each serum is tested either by the technique of Gard (immuno-inactivation test) or the method described by Wecker, in which the serum is incorporated in the overlay and a monkey-kidney monolayer is infected with the virus. Individual variations are found among the guinea pigs, since only a fraction of sera will show the desired distinctness of effect, when tested in appropriate dilutions, between different strains of the same type. Such sera may be pooled and used for the intratypic serodifferentiation test (IST).

In most of the results to be described the technique of Wecker has been employed. Monolayers of monkey kidneys were infected with a certain number of plaque-forming units of virus, and respective dilutions of serum were incorporated in the overlay. After 4-5 days of incubation, the plates were stained and the number and diameter of the plaques determined. Table 1 shows examples of IST applied to strains representing three types of virus. In each case, serum prepared against the attenuated strain was placed in the overlay and the homologous and heterologous virulent strains of the same type were tested under the same overlay. The results clearly indicate a marked decrease in the number of plaques produced by the homologous strain

TABLE 1. SEROLOGICAL IDENTIFICATION OF STRAINS OF POLIOVIRUS WITHIN THE SAME TYPE

TYPE	STRAIN	NEUTRALIZATION TEST WITH SERA PREPARED AGAINST THE VACCINE STRAINS*			
		NUMBER OF PLAQUES**		AVERAGE DIAMETER***	
		CONTROL	SERUM	CONTROL	SERUM
1	CHAT	13	1	5.0	<1.0
	Mahoney	8	5.3	9.0	4.0
2	Jackson	10	0	3.0	0
	MEF_1	6	4	7.0	2.0
3	Fox	8	0	5.0	0
	H24	5	4	7.0	3.0

* Anti-CHAT, anti-Jackson, and anti-Fox immune sera used in the test.
** Average.
*** In mm.

under its own serum overlay. This decrease was much less striking with heterologous virus of the same type. These findings are consistent and applicable to all three types. When the results were presented as the difference in the average diameter of plaques produced by homologous and heterologous virus, the data obtained are perhaps even more significant. Thus, the results of the intratypic serodifferentiation test are best expressed as the relationship of the size of the plaque produced by different viruses under serum prepared against one attenuated strain.

Markers as Detective Agents. In the mass vaccination campaigns conducted throughout the world, attenuated strains have been and will be administered to subjects during a period of incubation of poliomyelitis caused by a virulent virus. If such subjects become paralyzed, the burden of proof that their condition is caused by a wild virulent virus and not by the vaccine virus administered rests with those responsible for the vaccination. Thorough investigation of poliovirus strains isolated from non-vaccinated persons who live in the area of vaccination is also indicated, to prove that the strain causing paralysis is not a vaccine strain disseminated through the community after several passages through the human gut. An opportunity to use markers as detective agents arose during a vaccination campaign in Leopoldville, Belgian Congo. The epidemiologic and clinical observations in connection with this campaign were presented at this meeting last year by Drs. LeBrun, Courtois, and Plotkin and the progress report will be presented by the same team. As you will recall, the program of vaccination with Type 1 CHAT virus coincided with an epidemic of Type 1 poliomyelitis in the non-vaccinated areas of the city. As Dr. Plotkin will show, several infants were apparently fed the virus during the incubation period of a wild strain infection, and two of these children became paralyzed 6 and 20 days after they were fed the CHAT strain. Viruses isolated from the feces of these children, and from the stools of other paralyzed, non-vaccinated infants, were subjected to thorough laboratory investigations. Strains isolated from children vaccinated in Leopoldville who did not exhibit any signs of illness were included as controls.

In order to obtain more conclusive data on the serologic stability of the CHAT virus after passage through man, strains isolated from human subjects who were exposed to the CHAT virus during an immunization campaign in Moorestown, N. J. were employed in the test. Representative results from this study, which has been published elsewhere, are shown in Table 2. It will be observed that the diameter of the plaques observed under anti-CHAT serum overlay did not exceed 10 per cent the diameter of control

TABLE 2. INTRATYPIC SERODIFFERENTIATION TEST WITH VIRUSES REPRESENTING PASSAGE OF CHAT STRAIN THROUGH HUMAN INTESTINAL TRACT

VIRUS	HUMAN PASSAGE	% CONTROL PLAQUE DIAMETER UNDER ANTI-CHAT SERUM
CHAT		0
B 117	1	10
Q-2	3	0

plaques without serum overlay. No differences were observed between the first and third human passage of the CHAT virus.

The actual results of the intratypic serodifferentiation test applied to virus isolated in Leopoldville are shown on Table 3. Virus isolated from two healthy vaccinated infants showed serologic identity with the CHAT virus. In contrast, virus isolated from three infants who became sick after vaccination and from eight non-vaccinated paralytic cases, seem to be unrelated serologically to the CHAT virus, since the reduction in plaque diameter under anti-CHAT serum overlay did not exceed, in general, that observed with the heterologous Mahoney virus.

TABLE 3. MARKERS AS DETECTIVE AGENTS. INTRATYPIC SERODIFFERENTIATION TEST OF TYPE 1 POLIOVIRUSES ISOLATED FROM INHABITANTS OF LEOPOLDVILLE DURING VACCINATION CAMPAIGN WITH CHAT TYPE 1 VIRUS

SUBJECTS		% CONTROL PLAQUE* DIAMETER UNDER ANTI-CHAT SERUM OVERLAY
GROUP	CONDITION	
Vaccinated	Healthy	0
		10
	Sick	32
		36
		38
Non-Vaccinated	Sick	32
		36
		40
		43
		45
		48
		50
		50

* Positive control results (CHAT virus)—0%.
Negative control results (Mahoney virus)—40%

Another opportunity to investigate strains isolated during a vaccination campaign arose in Poland. Preliminary clinical and epidemiologic observations of this mass vaccination campaign, during which the entire country was immunized with the CHAT and Fox viruses, will be presented by Dr. Przesmycki. Strains isolated from six non-vaccinated children and three vaccinated children, all of whom who showed symptoms of disease, were submitted to our laboratory for identification, following preliminary typing in the Warsaw Laboratory. The results of the intratypic serodifferentiation test against anti-CHAT serum are shown in Table 4. They indicate clearly that all nine strains are as unrelated to the CHAT virus as was the Mahoney virus which was employed as the negative control in this test.

A summary of intratypic serodifferentiation tests with Type 1 poliovirus isolated during vaccination programs in Moorestown, Leopoldville, and Poland is given in Table 5. All seven strains isolated from subjects who were either fed the CHAT virus or were in familial contact with those fed the virus in Moorestown showed serologic identity with the CHAT virus. Similarly, Type 1 virus isolated from two individuals in Leopoldville who exhibited no symptoms follow-

TABLE 4. MARKERS AS DETECTIVE AGENTS. INTRATYPIC SERODIFFERENTIATION TEST OF TYPE 1 POLIOVIRUSES ISOLATED IN POLAND DURING THE VACCINATION CAMPAIGN

SUBJECTS		% CONTROL PLAQUE* DIAMETER UNDER ANTI-CHAT SERUM
GROUP	CONDITION	
Vaccinated	Sick	58
		61
		55
Non-Vaccinated	Sick	30
		44
		50
		71
		54
		75

* Positive control results (CHAT virus)—<10%
Negative control results (Mahoney virus)—44%

ing vaccination were serologically identical with the CHAT strain. In contrast, the remaining 11 strains isolated from vaccinated and non-vaccinated subjects who exhibited signs of poliomyelitis in Leopoldville and from nine subjects under similar clinical conditions in Poland were found to be serologically different from the CHAT virus.

The study of temperature markers is a valuable adjunct to IST. All the strains employed in our laboratory for vaccination purposes are cold variants—that is, they have much greater reproductive capacity at 35°-37° C. than at higher temperatures. None of the viruses was found to grow in monkey-kidney tissue culture maintained at 40° C. Some time ago a study was initiated to investigate the properties of strains isolated from the stools of healthy children and infants exposed to the CHAT strain by feeding or familial contact. As shown on Table 6, 18 strains were isolated from fecal material, and a first tissue-culture passage of these strains was inoculated in monkey-kidney tubes. Each set of the tissue cultures was divided for incubation at two different temperatures—37° and 40° C. The difference in the TCD_{50} was used to classify the strain as a cold or hot variant. Growth of 17 of the 18 strains, including nine isolated from premature infants fed CHAT virus, was inhibited at 40° C., indicating that the temperature marker of the original CHAT strain had been retained. The reproductive capacity of one of the strains was less markedly inhibited by the temperature

TABLE 5. SUMMARY OF RESULTS OF INTRATYPIC SERODIFFERENTIATION TEST OF TYPE 1 POLIOVIRUSES ISOLATED FROM EITHER HEALTHY OR PARALYZED, EITHER VACCINATED OR NON-VACCINATED SUBJECTS IN MOORESTOWN, LEOPOLDVILLE, AND POLAND

LOCATION	HUMAN SUBJECTS		RATIO** OF VIRUSES IDENTIFIED AS CHAT STRAIN
	"V" OR "N" *	CONDITION	
MOORESTOWN	V	HEALTHY	7/7
LEOPOLDVILLE	V	HEALTHY	2/2
	V	SICK	0/3
	N	SICK	0/8
POLAND	V	SICK	0/3
	N	SICK	0/6

* V = Vaccinated.
N = Non-Vaccinated.
** Denominator = Number of strains tested.
Numerator = Number of strains completely neutralized by anti-CHAT serum.

TABLE 6. MARKERS AS DETECTIVE AGENTS. TEMPERATURE MARKERS OF TYPE 1 STRAINS ISOLATED FROM HEALTHY INFANTS AND CHILDREN VACCINATED WITH CHAT VIRUS IN NEW JERSEY AND PHILADELPHIA

LOCATION	NUMBER OF PASSAGES THROUGH MAN	MARKER		
		TOTAL TESTED	COLD	HOT
MOORESTOWN	1	6	6	
	2	2	1	1(?)
	3	1	1	0
PGH *	1	9	9	0
TOTAL		18	17	1(?)

* Premature infants in Philadelphia General Hospital.

of 40° C. and the results were therefore classified as intermediate.

In view of this remarkable stability of the CHAT strain in relation to the temperature marker, similar studies were undertaken with Type 1 strains isolated in Leopoldville and Poland during large-scale vaccination campaigns with CHAT virus. The results of these investigations are shown in Table 7. Type 1 virus isolated from two symptom-free, vaccinated children in the Leopoldville area retained the cold character. In contrast, none of the remaining 11 strains isolated from vaccinated and non-vaccinated infants in Leopoldville, and none of the eight strains isolated from similar subjects in Poland, showed the cold character. Except for one Leopoldville strain, the reproductive capacity of these viruses was not inhibited by the temperature of 40° C. Results obtained with one strain seemed to classify its character as intermediate.

Development of New Attenuated Strains through Cultivation at Low Temperature. The attenuated strains of poliovirus now available can

TABLE 7. MARKERS AS DETECTIVE AGENTS. TEMPERATURE MARKER OF TYPE 1 STRAINS ISOLATED FROM HUMAN SUBJECTS DURING VACCINATION CAMPAIGN WITH CHAT VIRUS IN LEOPOLDVILLE AND POLAND

LOCATION	SUBJECTS		MARKER OF STRAINS		
	V or N	CONDITION	TOTAL TESTED	NUMBER COLD	NUMBER HOT
LEOPOLDVILLE	V	HEALTHY	2	2	0
	V	SICK	3	0	3
	N	SICK	8	0	8*
POLAND	V	SICK	3	0	3
	N	SICK	5	0	5

* One strain showed more intermediate characteristic.

be divided into two groups according to their genetic stability after passage through the human intestinal tract. One group comprises the relatively stable Types 1 and 2 strains. Of this group, the CHAT strain seems to show the highest degree of stability of characters after human passage, although it must be admitted that comparative studies with the LSc, 2ab strain, which showed a lesser degree of stability, have not been carried out in the same laboratory. To the second group belong all attenuated Type 3 viruses, none of which is stable after passage through the human intestinal tract. This may best be illustrated by the results shown in Table 8, where comparison is made of two markers—the temperature and *MS* markers—applied to the Type 1 CHAT virus and the Type 3 Wistar-Fox strain. It will be observed that 19 out of 20 strains of Type 1 virus isolated from individuals exposed to CHAT vaccine retained the original temperature marker, and nine of the same strains retained the *MS* marker—that is, reproduced poorly on an established line of monkey-kidney cells as compared to fresh monkey-kidney cells. Totally different results were observed with the Type 3 virus isolated from subjects exposed to the Wistar-Fox strain. Only a small fraction of individuals excreted virus resembling the original vaccine strain in its characters. The remaining strains changed their character. In addition, one of the strains—Lcon $12a_1b$—has shown a lower degree of immunizing capacity than the strains representing the other two types of attenuated virus. A search for an attenuated Type 3 virus with characteristics better than those presently available therefore seems to be indicated. Passage of virus in tissue cultures maintained at low incubation temperatures seems to be the method of choice, and this procedure was extended to investigations of possible attenuation of strains representing Type 1 and Type 2 virus as well. This work is now in progress and only preliminary results can be presented.

The Type 1 virus employed in this study was isolated by Fox and Gelfand from the stool of an asymptomatic case in Louisiana (W-1 strain). Following one passage in monkey-kidney cultures at 37° C., the virus was transferred serially through the same tissue-culture system kept initially at 25° C. and later at 23° C. Following 15 passages at low temperatures, the virus was plaqued at 23° C., and progeny of one plaque were subjected to further serial passages at 23° C. Although the original strain grew equally well on an established monkey-kidney cell line and on fresh monkey kidney, growth of the resulting cold variants on an established monkey-kidney line was inhibited after 13 passages at low temperature, and the virus thus assumed the *MS* character.

The cold strain called W-1 has been tested for neuropathogenicity for Rhesus monkeys at three different passage levels; the results are shown in Table 9. Groups of monkeys were injected intraspinally with undiluted tissue culture medium following the technique described by Melnick, and their central nervous system tissue

TABLE 8. COMPARATIVE STUDY OF TWO MARKERS OF CHAT (TYPE 1) AND WISTAR-FOX (TYPE 3) STRAINS EXCRETED BY VACCINATED SUBJECTS

VIRUS	MARKER	RATIO OF STRAINS* RETAINING ORIGINAL MARKER OF VACCINE STRAIN
CHAT	TEMPERATURE	19/20
	MS	9/9
WISTAR-FOX	TEMPERATURE	2/12
	MS	1/12

* Denominator = Number of strains tested.
Numerator = Number of strains showing the same marker as strain fed.

TABLE 9. STUDY OF PATHOGENICITY FOR RHESUS MONKEYS INJECTED INTRASPINALLY WITH TYPE 1 VIRUS (W-1 STRAIN) AT VARIOUS PASSAGE LEVELS AT LOW TEMPERATURES

TEMPERATURE OF TISSUE CULTURE	PASSAGE	TCD_{50} INJ.	RATIO OF MONKEYS SHOWING LESIONS L	C	B.S.
25° C	10	7.2	5/5	4/5	2/5
23° C	10 + 5	6.2	3/4	3/4	3/4
	10 + 5 PLAQUED*	6.7	1/4	1/4	0/4

* At 23° C.

was examined for histopathologic lesions after they were sacrificed 18-20 days after inoculation. It will be observed that the results obtained with the W-1 strain after 10 passages at 25° C., followed by five passages at 23° C., did not seem to distinguish this strain from other attenuated strains. Progeny of a plaque isolated after 15 passages at low temperature (23° C.) have proved remarkably less neurovirulent than other available attenuated strains.

The original W-1 virus and the plaqued cold variant were submitted to the intratypic serodifferentiation test using serum overlay prepared against the original strain. Results of this test, shown on Table 10, indicate serologic identity of the plaqued cold variant with the original virus.

The TN Type 2 strain—the first strain ever to be fed to a human being—was chosen to initiate passages of Type 2 strains at low temperatures. Two Type 3 strains were employed to initiate a series of passages at low temperatures. One was isolated by Gelfand and Fox from the feces of an asymptomatic child in Louisiana (W-3), and the other represents the first human passage of the Fox Type 3 strain which retained the cold character of the original virus. Following serial passages at low temperatures and change in the temperature and *MS* markers of the strains similar to those observed with the W-1 virus, neuropathogenicity tests were carried out in in-

TABLE 10. INTRATYPIC SERODIFFERENTIATION TEST BETWEEN THE W-1 ORIGINAL VIRUS AND ITS "COLD" VARIANT

PASSAGE AT 23–25°C	AVERAGE NUMBER OF PLAQUES	
	CONTROL OVERLAY	ANTI-W-I SERUM* OVERLAY
0	14	3
8	6	<1
MAHONEY CONTROL	4	3

* Serum prepared against original W-1 virus before its adaptation to cold.

traspinally injected monkeys. The data shown in Table 11 seem to indicate that the resulting cold variants show a low degree of neuropathogenicity as compared to the original strains, although the low concentration of virus in a preparation of the W-3 strain may account for some of the favorable results. After these preliminary investigations, the cold variants of the W-1 and TN strains were fed to non-immune infants and children. Although a relatively small number of subjects was involved, the results shown in Table 12 seem to indicate that after 13 passages at low temperature, the cold variant of the W-1 strain was as infectious for the human intestinal tract as any other Type 1 strain currently in use. On the other hand, the infectivity of the Type 2 TN virus after 19 passages at low temperature was not as high as that of other Type 2 strains available, since no intestinal infection was produced with virus doses below 10^5 TCD_{50}.

Type 1 strains isolated from three children fed the cold variant of the W-1 strain were passaged once in monkey-kidney tissue culture and studied for their temperature marker. A more elaborate procedure, similar to the one described by Lwoff, was employed. This involved one-cycle growth curves of intracellular virus at two different temperatures—37° and 40° C.—and the results are shown on Figure 1. It will be observed that the growth curves obtained at 40° C. with the vaccine strains CHAT and W1-P and the three strains isolated from human subjects fed W1-P—207, 210, and 211—are similar and show marked differences from the pattern of the Mahoney strain grown at the same temperature. As expected, there is little difference between the growth curves obtained when cultures were kept at 37° C. Growth-curve patterns established for virus R-1 isolated from a child fed the CHAT strain, as shown in Figure 2, resembled those obtained with W1-P virus and

TABLE 11. STUDY OF PATHOGENICITY FOR RHESUS MONKEYS INJECTED INTRASPINALLY WITH TYPES 2 AND 3 VIRUSES (THE TN AND W-3 STRAINS) AT VARIOUS PASSAGE LEVELS AT LOW TEMPERATURES

VIRUS	TEMPERATURE OF TISSUE CULTURE	PASSAGE	TCD_{50} INJ.	RATIO OF MONKEYS SHOWING LESIONS		
				L	C	BS
TN	25° C	15	8.2	2/4	2/4	2/4
W–3	25° C	5	5.7	1/4	2/4	2/4
	23° C	5	5.0	1/4	1/4	0/4

TABLE 12. RESULTS OF ORAL ADMINISTRATION TO CHILDREN OF W-1 AND TN STRAINS AFTER 13 AND 14 PASSAGES AT LOW TEMPERATURES

VIRUS	TCD_{50} FED	RATIO OF INTESTINAL INFECTIONS
W–1	7.7	1/1
	5.5	1/2*
	4.5	3/3
TN	5.7	5/7
	4.7	0/2
	3.7	0/2

* Failure occurred in a child with pyrexia at the time of virus feeding.

its first human passage strains. These preliminary data seem to indicate that the cold variant of the W1-P strain retains its infectivity for the human intestinal tract, as do other attenuated Type 1 strains, and does not undergo marked changes in its growth characteristics under different temperatures after one passage through the human intestinal tract. It must be emphasized that these are very preliminary results, obtained on a small number of individuals and subject to possible revision when more extensive data are obtained. Similar data are now being sought for the Type 3 virus, where development of a new and improved attenuated strain is much more imperative than in the case of the two other types of poliovirus.

Inquiry into the Nature of MS and Temperature Markers. Although genetic markers for poliovirus and other viruses have been employed in laboratory investigations for some time, the nature of these markers seems to be somewhat illusive. As shown in Table 13, the *MS* cell monolayer infected with CHAT virus will yield much smaller quantities of virus than parallel infection of fresh monkey-kidney culture. However, if, instead of intact virus, infectious nucleic acid isolated from the CHAT strain is used as the inoculum, the yield from *MS* cells will be greater than from monkey-kidney cells and similar to that obtained with either intact virus of the virulent Mahoney strain or infectious nucleic acid isolated from this strain. These results seem to indicate that in the process of attenuation the protein coat loses some of its ability to attach itself to the receptors and penetrate the cells of the established monkey-kidney line. In contrast, the nucleic acid part of the attenuated strains, which does not seem to need receptors to penetrate the cells, infects the *MS* cells at the same rate as a virulent intact virus. Thus results obtained with the CHAT strain and the *MS* cells are somewhat similar to those of Holland *et al.*[6] for cell systems which are resistant to poliovirus. Obviously, the parallel cannot be drawn too closely, since ultimately the attenuated poliovirus can be propagated in the *MS* line, in contrast to

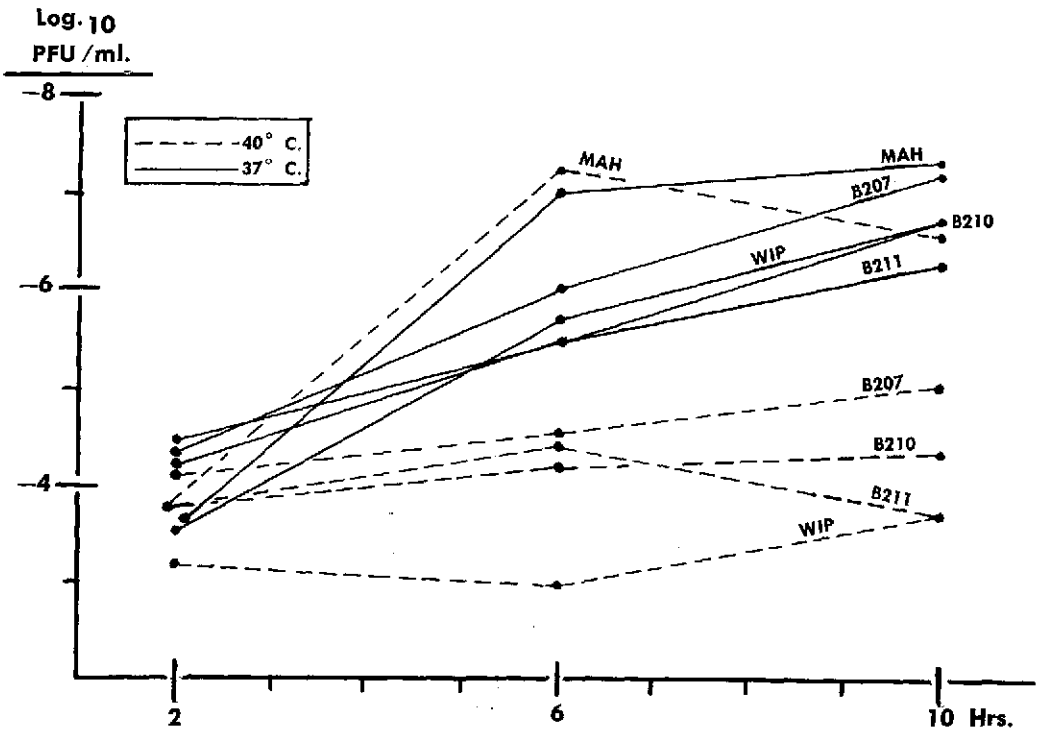


FIG. 1

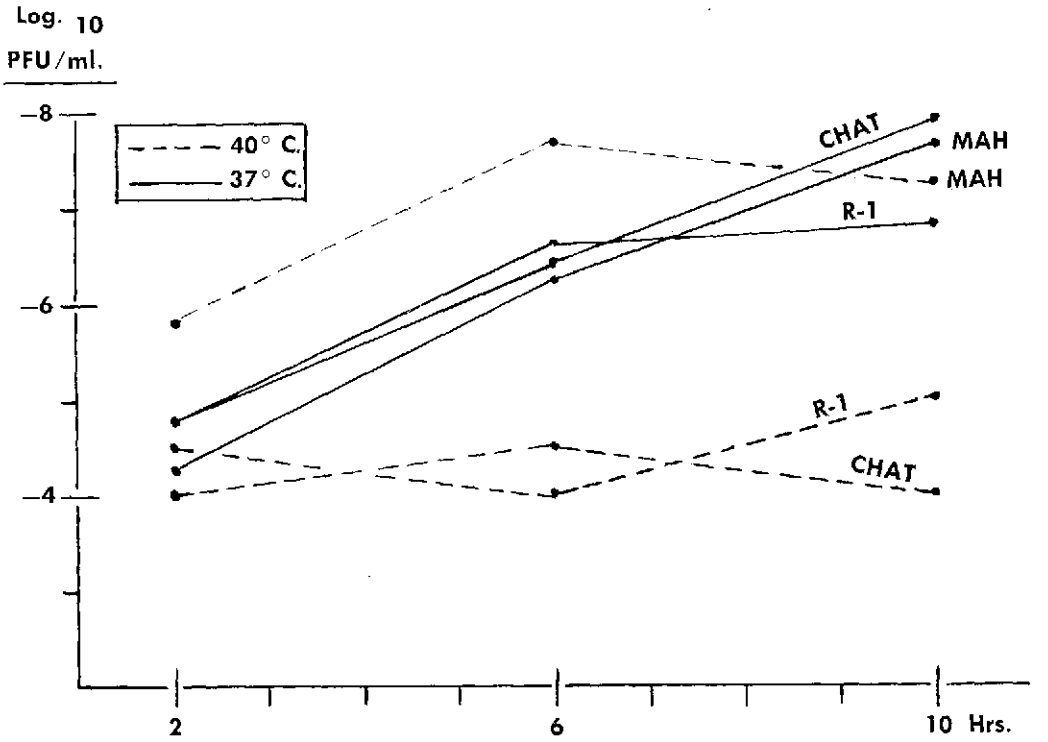


FIG. 2

TABLE 13. COMPARATIVE INFECTIVITY OF INTACT VIRUS AND INFECTIOUS RIBONUCLEIC ACID FOR THE MS AND MK CELLS

VIRUS	INOCULUM	RATIO OF TCD_{50} OF MS TO MK
CHAT	INTACT VIRUS	1 : 6
	RNA	3.3: 1
MAHONEY	INTACT VIRUS	3 : 1
	RNA	3.7 : 1

the system used by Holland, where infectious nucleic acid will yield intact virus non-infectious for the cell system into which it was introduced. Similar results were obtained when intact CHAT virus was used to infect *MS* cells in the presence of deuterium oxide (heavy water) in the medium. Substitution of hydrogen by deuterium in living systems has been studied for some time in various laboratories and has been found to have distinctive biologic effects. In the field of virus infections, the burst size of T7 bacteriophage was increased when the phase was permitted to multiply in *E. coli* grown in D_2O medium instead of H_2O medium. The data presented in Table 14 seem to indicate that the plating efficiency of CHAT virus on *MS* cells grown in

TABLE 14. EFFECT OF D_2O IN MEDIUM ON PLATING EFFICIENCY OF CHAT VIRUS ON MS CELLS

EXP.	MEDIUM	NO. OF PLAQUES PRODUCED ON CELL MONOLAYERS	
		MS	MK
1	H_2O	0	14
	D_2O*	14	N. T.
2	H_2O	1	33
	D_2O*	20	N. T.

N.T. = Not tested. * 40-50% D_2O in medium.

D_2O medium is markedly increased and similar to that observed in fresh monkey-kidney cells grown in H_2O medium. Thus, the substitution of H_2 ions by deuterium in the tissue culture system seems to increase the plating efficiency of *MS* cells to between 50 and 100 per cent.

As mentioned before, the cold CHAT strain cannot be grown at 40° C. However, it seemed to be of interest to verify whether infectious nucleic acid is produced in cultures kept at these temperatures shortly after infection with the CHAT strain. In this experiment monkey-kidney tissue-culture monolayers were infected respectively with CHAT and Mahoney virus and incubated for 6-8 hours at 37° and 40° C. Following this period of incubation cultures were harvested and RNA extracted. The infectivity of the extracted RNA is shown in Table 14a. It may be observed that although the yield of infectious RNA from the virulent Mahoney virus was similar regardless of the temperature at which cultures were incubated, the yield of CHAT RNA from cultures kept at 40° C. was very small compared to that obtained from cultures kept at 37° C. These results seem to indicate that a cold variant, when kept at high temperatures, appears to lose the capacity to produce even an infectious nucleic acid.

As shown in Table 15, incorporation of D_2O into growth medium and agar overlay of monkey-kidney monolayers infected with the CHAT virus and kept at 40° C. resulted in formation of plaques in numbers representing 10 to 63 per cent of those observed at 37° C. Progeny of the

TABLE 14a. INFECTIVITY OF RNA EXTRACTED FROM "HOT" MAHONEY AND "COLD" CHAT VIRUS GROWN FOR 8 HOURS AT TWO DIFFERENT TEMPERATURES IN MONKEY-TISSUE CULTURE

VIRUS	TEMPERATURE	NO. OF PLAQUES PRODUCED BY THE EXTRACTED RNA
MAHONEY	37° C	39
	40° C	39
CHAT	37° C	43
	40° C	4

TABLE 15. EFFECT OF D_2O ON GROWTH OF CHAT VIRUS IN MONKEY-KIDNEY CELLS AT 40° C.

EXP. NO.	MEDIUM	NUMBER OF PLAQUES PRODUCED IN CULTURES KEPT AT: 37° C.	40° C.
1	H_2O	32, 48	0, 0
	D_2O	30, 33	3, 5
2	H_2O	24, 29	0, 0
	D_2O	19, 21	11, 14

deuterated CHAT virus grown at 40° C. seemed to show greater replication of 40° in standard medium than progeny of the same virus grown in deuterated medium at 37° C., where no difference in plating efficiency between the two systems was observed.

Experiments are now in progress to determine the mechanism of deuterium use mediated through the increased burst size. The results may perhaps elucidate the nature of some of the factors responsible for the genetic markers of the virus. The data should certainly indicate which part of the virus molecule plays a prominent part in the determination of one or another marker.

Finally, since tests for temperature markers have become important tools for the study of polioviruses, Dr. Richard I. Carp, of our laboratory, has been developing a temperature marker test which would be more sensitive than the comparative tube titration tests and less cumbersome and elaborate than the one-cycle growth curve test. In this test, monolayers of monkey-kidney cells are exposed to various concentrations of test viruses, and a certain number of plaque-forming units of the viruses to be tested are incubated for different time intervals at 40° C. The plates are then removed from the 40° C. incubator and placed in a 37° C. incubator. The number of plaques is read after an incubation period of 3-4 days and compared with the number of plaques developed on the same systems kept throughout at 37° C. Table 16 shows data related to four strains investigated in this manner. You may observe that only six plaques were formed by CHAT virus in monolayers kept

TABLE 16. A MODIFIED TEST FOR TEMPERATURE MARKER

STRAIN	HUMAN PASSAGE	NUMBER OF PLAQUES ON PLATES KEPT AT 40° C.*FOR HOURS: 0	18	30	42
CHAT		23	6	0	0
CHAT Q-1	1	4	0	1	0
SICKLE		18	48	55	49
MAHONEY		18	71		92

* And then incubated at 37° C for 3-4 days.

for 18 hours at 40° C., and none when cultures were kept at 30 and 42 hours at 40°, as compared to 23 plaques formed by the same virus when cultures were incubated at only 37° C. Similar results were obtained with the CHAT Q-1 virus, representing first human passage of the CHAT strain. In contrast, the yield of virulent Mahoney virus seemed to increase in parallel with the time the plates were kept at 40° C., and the results obtained with the Sickle strain lie somewhere in between. Once the conditions of the test become stabilized in relation to other markers, it may become a valuable tool for simplifying the laboratory procedures related to temperature markers.

REFERENCES

1. McBride, W. D.: Antigenic analysis of polioviruses by kinetic studies of serum neutralization. *Virology* **7:** 45-58, 1959.

2. Wenner, H. A., Archetti, I., and Dubes, G. R. Antigenic variations among Type 1 polio viruses. *Am J. Hyg.* **70:** 66-90, 1959.

3. Wenner, H. A., Kamitsuka, P., and Lenahan, M.: Comparative study of Type 2 poliomyelitis viruses; antigenic difference relating to 13 Type 2 strains. *J. Immun.* **77:** 220-231, 1956.

4. Gard, S.: Immuno-inactivation of poliovirus. *Arch. Virusforsch.* **7:** 449-460, 1957.

5. Wecker, E.: A simple test for serodifferentiation of poliovirus strains within the same type. *Virology* **10:** 376-379, 1960.

6. Holland, John J., McLaren, Leroy C., and Syverton, Jerome T.: The mammalian cell-virus relationship. IV: Infection of naturally insusceptible cells with enterovirus ribonucleic acid. *J. Exp. M.* **110:** 65-80, 1959.

DISCUSSION

CHAIRMAN BURNET: The paper presented by Dr. Koprowski is now open for discussion. Dr. Dick.

DR. DICK: I would like to request some information. In using this intratypic serological marker, how can one determine that the virus has not undergone an antigenic change in multiplication in the gut?

The other question concerns the *T* marker: Generally, what experience have people had in finding *T* negative viruses in wild paralytic strains? We have not done very much of this yet, but we do have some evidence that some wild paralytic strains are *T* minus. Therefore, if you find *T*— wild paralytic strains, and if you get an antigenic change neither the *T* marker nor the intratypic serologic marker is of much use in sorting things out.

DR. KOPROWSKI: The number of wild strains studied in our laboratory for the *T* marker is relatively small, and of all strains examined one showed intermediate *T* characters and the remaining strains were all "hot."

As far as antigenic stability of CHAT strain is concerned, viruses isolated from the healthy infants and children fed CHAT virus either in the Belgian Congo or in the U.S.A. were identical serologically (in the intratypic serodifferentiation test) with the CHAT virus. We found no exception to this observation. This obviously refers to a study in which anti-CHAT serum was employed in the test. We have not as yet explored the use of a serum prepared against a wild strain in a test with CHAT-virus-derived human passage strains. However, results published by McBride suggest that a specific reaction may also be encountered.

DR. MELNICK: I would like to refer Dr. Dick to Table 9 of our paper.* In it I have indicated the results of such *T* tests on wild viruses. We found that, of 64 wild viruses tested, 8 were negative for the *T* characteristic; 5 were plus-minus, and 51 were positive. Dr. Sabin has also carried out such tests.

I should like to address a question to Dr. Koprowski in connection with the antigenic typing of viruses. Would he say a word about the number of bottles or plates which he used per dilution? In order to get significant differences, it is important to know the total number of plaques counted, and one could not determine this from the averages which Dr. Koprowski showed in his paper.

I should also like to know the number of plaques that were counted in order to determine the average size, and how these plaques were followed during the incubation period. If plaques are counted only on day 5, then some plaques are three or four days old and others are perhaps only one day old. This will greatly influence the results.

DR. KOPROWSKI: We used three to five plates with a predetermined dilution of serum. The number of plaques counted varies, let us say from 40 to about 60 or 70, and from this we obtain an average value.

Dr. Wecker, who originally developed the use of serum in the overlay, has counted plaques at different days after infection of monolayers and found little difference in plaque counts between the third and fifth day.

DR. SABIN: Since the question of the reproductive capacity of naturally occuring poliovirus at higher temperatures has been raised several times, I should like to call attention to the results of a rather extensive study shown in Table 1.

These are results of tests on a total of about 260 strains derived from patients with disease, without disease, and under various other circumstances.

To summarize, while the majority of naturally occurring polioviruses do possess the capacity for multiplying at 40° C., there are exceptions to the rule, even in the central nervous system of the fatal cases. I should point out that I went back and studied the original central nervous system tissue suspensions—thinking that the virus might have undergone a change after one passage in monkey-kidney tissue culture—and found that the virus in the original central nervous system

* See p. 23.

TABLE 1. INCIDENCE OF STRAINS WITH CAPACITY TO PROPAGATE AT 40° C (T/40+) AMONG NATURALLY OCCURRING POLIOVIRUSES OF DIVERSE ORIGIN AND AMONG VIRUSES EXCRETED BY PERSONS AFTER INGESTION OF ATTENUATED POLIOVIRUS VACCINES

SOURCE OF STRAINS	NO. TESTED				NO. T/40			
	TYPE 1	TYPE 2	TYPE 3	TOTAL	TYPE	+	±	—
CLINICAL POLIOMYELITIS								
STOOLS - EARLY AFTER ONSET	29	3	4	36	All	36	0	0
CNS - FATAL CASES	15	-	1	16	All	13	3	0
STOOLS OF HEALTHY CHILDREN	20	17	21	58	1	17	2	1
DURING NON-EPIDEMIC PERIODS					2	13	4	0
NO KNOWN CONTACT WITH CASES					3	12	7	2
STOOLS OF VACCINATED	44	38	42	124	1	0	0	44
					2	1	1	36
					3	15	20	7
STOOLS OF VACCINE CONTACTS	5	-	27	32	1	0	0	5
					3	26	1	0

tissue would not multiply at 40° C. and yet was neurovirulent for the monkey. On the other hand, there were *rct*/40+ strains of very low virulence for the monkey.

DR. VOROSHILOVA: We have studied the temperature marker of the strains which were isolated from the patients in Karaganda (40 of Type 1, 12 of Type 2, and 5 of Type 3.) Thirty-four strains of Type 1 were isolated from children that had not been vaccinated. Thirty-one of them possessed a $T+$ marker and three, a $T\pm$ marker. There was none of the $T-$ strains.

Of 12 strains of Type 2, all had marker $T+$. Of five strains of Type 3, one was $T+$ and four were $T-$. In one of those cases the child had no contact with vaccinees because this took place before the vaccination campaign. Perhaps in three cases alone there might have been some contagion from children who had been vaccinated.

Of the six strains of the Type 1 isolated from vaccinated children, three had $T+$, one $T\pm$, and two had a $T-$ marker.

On the two strains of the Type 2 isolated from vaccinated children, both belonged to the $T-$ type.

On the four strains of the Type 3, one had $T+$, two $T\pm$, and one a $T-$ marker.

In this way, of all the strains isolated from non-vaccinated children, only one had definitely the $T-$ marker. Most of the others had $T+$ and three strains of the Type 1 $T\pm$ marker.

Thus, it is obvious that this is the exception which has been mentioned by Dr. Sabin.

CHAIRMAN BURNET: The next paper will be presented by Dr. Pette on "Experimental Studies on Animals with Attenuated Poliovirus (Cox and Sabin Strains). This is to be followed by Dr. Sweet's and Dr. Hilleman's paper on "Detection of a 'Non-Detectable' Simian Virus (Vacuolating Agent) Present in Rhesus and Cynomolgus Monkey-Kidney Cell Culture Material." The discussion on these papers will take place after these presentations.

5. EXPERIMENTAL STUDIES ON ANIMALS WITH ATTENUATED POLIOVIRUS (COX AND SABIN STRAINS)

H. PETTE, H. LENNARTZ, G. MAASS, L. VALENCIANO, AND K. MANNWEILER

Institute for the Research of Poliomyelitis and Multiple Sclerosis, Hamburg, Germany

DR. PETTE (*presenting the paper*): Conflicting reports on the extent of pathogenicity of attenuated strains of poliovirus suggested this investigation on the results of the experimental infection of monkeys with such strains. The polio strains of Sabin* and Cox* were used; external circumstances did not permit the inclusion of Koprowski's strains in this study.

Material and methods. Rhesus monkeys weighing 1500 to 3000 g. were divided into groups of 4 to 5 animals and subjected to intraspinal and intracerebral inoculation with virus strains (undiluted and dilutions 1:10 and 1:100). At the beginning of the experiments the animals received aureomycin daily for 10 days (in the examination of Type 1 strain of Cox terramycin was used) to prevent enteritis.

Technique of inoculation. The technique described by Murray was employed, i.e., the intraspinal injection of 0.2 ml. between the first and second lumbar vertebra into the lumbar intumescence, or the bilateral intrathalamic injection of 0.5 ml. The injections were made under ether anesthesia.

The strains used including the virus concentrations found by us, are recorded below.

For the dilution of the virus strains examined TCM 199 was used. Following a 21-day period of observation the monkeys were sacrificed.

Virus isolation.

(a) *From feces:* Before the beginning of the experiment and thereafter weekly stools were taken by rectal swabs, prepared in usual manner and inoculated into cultures of monkey-kidney tissue.

(b) *Central nervous system:* At autopsy material was taken from the lumbar and cervical cord, brain stem and precentral gyrus. A 10 per cent suspension was examined for poliovirus in monkey-kidney tissue cultures.

Virus titration. Tenfold dilution series, inoculation dose per tube 0.1 ml.; in general five test tubes per dilution were used. Calculations of virus titer were made according to Reed and Muench. The determination of plaque-forming units was carried out in bottles according to the technique of Hsiung and Melnick.

Antibody determination. Blood was drawn for serological examination at the beginning and thereafter once weekly during the experiment. Neutralizing antibodies were determined with the color test according to the method of Salk *et al.*

Observation of the animals. The animals

	TYPE	STRAIN	PFU/1.0	$TCID_{50}/1.0$
Sabin	1	L Sc, 2 ab	$10^{6.7}$	$10^{6.5}$
	2	P 712, Ch, 2 ab	$10^{7.0}$	$10^{7.0}$
	3	Leon 12 a$_1$b	$10^{6.7}$	$10^{6.3}$
Cox	1	7 — 1231 — 166	$10^{6.6}$	$10^{6.2}$
	2	7 — 1232 — 243	$10^{6.2}$	$10^{6.0}$
	3	7 — 1233 — 344	$10^{7.0}$	$10^{7.3}$

* We are grateful to Prof. Verlinde of the University of Leiden, for supplying us with the virus strains of Sabin, and to Dr. Cox of Lederle Laboratories, Pearl River, N.Y., for the Cox strains.

were examined daily with special care regarding motor function and reflex behavior, particularly the patellar reflexes.

Histological techniques. Brain and spinal cord were removed and fixed in 10 per cent formalin solution for histological examination. Paraffine blocks, staining according to Nissl, van Gieson, Heidenhain and with hematoxylin-eosin. The lumbar and cervical intumescence were imbedded in six blocks, from each block one section plane was examined. In the same manner three levels of the thoracic cord were examined, from the brain stem the upper and lower portion. Both sides of the precentral gyrus (apical portion) were examined. Grading of lesions from 1 — 4+ and determination of the extent of lesions in the spinal cord were carried out according to the method of Melnick.

Findings.

(a) *Observation of the animals:* Following intracerebral inoculation traumatic disturbances occurred in three cases. During the further period of observation weakness of the extremities was found in none of the animals inoculated intracerebrally. Following intraspinal inoculation all of the animals, in which regular injections had been made into the lumbar cord, developed paralyses immediately after the inoculation, with an increase during the following 1-2 days in some cases. Essentially, these traumatic paralyses consisted in motor weakness of the flexor of feet and toes, more rarely of the legs. It proved difficult to distinguish these traumatic paralyses from those caused by the poliovirus. For this reason, motor weakness appearing within 48 hours after injection was considered as being of traumatic origin, whereas paralyses developing subsequently were attributed to virus action. The latter lesions also consisted in motor weakness of the leg and foot musculature, more seldom of the upper leg and thigh; all degrees from slight paresis to complete paralysis were encountered. Slight paresis of the upper extremities were seen in four cases following I.S. inoculation with Type 1 Cox's strain.

In general the motor weakness described reached maximum development about the 10th day after injection; the general condition of the animals remained good in all cases. The arrest of the process was occasionally manifested by a return of the patellar reflex. In one monkey (No. 99), inoculated with Type 2 Cox's strain, an ascending paralysis of Landry type followed I.S. injection of virus. The animal was sacrificed in moribund condition six days after inoculation.

(b) *Pathological anatomy:* The quality of histologic alterations corresponded to those found in not too severe poliomyelitis with a destruction of nerve cells and inflammatory reaction. The slight extent of active neuronophagia may be attributed to the duration of the process.

TABLE 1.

TYPE 1

	COX (6.5PFU/1.0)				SABIN (6.7PFU/1.0)			
	L.	C.	B.S.	C.A.	L.	C.	B.S.	C.A.
				I.S. Inoculation				
10^{0}	3/3	3	3	0	5/5	0	0	0
10^{-1}	4/4	3	2	1	4/4	0	0	0
10^{-2}	5/5	5	1	1	3/3	2	1	0
				I.C. Inoculation				
10^{0}	0/5	0	0	0	0/4	0	0	0
10^{-1}	0/5	0	0	0	0/5	0	0	0
10^{-2}	0/5	0	0	0	0/5	0	0	0

No. of monkeys with polio lesions / No. of inoculated monkeys

In the animals that died intercurrently nueronophagia as well as acutely damaged ganglian cells were found. The neuronophagia was of leucocytic and microglial nature. In the course of the inflammatory reactions pronounced diffuse and nodular proliferations of microglia as well as perivascular infiltrations of lymphocytes and plasma cells were seen.

The topography of alterations, partly extending up to the precentral gyrus, was characteristic of poliomyelitis. In cases in which the lumbar and cervical cord were involved, the thoracic cord usually also contained lesions, even though less pronounced. The distribution of specific alterations thus corresponded to the typical distribution in poliomyelitis. The animals in which the virus had not been injected into the spinal cord grey matter showed no lesions of poliomyelitis. Histologically the following differences in neuropathogenicity of the strains examined were found:

Type 1 I.S. inoculation. All of the animals showed alterations in the lumbar section which obviously were not merely of traumatic nature. The alterations found with the Sabin strain were not as extensive and severe as with Cox's strain. Here the alterations reached up to the brain stem in six cases, in two animals up to the precentral gyrus. With the Sabin strain the process extended up to the cervical cord twice and once up to the brain stem. Following I.C. inoculation, alterations were missing in both groups.

Type 2 I.S. inoculation. In all animals the alterations of the lumbar region already described were found. With the Sabin strain an extension of the process up to the brain stem was seen in two animals. With the Cox strain the process almost invariably extended up to the brain stem, partly up to the precentral gyrus. Clinically a slight paralysis of the arms was seen in four cases. Following I.C. inoculation there were no histological alterations with the Sabin strain. With Cox's strain alterations were found in most of the animals at the predilection sites of poliomyelitis. Intensity and extent of the lesions showed no significant relationship to the virus concentrations administered. Histological findings were as described above, motor weakness of extremities could not be observed with certainty.

Type 3 I.S. inoculation. In the lumbar area all of the animals had the usual lesions. With the Sabin strain the process in three animals extended up into the cervical cord, in two others lesions of the central gyrus were found.

With Cox's strain 5 of the 13 animals inoculated showed alterations characteristic of polio up to the precentral gyrus.

Following I.C. inoculation there were no pathological changes with the Sabin strain. Following inoculation with the Cox strain alterations reaching down to the lumbar area were found in three animals.

TABLE 2.

TYPE 2

	Cox (6.2PFU/1.0)				Sabin (7.0PFU/1.0)			
	L.	C.	B.S.	C.A.	L.	C.	B.S.	C.A.
				I. S. Inoculation				
10^{0}	4/4	4	4	4	4/4	1	1	0
10^{-1}	5/5	5	5	3	4/4	0	0	0
10^{-2}	5/5	5	4	4	4/4	1	1	0
				I. C. Inoculation				
10^{0}	3/5	3	4	3	0/3	0	0	0
10^{-1}	4/5	4	4	1	0/5	0	0	0
10^{-2}	3/5	3	3	3	0/5	0	0	0

No. of monkeys with polio lesions / No. of inoculated monkeys

TABLE 3.

	TYPE 3							
	COX (7.0PFU/1.0)				SABIN (6.7PFU/1.0)			
	L.	C.	B.S.	C.A.	L.	C.	B.S.	C.A.
	I. S. Inoculation							
10^{0}	5/5	2	3	2	4/4	1	1	1
10^{-1}	4/4	2	2	1	4/4	1	1	1
10^{-2}	4/4	2	2	2	5/5	1	0	0
	I. C. Inoculation							
10^{0}	2/5	2	2	0	0/4	0	0	0
10^{-1}	1/5	1	0	0	0/5	0	0	0
10^{-2}	0/5	0	0	0	0/5	0	0	0

No. of monkeys with polio lesions / No. of inoculated monkeys

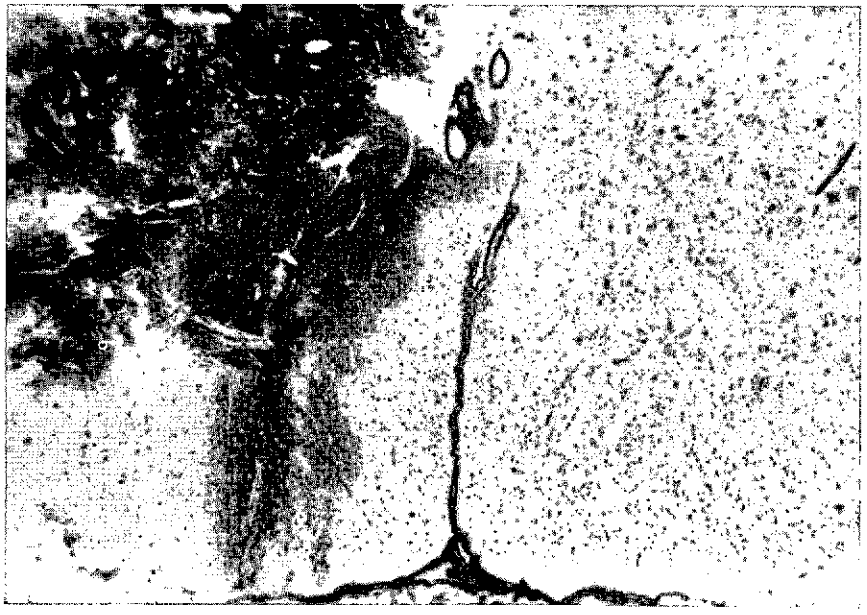


FIG. 1. Inoculation of india ink into the lumbar cord.

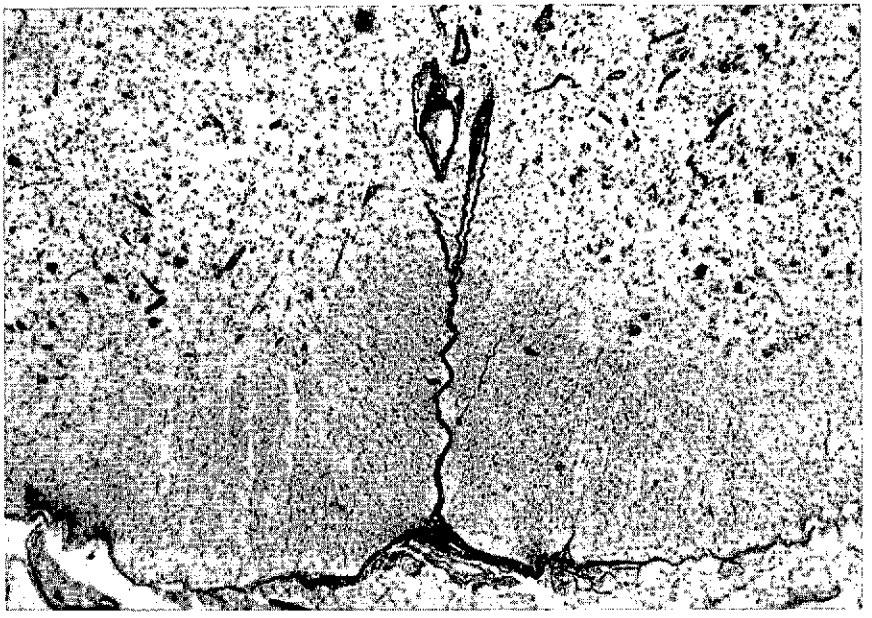


FIG. 2. Same, more distant of site of inoculation.

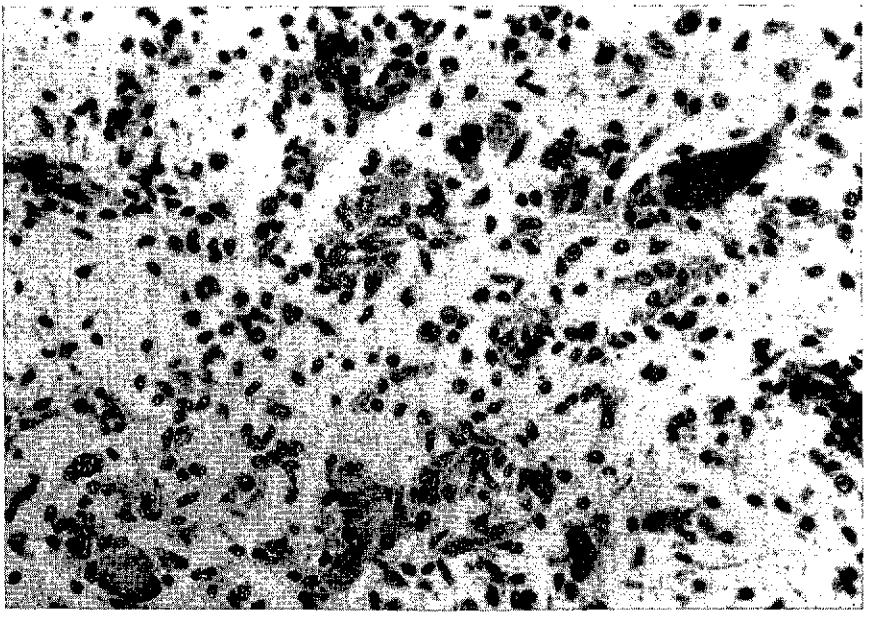


FIG. 3. Sabin's Type 3 (intraspinal inoculation), lumbar cord. Inflammatory reaction in the anterior horn.

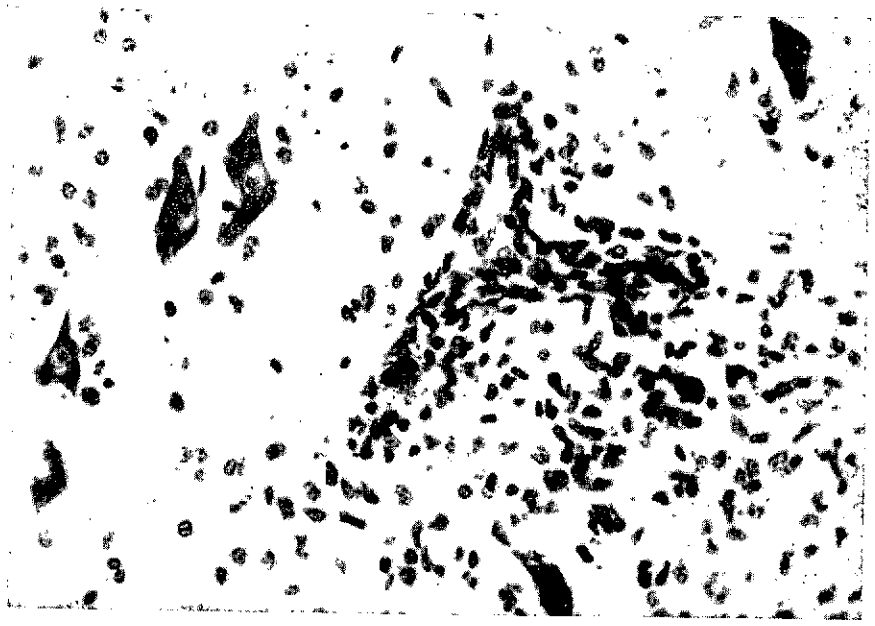


FIG. 4. Sabin's Type 3 (intraspinal inoculation), cervical cord. Perivascular and focal infiltrative lesions, damaged neurons.

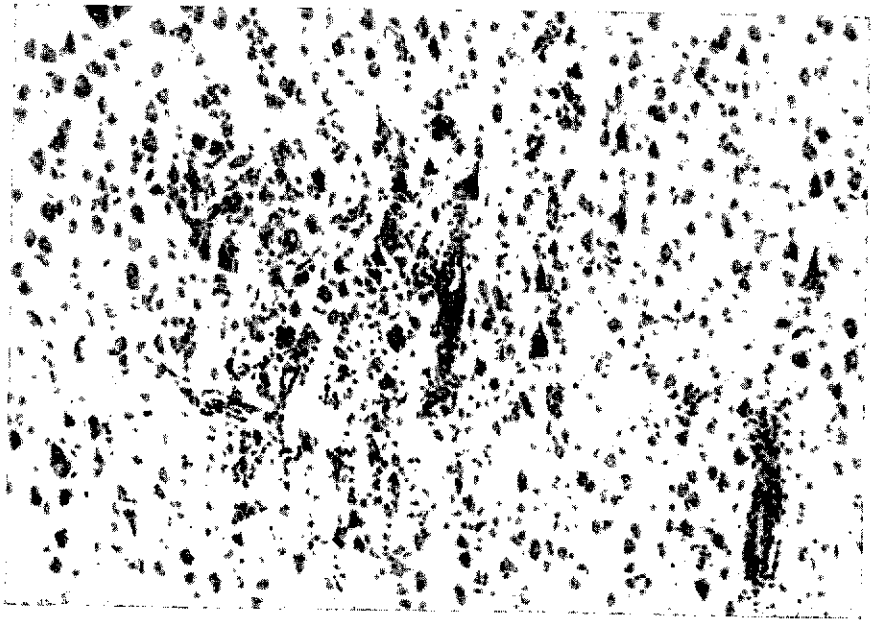


FIG. 5. Sabin's Type 3 (intraspinal inoculation), precentral gyrus, inflammatory lesions.

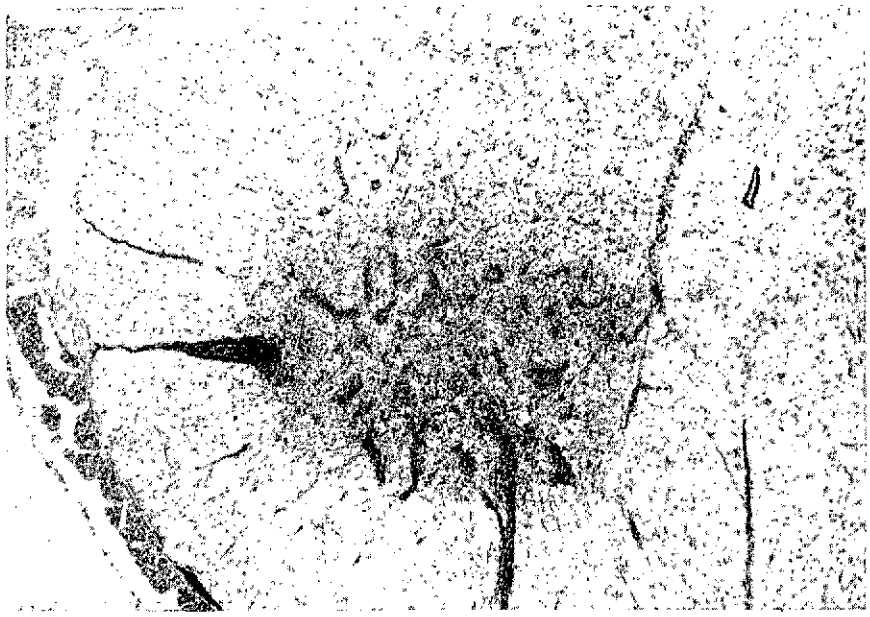


FIG. 6. Cox's Type 2 (intraspinal inoculation), lumbar cord. Inflammatory reactions, neuronal lesions.

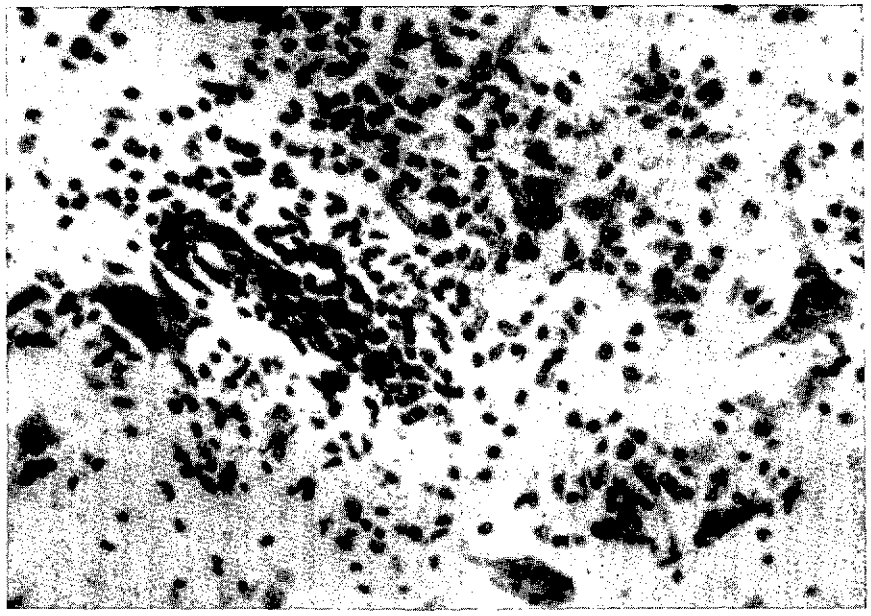


FIG. 7. Cox's Type 2 (intraspinal inoculation), cervical cord. Perivascular and focal infiltrative lesions and neuronal lesions in the anterior horn.

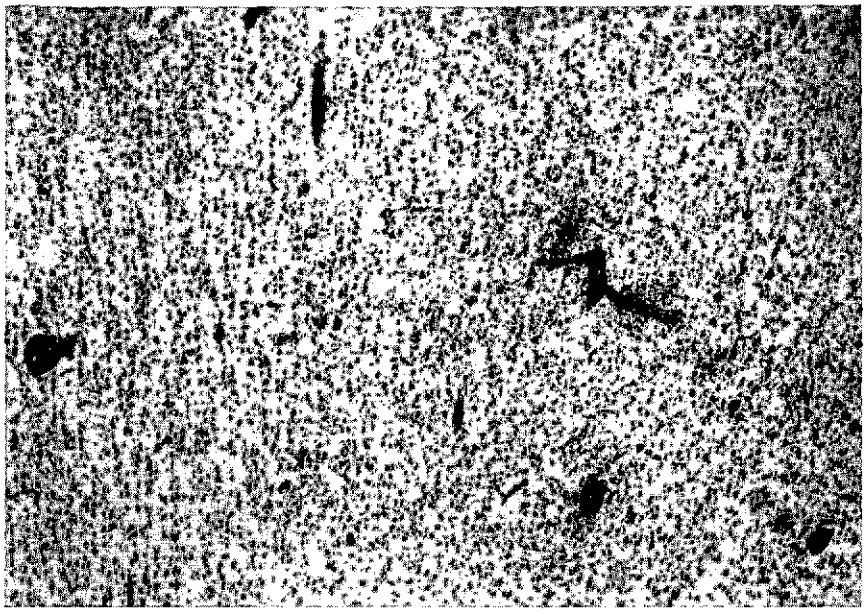


FIG. 8. Cox's Type 2 (intraspinal inoculation) precentral gyrus. Inflammatory lesions.

TABLE 4.

	TYPE	ROUTE	DILUTION		
			10^{0}	10^{-1}	10^{-2}
Cox	1	I.S.	2/3	2/4	3/5
		I.C.	0/5	0/5	0/5
	2	I.S.	2/4	2/5	4/5
		I.C.	3/5	2/5	3/5
	3	I.S.	1/5*	0/4	0/5
		I.C.	0/5	1/5*	0/5
Sabin	1	I.S.	0/5	0/4	0/3
		I.C.	0/4	0/5	0/5
	2	I.S.	0/4	0/4	0/4
		I.C.	0/3	0/5	0/5
	3	I.S.	1/4	1/4	0/5
		I.C.	0/4	0/5	0/5

No. of virus isolations/No. of inoculated monkeys.

(c) *Virus isolation from CNS:* With Types 1 and 2 of the Sabin strain no virus could be isolated from the central nervous system of any of the animals (lumbar and cervical cord, brain stem, precentral gyrus) at the end of the observation period. With Type 3 isolation attempts were successful in two cases. With Cox's Type 1 strain, virus could be isolated from the CNS of the monkeys inoculated intraspinally in numerous cases, not however in animals following I.C. inoculation. With Cox's Type 2, virus was isolated in more than half of the cases at the end of the observation period; with Type 3 in only two monkeys who died during the observation period (indicated by an asterisk, see Table 4).

In the monkeys found positive, virus isolation was successful in one or more; seldom, however, in all areas of the central nervous system examined. The virus concentration was lower than that found in corresponding areas after inoculation of virulent strains. A comparison of the frequency of positive virus findings with the severity of histological lesions in general showed parallel results. Virus isolation was successful especially in those cases in which motor weakness was still pronounced during the second or third week of the observation period. Particularly in the animals dying intercurrently poliomyelitis virus was occasionally also found in the absence of characteristic histological alterations.

The virus strains isolated from the central nervous system were examined with reference to tissue-culture markers *d*, *T*, and *MS*. These examinations appeared promising since in addition to possible mutations of the virus inoculated the selective effect of central nervous system for neurotropic poliovirus particles also had to be considered. With all strains in which an examination was possible, some of the cases showed alterations of one or two markers in comparison to the initial material. In no instance did these cases approach the behavior of the virulent strains used for controls, however. Compared with the other strains, the low number of changes in genetic behavior found with Cox's Type 2 strain was remarkable.

(d) *Antibody formation in animals:* With all strains there appeared to be a relation between the production of antibodies and the virus concentrations administered. There was no difference in the results with I.S. and I.C. inoculation, however. Antibody formation did not seem to

TABLE 5.

	TYPE	ROUTE	DILUTION		
			10^{0}	10^{-1}	10^{-2}
Cox	1	I.S.	2/3	0/4	0/5
		I.C.	3/5	1/5	0/5
	2	I.S.	2/4	1/5	0/5
		I.C.	3/5	3/5	0/5
	3	I.S.	1/5	1/4	0/5
		I.C.	1/5	2/5	0/5
Sabin	1	I.S.	0/5	1/4	0/3
		I.C.	2/4	2/5	0/5
	2	I.S.	4/4	2/4	0/4
		I.C.	1/3	2/5	1/5
	3	I.S.	1/4	0/4	0/5
		I.C.	0/4	0/5	0/5

No. of monkeys with antibodies/No. of inoculated monkeys.

depend on the extent and occurrence of central nervous system lesions, i.e., animals in which I.S. inoculation did not reach the grey substance of the lumbar cord, also showed antibody formation. Differences between the strains examined seem to occur only with regard to Type 3, of which Sabin's strain led to antibody production in only one case (see Table 5).

The antibody titer was partly high and in some cases exceeded a dilution of 1:512. The various strains showed no difference in the height of titers.

(c) *Virus isolation from feces:* 627 specimens of feces were examined before and during the experiment. In 206 cases a cytopathogenic effect could be found in tissue cultures (43 cases showed the type of cytopathogenic effect found with adenovirus, in 159 cases it had not yet been possible to determine the type, in 4 cases poliovirus was isolated).

Discussion. Our findings with regard to the neuropathogenicity of the attenuated virus strains of Sabin and Cox are in agreement with the results of Murray and Melnick, i.e., all three of Sabin's intraspinally inoculated strains are less neuropathogenic than those of Cox. This difference was most striking in the Type 2 strains, whereas there was no difference following intracerebral inoculation of Type 1 strains of Sabin and Cox.

A comparison with Murray's findings regarding the extent of neuropathogenicity showed that in our examinations Cox's Type 1 led to less pronounced histological lesions in the central nervous system. Results with Types 2 and 3 of Cox tallied quite well with the findings of Murray. With Sabin's Types 2 and 3 strains, the alterations found by us were similar to Murray's findings. This was also the case with Type 1 following I.C. inoculation. Following I.S. inoculation, the alterations found by us were less pronounced.

A comparison of our findings with those of Cabasso using the Cox strains revealed a higher degree of neuropathogenicity following I.S. inoculation of Type 1. There was no difference in results following I.C. inoculation. With Type 2 we found histological alterations more frequently than Cabasso following I.S. as well as I.C. inoculation. A comparison of results with Type 3 was not possible.

An attempt to isolate the virus from the central nervous system after the end of the observation period showed striking differences between the various strains. The rate of successful isolations essentially paralleled the neuropathogenicity found by histological examination. The examination of genetic properties of isolated strains using markers *d*, *T*, and *MS* occasionally showed slight alterations of some characteristics in comparison to the initial material. Cox's Type 2 seems to possess the greatest stability with respect to the examined genetic markers.

There was no difference in the production of antibodies following I.C. and I.S. inoculation; particularly, there was no relationship with the occurrence and extent of histological lesions in the central nervous system. Noteworthy was the fact that antibody production was rare following inoculation of Sabin's Type 3 strain. No connection was found between antibody production and the frequency of isolation of non-poliovirus from the feces of the animals. The antibody formation is regarded as proof of a multiplication of virus outside of the central nervous system, with the spreading of the virus obviously taking place directly via the inoculation trauma. The validity of this statement was supported by the results of experiments on the inoculation of india ink into the central nervous system.

Finally we would like to raise the question of whether, in addition to the usual methods of testing the neuropathogenicity of virus, other criteria might also be useful in assessing the suitability of strains for human vaccination. Of these, one might name the obvious multiplication of virus in the central nervous system, the ability to multiply outside of the central nervous system and the genetic stability of virus strain following passage in neural and extraneural tissue.

CONCLUSION

By intracerebral and intraspinal inoculation of monkeys with the attenuated poliovirus strains of Sabin and Cox it was shown that all three of Sabin's strains are less neuropathogenic than those of Cox. These results are in agreement with the findings of Murray and Melnick.

At the end of the observation period of 21 days, the results of virus isolations from the central nervous system paralleled those on neuropathogenicity as manifested by histological alter-

ations. In tests with genetic markers *d*, *T*, and *MS* the strains isolated revealed only occasional and slight alterations in comparison to the initial material. While following passage through the central nervous system they did not present in one single instance the characteristics of virulent strains.

In tests on antibody formation following intraspinal and intracerebral inoculation, antibodies were seldom produced following the use of Sabin's Type 3 strain. The possibilities for determining neuropathogenicity and the question of further criteria for the evaluation of these vaccines are discussed.

6. DETECTION OF A "NON-DETECTABLE" SIMIAN VIRUS (VACUOLATING AGENT) PRESENT IN RHESUS AND CYNOMOLGUS MONKEY-KIDNEY CELL CULTURE MATERIAL. A PRELIMINARY REPORT

B. H. SWEET AND M. R. HILLEMAN

Division of Virus and Tissue Culture Research,
Merck Institute for Therapeutic Research,
West Point, Pennsylvania

DR. HILLEMAN (*presenting the paper*): A number of viral agents have been recovered from the tissues and excreta of monkeys. [1-6] Certain of these agents are present in high frequency in kidney-cell cultures of these animals. These agents are currently called simian or ECMO viruses. Hull [5] has classified the simian viruses into four groups (CPE groups 1, 2, 3, and 4) based on the kind of cytopathic change induced in monkey-kidney cell cultures infected with these agents. Twenty-eight of these viruses were precisely separated serologically into types and, additionally, 24 "unidentifiable" viruses were recorded. Malherbe and Harwin [4] distinguished seven distinct types among the SA viruses they recovered from vervet monkey materials.

The simian viruses of monkey kidney origin present a real problem in the virus application of the cell culture technique. Thus, indigenous simian virus may contaminate or even exclude the inoculated virus on passage in monkey-kidney cell culture. Virus-infected culture fluids used to prepare killed poliomyelitis or adenovirus vaccine are commonly contaminated with simian agents and assurance must be provided for the inactivation of both the intended and the simian viruses in these vaccines. Testing for such "intruding" viruses may be further complicated by the presence of such agents in the cultures used to perform the tests. Finally, such simian viruses must be definitively excluded from any live virus vaccine intended for routine use in man since the long-term effect of human infection with these agents is unknown and since data on short-term effect are either meager or entirely lacking.

All of the presently reported simian viruses derived from monkey-kidney cell culture have been detected in kidney-cell cultures of the same monkey species, either in the primary culture itself or on further passage. Thus, all of the agents previously reported have been readily detectable and can be excluded on the basis of rigid testing in monkey cell cultures. The question has often been raised concerning hypothetical "non-detectable" simian viruses, i.e., those agents which might be present in monkey kidneys but which cannot be detected by current procedures.

During the past two years, our virus research work has repeatedly uncovered what appears to be a new simian virus of Rhesus and Cynomolgus monkey kidney origin and which does not cause significant cytopathic change in kidney-cell cultures of these same species. The agent does, however, cause very marked and distinctive cytopathic changes in kidney-cell cultures of the African green monkey, *Cercopithecus aethiops* [grivet, according to Sanderson [7]], obtained from equatorial East Africa. In our laboratory, we have referred to this virus as the "vacuolating agent", because of the prominent cytoplasmic vacuolation seen in infected cell cultures. Dr. Hull [8] has suggested that this vacuolating virus be designated S.V. 40 and be included in Hull's C.P.E. Group 4.[8]

Studies in our laboratory of the vacuolating agent have been directed mainly toward its elimination rather than its emulation. Hence, certain aspects of the work are scanty or still in progress. The present report, which is preliminary, records the findings to date.

ORIGIN OF STRAINS OF VACUOLATING VIRUS

Vacuolating virus is readily recovered from contaminated virus seed stocks by passage of the virus seed, in the presence of its homologous

antiserum, in cell cultures of green monkey kidney. Under these conditions, the contaminating vacuolating virus "breaks through" and is identified in serum neutralization tests using anti-vacuolating agent serum prepared in rabbits. For the studies reported here, the green monkey-kidney cell cultures were prepared by overnight trypsinization of the kidneys at 4° C. and cultivation in lactalysate medium containing 2 per cent heat-inactivated calf serum. For virus propagation, the cultures were washed and maintained in medium 199 containing 2 per cent chicken serum.

More than 20 strains of vacuolating virus have been recovered in our laboratory to date. Table 1 shows the origin of eight of the strains examined most extensively to the present time. Vacuolating virus has appeared as a contaminant in the seed stocks of Types 1-7 adenovirus. These seeds were prepared in cultures of Rhesus or Cynomolgus monkey kidney and were obtained from Dr. R. Huebner or brought to this laboratory by one of us (M.R.H.) from the Walter Reed Army Institute of Research. Additionally, vacuolating virus has been recovered from seed stocks of Myxovirus para-influenza 1 and 3, from the SA virus, and from the respiratory syncytial agent received from Dr. R. Chanock or Dr. K. Habel.

Strains S 207, S 211, and S 215 were derived from Types 1, 2, and 3, respectively, of Sabin live attenuated polio vaccine. In each instance, the poliovirus was neutralized by homologous polio immune serum prepared in rabbits immunized with Syverton's HeLa lines of poliovirus propagated in human stable amnion cell culture. The infectivity titer of the vacuolating virus in each preparation ranged from $10^{-3.7}$ to $10^{-4.5}$. These isolates of vacuolating agent were identified in serum neutralization tests with rabbit antisera against prototype strain 776. At the time of the initial isolations of vacuolating virus, the Sabin vaccines were also passaged in the presence of a mixture of homologous poliovirus and vacuolating agent antiserum. These antisera suppressed both the poliovirus and the vacuolating agent and showed the absence of other detectable viruses which might have been present in the Sabin materials.

Injection rate in normal monkey-kidney cell cultures. During a one-month period, 10 Rhesus and 10 Cynomolgus kidney-cell culture lots prepared for use in ordinary Salk vaccine production were examined for presence of the vacuolating virus. Each lot of monkey kidney was derived from a pool of kidneys from two or three monkeys. Ten days after planting, the cultures were changed to medium 199 and held for an additional 10 days. No definitive change typical of the vacuolating agent was seen. On the tenth day, culture fluid from each lot was passed to green monkey-kidney cell cultures. Seven of the 10 Rhesus-cell lots yielded the vacuolating virus. One of the cell lots was infected with a foamy-like virus [1] and two appeared free of contaminating agents. The infectivity titers of the inapparent vacuolating virus in the "normal" Rhesus monkey-kidney

TABLE 1. ORIGIN OF EIGHT STRAINS OF VACUOLATING VIRUS

STRAIN	RECOVERED FROM	QUANTITY OF VACUOLATINGL VIRUS IN ORIGINA MATERIAL (INFECTIVITY TITER)
776	Adenovirus Type 1, Seed Stock	—
175	Adenovirus Type 5, Seed Stock	—
S 207	Sabin Type 1, Live Attenuated Oral Polio Vaccine, Strain LSc	$10^{-4.5}$
S 211	Sabin Type 2, Live Attenuated Oral Polio Vaccine, Strain P712	$10^{-3.7}$
S 215	Sabin Type 3, Live Attenuated Oral Polio Vaccine, Strain Leon	$10^{-4.5}$
953	Uninfected "Normal" Rhesus Monkey-Kidney Cell Culture	$10^{-5.5}$
1095	Uninfected "Normal" Rhesus Monkey-Kidney Cell Culture	$10^{-6.0}$
584	Uninfected "Normal" Cynomolgus Monkey-Kidney Cell Culture	$10^{-5.5}$

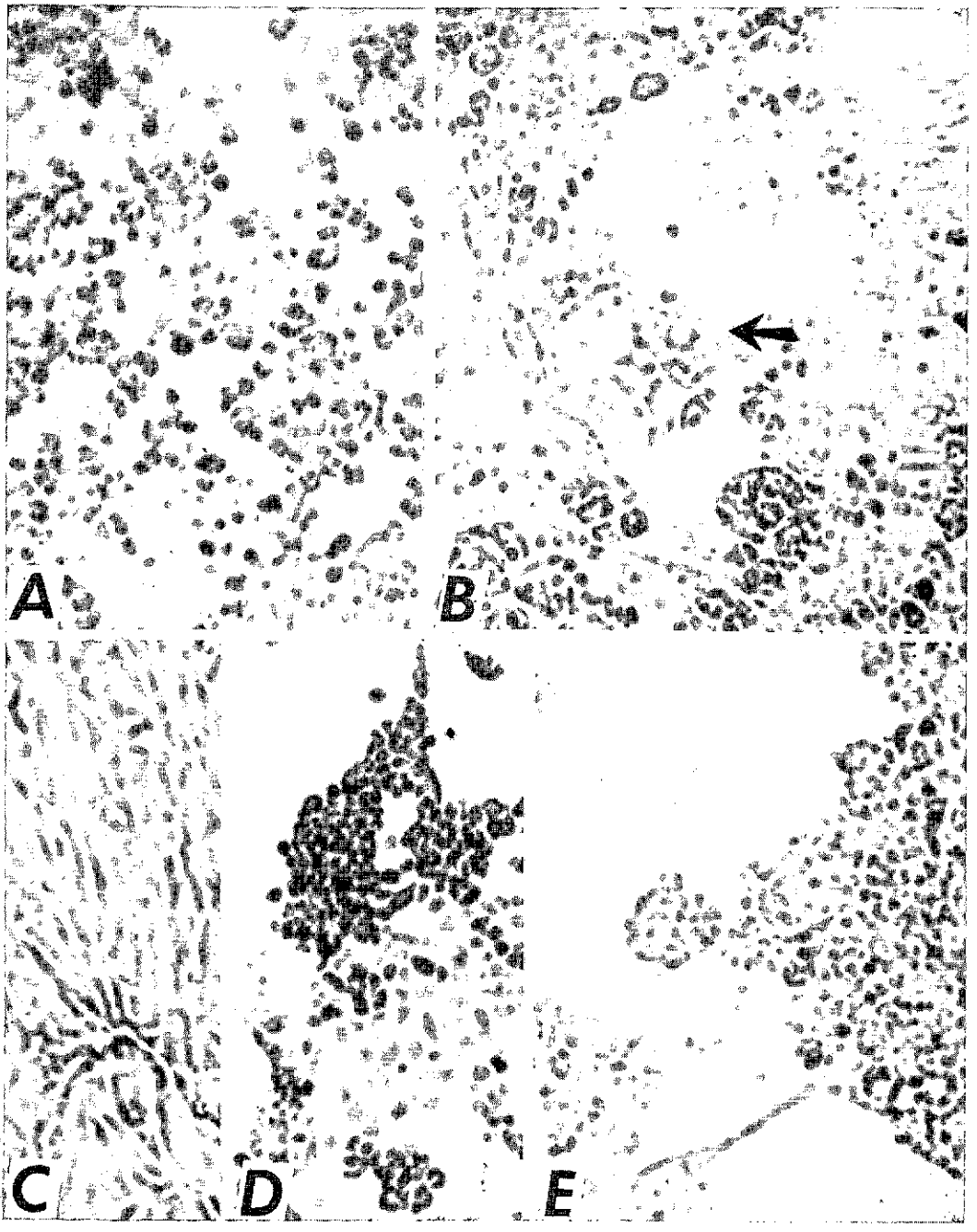


FIG. 1. Cytopathic changes in wet preparations, infected green monkey-kidney cell cultures, caused by strain 1095 of vacuolating virus. A.—Infected culture, day 4, 112 x mag'n. B.—Same culture 400 x mag'n. (arrow points to cell with vacuoles). C.—Uninoculated control culture, day 6. D.—Infected culture, day 6, 112 x mag'n. E.—Same culture, 400 x mag'n.

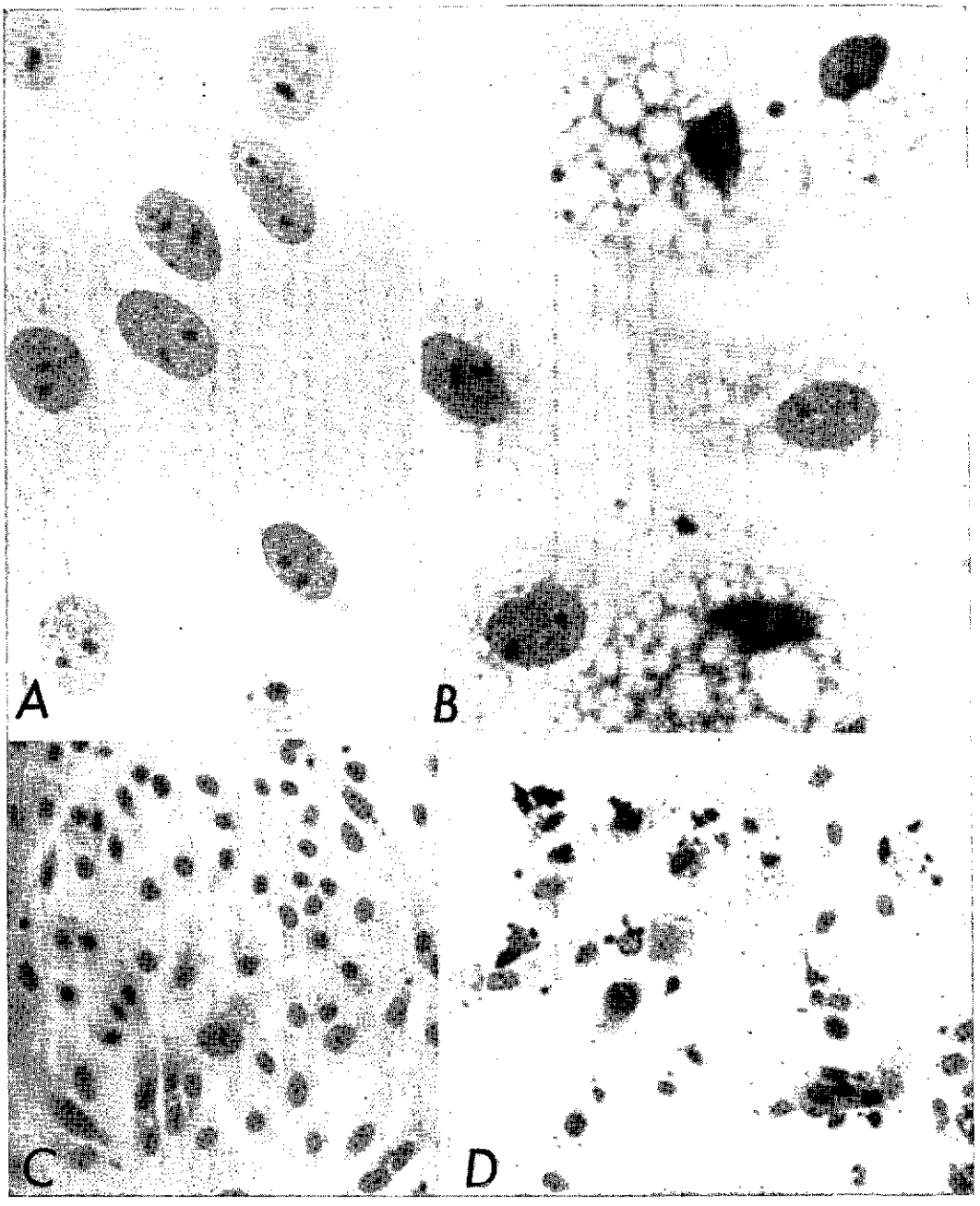


FIG. 2. Cytopathic changes in hematoxylin-eosin stained infected green monkey-kidney cell cultures, caused by strain 1095 of vacuolating virus. A.—Uninoculated control culture, day 5, 840 x mag'n. B—Infected culture, day 5, 840 x mag'n. C.—Uninoc. control culture, day 5, 198 x mag'n. D.—Infected culture, day 5, 198 x mag'n.

cell culture were found to be as high as $10^{-6.0}$ in 0.2 ml.

Only one of the 10 Cynomolgus cell-culture lots revealed the vacuolating virus. However, definitive tests of these lots for vacuolating virus were not possible since eight of the lots were heavily contaminated with what appeared to be the foamy virus. Thus, vacuolating agent, if present, may have been overgrown or excluded by foamy virus.

Cultures of African green monkey-kidney prepared in our laboratories have been found remarkably free of the vacuolating agent. In the course of use of more than 1000 lots of individual monkey-cell cultures, presence of vacuolating agent was suspect for only four lots and proved for only one lot.

PROPERTIES OF THE VACUOLATING VIRUS

Cytopathic effect. Figures 1 and 2 show, respectively, the outstanding changes caused by vacuolating virus as observed in wet culture and in stained preparations. Green monkey-kidney cell cultures infected with about 1000 TCD_{50} of vacuolating virus show beginning changes on day 3 or 4. At this time, some of the cells may appear rounded or shrunken and may exhibit a darkened cytoplasm. A few of the cells may show beginning vacuolation of the cytoplasm. With increased time, the typical changes develop. These consist predominantly of enlargement of cells which are spread out on the glass or ballooning of free cells. The cytoplasm of these cells becomes filled with vacuoles which appear highly refractile in wet preparations and which appear as "holes" with intensely stained boundaries in stained preparations. The nucleus may appear normal or disorganized internally. Limited fusion of the cytoplasm of adjoining cells may occur but, predominantly, each cell maintains its own integrity. Thus, syncytia and giant cells, such as occur with measles or foamy virus infection, are not prominent in vacuolating agent infection. Within 5 to 10 days, the infected cells aggregate together and detach from the glass leaving behind only small islands of normal or degenerated cells.

No definitive cytoplasmic or nuclear inclusion bodies have been observed to the present in H and E stained preparations of cells infected with the vacuolating virus. The cytopathic changes caused by the vacuolating agent appear quite distinct from those described for Hull's four groups of simian viruses and for Malherbe's and Harwin's seven simian agents.

Antigenic relationships. The strains of vacuolating virus studied to date appear to comprise a single immunologic group. As shown in Table 2, antisera against vacuolating agent strains 776 and 175 neutralized the homologous viruses and all of three additional strains as well. Additionally, all three strains of Sabin vaccine origin were neutralized by strain 776 antiserum.

Strain 776 of vacuolating virus was not neutralized by antiserum against group 4 SV-6, SV-26, or SV-29 antisera furnished by Dr. Hull. Additionally, it was not neutralized by Myxovirus parainfluenza 1 or 3 antisera or by such SA, SV-5 or foamy virus antisera as were im-

TABLE 2. ANTIGENIC RELATIONSHIPS OF SELECTED STRAINS OF VACUOLATING VIRUS AS MEASURED IN SERUM NEUTRALIZATION TESTS

VIRUS STRAIN TESTED	TITER OF SERUM AGAINST STRAIN	
	776	175
776	4096 or >	800
175	4096 or >	1600
1095	4096 or >	2048
953	4096 or >	4096 or >
584	4096 or >	4096

mediately available to us. These tests are being confirmed with certified sera. Vacuolating virus strains 776, 175, 953, 1095, and 584 were neutralized by pooled antiserum against Hull's group 1 B (S.V. 20, 23, 25, and 27) and the last three strains by group 3 (S.V. 12, 28, 59). None was neutralized by group 2 (S.V. 2, 16, 18, 19) or group 4 (S.V. 5, 6, 29) antisera. In these tests, only a negative finding has significance since the antisera employed were prepared using virus grown in Rhesus or Cynomolgus kidney-cell cultures, which might also have contained vacuolating virus.

Host cell range. Vacuolating virus strain 776 titering $10^{-5.0}$ in green monkey-kidney culture was tested in the various primary and line cell cultures shown in Table 3. No definitive cytopathic change referable to the virus was observed in any of these cultures, other than green monkey kidney, when observed for 8 to 12 days. The virus was shown to persist for at least 10 days in HeLa or stable amnion cell cultures, but without increase in titer. Titers as high as $10^{-8.5}$ have been obtained with vacuolating virus propagated in green monkey-kidney cultures.

Miscellaneous physical and biological properties. Certain of the properties of the vacuolating agent, not previously discussed, are shown in Table 4. Studies are in progress to determine the inactivation kinetics for the virus with 1:4000 formalin in the Salk vaccine production procedure.

TABLE 3. HOST CELL RANGE FOR CYTOPATHIC EFFECT OF VACUOLATING AGENT STRAIN 776

KIND OF CELL	CYTOPATHOLOGY
Primary Cell Cultures:	
Green Monkey Kidney	Positive
Rhesus Monkey Kidney	Negative
Rabbit Kidney	"
Human Amnion	"
Line Cell Cultures:	
HeLa	"
Stable Amnion	"
Chang Liver	"
Girardi Human Heart	"

TABLE 4. MISCELLANEOUS PHYSICAL AND BIOLOGICAL PROPERTIES OF VACUOLATING AGENT, STRAIN 776

1. Filterable through Selas 03 and Seitz S1 filters.
2. Infected cultures fail to hemadsorb or cause hemagglutination of guinea pig, chicken, or human "O" erythrocytes at 4°C. or at 25°C.
3. Resists treatment for 18 hours with an equal volume of diethyl ether.
4. Infectivity destroyed by treating with 1:2000 formalin for 48 hours at 37°C.
5. Relatively heat stable. Heating at 56°C. for one hour reduces infectivity titer about 30-fold.
6. Stable on storage at −20°C. and at −70°C.
7. Induces homologous neutralizing antibody in rabbits immunized with the agent.

OCCURRENCE OF ANTIBODY AGAINST VACUOLATING VIRUS IN HUMAN AND MONKEY SERA

Tests of various human and monkey antisera were carried out with the results shown in Table 5.

None of the sera from six animals in two lots of normal African green monkeys neutralized the virus. Twelve of 18 antisera from normal Rhesus monkeys neutralized the agent. This included six monkeys especially caught, in India, in a group of about 350 and flown to the U.S.A. without ever being "gang-caged". The animals were housed in single isolated cages after arrival and tested eight months after receipt.

One lot of human gamma globulin was found free of antibody when tested diluted 1:50.

Dr. Sabin sent us, for test, sera from five children, each fed Sabin live poliovirus vaccine Types 1, 2, or 3 on a total of six occasions and sera from five normal children. None of these sera showed antibody against the vacuolating virus at a dilution of 1:5.

Tests were conducted with sera from 10 military recruits in the first field trial[9] of adenovirus vaccine at Fort Dix, N. J., in 1956. Three of four recruits who had received two doses of adenovirus vaccine showed antibody against the vacuolating virus while none of six non-vaccinated persons showed antibody. Tests of pre- and post-vaccination sera from the same individuals are currently in progress. Appearance of antibody against vacuolating virus in such

TABLE 5. TESTS FOR NEUTRALIZING ANTIBODY AGAINST VACUOLATING AGENT STRAIN 776 IN HUMAN AND MONKEY SERA

No. Positive No. Tested	Description of Sera
0/6	Normal African green monkey, diluted 1:5
12/18	Normal Rhesus monkey, diluted 1:5
0/1	Phil. Serum Exchange, human gamma globulin, lot G50B, diluted 1:50
0/5	*Children fed Sabin live oral polio vaccine on six occasions, serum diluted 1:5
0/5	*Normal children, serum diluted 1:5
0/6	Normal military recruits, serum diluted 1:5
3/4	Military recruits given two doses of formalin-killed adenovirus vaccine. Post-vaccination sample, serum diluted 1:5

* Sera tested for Dr. A. Sabin.

vaccinated persons is not surprising since the adenovirus seed stocks used to prepare the vaccine have been found to be contaminated with the vacuolating virus.

SUMMARY AND COMMENT

The vacuolating virus appears to be a common and essentially ubiquitous contaminant of Rhesus monkey-kidney cell cultures, a likely common contaminant of Cynomolgus kidney cultures, and a relatively infrequent contaminant of African green monkey kidney.

The virus appears different from Hull's [3] four simian virus C.P.E. groups and from Malherbe's and Harwin's [4] seven agents, based on the distinctive vacuolating type of cytopathic change in infected cells. Failure of the vacuolating virus to cause cytopathic changes in Rhesus or Cynomolgus monkey-kidney cell cultures further distinguish the agent from Hull's groups.

Resistance of the virus to ether and failure of hemagglutination and hemadsorption distinguish the agent from the Myxoviruses.

The vacuolating agent appears to be just "one more" of the troublesome simian agents to be screened for and eliminated from virus seed stocks and from live virus vaccines. Lack of antibody response in human subjects fed polio vaccine containing vacuolating agent suggests lack of massive proliferation of the virus under the conditions employed although absence of human infection to some degree could not be excluded.

The detection in green monkey kidney of this common inapparent virus infection of Rhesus and Cynomolgus monkey kidney represents the first instance of demonstration of a "non-detectable" indigenous agent in the monkey-kidney culture system. Although new to simian viruses and monkey kidney, it is neither new nor unique to other viruses and cell culture systems. Demonstration of the vacuolating agent raises the question of possible presence of other indigenous and inapparent monkey kidney agents which might be detected under different methods of testing. It also raises the question of the extent to which proliferation of viruses inoculated into monkey-kidney cell cultures may be interfered with owing to the presence of such inapparent indigenous viral agents.

REFERENCES

1. Rustigian, R., Johnston, P., and Reihart, H.: *Proc. Soc. Exp. Biol.* **88**:8-16, 1955.
2. Hull, R. N. and Minner, J. R.: *Ann. N. York Acad. Sc.* **67**:413-423, 1957.
3. Hull, R. N., Minner, J. R., and Mascoli, C. C.: *Am. J. Hyg.* **68**:31-44, 1958.
4. Malherbe, H. and Harwin, R.: *Brit. J. Exp. Path.* **38**:539-541, 1957.
5. Cheever, F. S.: *Ann. N. York Acad. Sc.* **67**: 427-429, 1957.
6. Kalter, S. S.: *Bull. World Health Org.* **22**: 319-337, 1960.
7. Sanderson, I. T.: *The Monkey Kingdom.* Garden City, N. Y., Doubleday & Co., Inc., 1957.
8. Hull, R. N.: Personal communication.
9. Hilleman, M. R., Stallones, R. A., Gauld, R. L., Warfield, M. S., and Anderson, S. A.: *Proc. Soc. Exp. Biol.* **92**:377-383, 1956.

DISCUSSION

CHAIRMAN BURNET: The papers presented by Dr. Pette and Dr. Hilleman are now open for discussion.

DR. BARON: As regards the low isolation rate of viruses from the central nervous system of the monkeys inoculated with the attenuated virus in Dr. Pette's study, we have had similar experiences, that is, virulent strains resulted in a very high isolation rate of virus, while the avirulent strains gave a very low isolation rate, approximating an over-all average of 30 per cent.

On studying this further, and as we have reported in the recent Federation Proceedings, an inhibitory material was found in the central nervous system of these suspensions of central nervous system material as well as in CNS suspensions from normal monkeys. This inhibitor was able to inhibit one, two, sometimes three, $\log s_{10}$ of virus. Upon dilution of some of the originally negative central nervous system suspensions from monkeys with poliovirus lesions, we found that the virus was able to be isolated, but not in all cases.

We are currently working on methods to destroy the inhibitor selectively and leave the virus intact. I think the point to be made is that there may be a higher virus isolation rate once the inhibitor in the central nervous system suspension has been eliminated.

CHAIRMAN BURNET: Are there further comments? Dr. Gear.

DR. GEAR: As Dr. Hilleman has pointed out, Dr. Malherbe, who is in charge of our safety testing at the Poliomyelitis Research Foundation, has taken a particular interest in the simian viruses. These are now of increasing importance and interest, and it may be recalled that he and Harwin have described eight such viruses in the South African vervet monkey, namely, SA—which stands for simian agent, though perhaps we should add South African in this particular instance—SA-I, which is the foamy agent; SA-II, which is the measles-like virus agent; SA-III, which is similar to, or identical with ECHO-X virus; SA-IV and SA-V, both of which produce changes somewhat similar to those of the poliovirus but are not polioviruses; SA-VI, which is a cytomegalic inclusion or salivary-gland disease virus, presumably of monkeys; SA-VII, an ECHO-like virus; and SA-VIII, which is similar to the Herpes "B" virus.

In batches of vaccine, prepared recently from Sabin's strain at the Laboratory of the Poliomyelitis Research Foundation, it has been possible to demonstrate the foamy agent in about 100 per cent of the batches prepared from five or more monkeys, and in about 50 per cent of kidneys from single monkeys.

Of some interest is the increasing proportion of positive findings resulting from lengthening the time of observation from three weeks to four, and of subinoculating onto test tubes.

For example, in one particular batch, out of 12 bottles under test at three weeks, only one showed the foamy agent. At four weeks, seven showed the foamy agent; then, by subinoculating onto tubes, an additional two were picked up.

In addition to the foamy agent, there have been found several isolations of the measles-like virus, and one can imagine that if we fed measles virus on a large scale to fairly young infants then there might be a severe epidemic of measles.

Aside from the trouble with these agents, it might be worth while to mention that in the ECHO-XVI strains, which were received from Dr. Sabin, another virus was isolated which we have not isolated so far from South African monkeys and which crosses with Hull's SV-VI and Hull's SV-XVIII. Presumably, it was in the original material.

In addition to the trouble with the monkey kidneys, there has been similar trouble with dog kidneys, most of which so far have contained a virus which resembles distemper virus in appearance. Even the young puppies we are now using have had quite a proportion of this virus.

One cannot assume, of course, that these viruses are harmless. One would agree with

Dr. Koprowski's statement at the first session that their importance should not be exaggerated. However, in view of the possible presence of viruses, such as the "B" virus and measles virus, it is important to eliminate these viruses if that can be done, and it can be done to some extent by using cultures from single monkeys as lots.

It has also been found that treatment of the suspensions with chloroform and with ether will destroy the activity of the foamy agent, the measles agent, and "B" virus. Of course, it does not eliminate the polio-like viruses, the ECHO viruses, or the adenoviruses, or, in this particular case, the virus that Dr. Hilleman has described.

I should emphasize that one should not assume that these viruses are harmless. One should take every precaution to eliminate them or to prove that they are harmless before presuming that they are.

DR. MURRAY: Dr. Hilleman provided our laboratory with some materials in connection with his vacuolating agent. Dr. Baron has actually been carrying out this work personally and can answer questions concerning any of the details. However, I would say that, short of actual neutralization tests, we have been able to confirm the results that Dr. Hilleman has presented.

In this respect, I would say that we were fortunate in having in stock a number of vervet monkeys which were kindly supplied to us by Dr. Gear some years ago. Otherwise, we would not have been able to carry this out.

To digress for a moment on the question of eliminating some of these agents, I was interested in Dr. Gear's remarks and would like to mention, in case some of you did not pick this up, that recently Dr. Hiatt of our Division has carried out some work on the differential destruction, particularly of B-virus, using the phenomenon of the photodynamic action of certain dyes. This seems to be quite successful, at least from the point of view of reducing the titer of B-virus, without simultaneously reducing the titer of the poliovirus component of the virus fluids.

DR. DULBECCO: In view of what has been said about the presence of these viruses and the implications of the many kinds they can have, I wonder whether those who make the vaccine should not consider purifying the virus.

With modern technology, I believe, purification could, without tremendous effort, be achieved by a combination of various types of chromatography and equilibrium density gradient sedimentation.

In fact, poliovirus happens to be probably one of the animal viruses of highest density. By equilibrium density gradient sedimentation one could remove essentially everything except the polio-like viruses which, from what we heard at the first session, could be separated by chromatography.

DR. SABIN: I think that a little history on the 10 sera that I sent to Dr. Hilleman may be of interest. Five of the sera were from children who had no Salk vaccine and five from triple-negative children who were fed the three types of vaccine seriatim—first in 1957 and then in 1959. After the initial feedings, poliovirus predominated, and if this other virus could multiply in man it might conceivably have been suppressed.

But the second series of feedings, which were carried out two years later, three in a row, were not associated with multiplication of poliovirus in the intestinal tract and presumably this virus should have had a clear field for multiplying—if it possesses the capacity to multiply in the human intestinal tract.

Accordingly, the absence of any neutralizing antibodies in the sera of these five children is an indication that there was not sufficient multiplication to produce antibodies. The stools of all these children were preserved, and we prepared to test them for evidence of actual multiplication in vervet monkey-kidney cells.

But until recently, Dr. Hilleman was misinformed about the type of monkey he was dealing with. He had told us to get vervet monkeys, then he telephoned a short time ago and said the monkeys he was working with were not vervets but grivets.

I have heard Dr. Murray speak of having obtained the Hilleman virus effect in cultures from monkeys which he calls vervets. Perhaps this virus is also pathogenic for vervet monkey-kidney cultures. The difference, according to Dr. Hilleman, is that the vervets come from

West and South Africa, and that the grivets come from Equatorial East Africa.

By the end of this week, before this Conference is over, we shall have our own data to indicate whether or not Hilleman's virus produces a CPE in vervet kidney cultures and also whether the stools of the children have this agent in them.

With increasing numbers of simian agents, it is important to ask whether a particular agent multiplies in the human being. If it does not multiply, it is not important. As Dr. Koprowski has said, it may be that many things we eat may have viral agents that we do not know about. However, if they do not multiply they are of no consequence.

I often wonder whether the so-called foamy virus, which we encounter so frequently, actually multiplies in human beings. I think somebody ought to test it.

Discussion with British manufacturers of the oral vaccine indicated that the foamy virus is the chief troublemaker in getting cultures that can be used. They found that even when you use single monkeys, and even when you use quarantined monkeys, which seems to eliminate most of the simian viruses—they have not been able to detect any of the others except perhaps in 1 per cent of the quarantined monkeys—the foamy virus remains. It is precisely as Dr. Gear has described it; depending on how long you keep the cultures, the incidence increases. Some decision will be required as to when to draw the line, particularly when the harvest culture fluid itself is by the most sensitive test free of any demonstrable foamy agent.

As regards this foamy agent, I think I would like to mention here the experience of Professor Chumakov, as pointed out to us by him at the last meeting in Moscow. As long as they used Indian monkeys they had the same problem with foamy virus as other people have been encountering. But since they have started importing monkeys from China and from North Viet Nam, their tests have indicated that only about 3 per cent of individual monkeys that were worked up have presented problems of foamy agents.

In conclusion, I think the most important thing we have to decide is whether or not the new agent multiplies in the human being. If it does not multiply, it is just like putting it into the sewer in an indirect way.

DR. HILLEMAN: In reply to Dr. Sabin's remarks, I should like to state that probably it is only the monkeys that understand the classification of the green monkey. This is a very confused situation. All the green monkeys belong to the species *Cercopithecus aethiops*, but there are 20 subspecies or races. By common parlance the South African green monkeys are usually called vervets, the Northeast African greens are grivets, and the East African greens are called African green monkeys.

I wonder if we could ask Dr. Baron to recount in a few words what he has found to date with the various live poliovirus vaccines and other materials he is testing for the presence of the vacuolating virus. I think it is important and I think Dr. Baron should have something to say about it.

DR. BARON: I really cannot add very much to what Dr. Murray has said other than that we have tested for vacuolating agent representative seed lots of the various attenuated viruses proposed. We have observed a cytopathogenic effect which is identical to that produced by the strain which Dr. Hilleman has given us, i.e., strain 776.

Finally, I might add that we now have on test or plan to test stool samples and pre- and post-feeding sera from persons fed vaccines which presumably contained a vacuolating agent.

DR. SABIN: What are the monkeys?

DR. BARON: South African vervet monkeys.

DR. SABIN: In view of that statement I think it is important to note that Dr. Gear's laboratory has been making the vaccine from vervet monkeys, has tested the production lots in vervet monkeys, and has not encountered this agent. Is that correct or not? Would Dr. Gear please comment on this, since he has used a monkey which is susceptible to this agent?

DR. GEAR: Until today, we were not aware of this agent. It is possible that we did not

recognize its effects in the cultures, but of course its effect would be masked by the destruction of the cells resulting from the poliovirus infection. But we did not come across any marked cytopathogenic effect in the culture bottles.

CHAIRMAN BURNET: I should like to ask Dr. Kirschstein to present her paper on "Laboratory Investigations of the Attenuated Poliovirus Vaccine Strains. I. Neurovirulence after Intramuscular Inoculation of Monkeys."

7. LABORATORY INVESTIGATIONS OF THE ATTENUATED POLIOVIRUS VACCINE STRAINS

I. NEUROVIRULENCE AFTER INTRAMUSCULAR INOCULATION OF MONKEYS

R. KIRSCHSTEIN, G. BORMAN, S. BARON, R. FRIEDMAN, R. MURRAY, AND G. HOTTLE

Division of Biologics Standards
National Institutes of Health, Bethesda, Maryland

DR. KIRSCHSTEIN (*presenting the paper*): At the First International Conference on Live Poliovirus Vaccines last year we presented a comparison of the neurovirulence of the attenuated poliovirus vaccine strains when inoculated directly into the central nervous system (CNS) of monkeys [1]. As you heard this afternoon, Dr. Pette confirmed our observations.

The significance of those results and the correlation of neurovirulence after that type of inoculation with neurovirulence in man is not really known. Therefore, it appeared that a study of the neurovirulence of the attenuated strains after inoculation extraneurally might be more meaningful and add to our knowledge of the invasiveness of these strains.

Preliminary studies of these strains after intramuscular inoculation have been performed before by Sabin in 1957 [2] and by ourselves and Melnick in our laboratory [3] in 1958.

Monkeys: The majority of monkeys used in this study were Rhesus (Macaca mulatta). A small number of Cynomolgus (Macaca irus philipinensis) monkeys were also used. The animals were in overt good health and weighed between 4-10 lbs. initially. The animals were observed daily for signs of poliomyelitis and were sacrificed at 14-20 days. They were housed in stainless steel double-decked cages with 2 or 3 per cage.

Virus strains: The virus strains were obtained from Dr. Koprowski, from Lederle Laboratories and from Dr. Sabin. The titers and identification of the virus strains are seen in Table 1. The virus fluids were titered as described in our paper at the first Conference. On the whole the titers were somewhat higher than those we obtained last year.

Inoculation: The monkeys were inoculated into the right thigh, or upper arm muscles with 1 ml., 5 ml., or 10 ml. amounts of undiluted virus fluids or 1 ml. amounts of material diluted 1:10.

Examination of Tissues: The methods followed were those used in the safety test for inactivated poliomyelitis vaccine and were de-

TABLE 1. STRAINS OF POLIOVIRUS STUDIED

Koprowski group		
Type 1	Wistar-CHAT Pool 13	$10^{7.2}$ PFU/ml.
Type 2	TN 19 Pool 1	$10^{6.0}$ PFU/ml.
Type 3	WFX Pool WY 13	$10^{7.1}$ PFU/ml.
Lederle group		
Type 1	Lederle-SM, # 7-1231-166	$10^{7.9}$ PFU/ml.
Type 2	Lederle-MEF-1, # 7-1232-243	$10^{6.5}$ PFU/ml.
Type 3	Lederle-Fox, # 7-1233-344	$10^{7.0}$ PFU/ml.
Sabin group		
Type 1	L Sc, 2 ab	$10^{7.7}$ PFU/ml.
Type 2	P 72, Ch, 2 ab	$10^{7.3}$ PFU/ml.
Type 3	Leon, 12 a, b	$10^{7.9}$ PFU/ml.

scribed in detail in our report to the First Conference.[1]

Each section, as reported previously, has been evaluated individually and the lesions graded as minimal, mild, moderately severe, or severe. Since there has been much discussion of grading systems and many attempts at quantitation of lesions [4,5], a description of our grading system and a discussion of its usefulness is presented.

A severe lesion is one in which the neurons of both anterior horns of the spinal cord section or of the motor nuclei affected in the brainstem sections are totally or almost totally involved.

A moderately severe lesion is one in which (1) the extent and degree of neuronal damage is not as great as in the first group, is present in both the hemisections of the cord or in all the brainstem nuclei affected, or in which (2) the involvement may be severe but confined only to one hemisection of the cord, or to only several of the brainstem nuclei that might be affected.

A mild lesion is one in which there are only a few neurons damaged in one or both hemi-

TABLE 2. NEUROVIRULENCE FOLLOWING INTRAMUSCULAR INOCULATION OF RHESUS MONKEYS WITH TYPE 1 ATTENUATED POLIOVIRUS

Inoculum-P.F.U.	Clinical Signs	Histopathology		
		Lumbar	Cervical	B.S.
		Koprowski Type I		
$10^{8.7}$-$10^{8.9}$	-	-	-	-
$10^{7.7}$-$10^{7.9}$	2/4*	2/4	1/4	2/4
$10^{6.7}$-$10^{7.2}$	0/12	1/12	1/12	1/12
$10^{6.2}$-$10^{6.4}$	0/2	0/2	0/2	0/2
		Lederle Type I		
$10^{8.7}$-$10^{8.9}$	4/4	4/4	4/4	4/4
$10^{7.7}$-$10^{7.9}$	5/14	9/14	9/14	9/14
$10^{6.7}$-$10^{7.2}$	0/4	0/4	0/4	0/4
$10^{6.2}$-$10^{6.4}$	-	-	-	-
		Sabin Type I		
$10^{8.7}$-$10^{8.9}$	0/4	3/4	0/4	0/4
$10^{7.7}$-$10^{7.9}$	0/14	5/14	2/14	1/14
$10^{6.7}$-$10^{7.2}$	0/4	0/4	0/4	0/4
$10^{6.2}$-$10^{6.4}$	-	-	-	-

* No. of monkeys having findings/No. inoculated.

sections of the cord or only one or two nuclei of the brainstem sections affected.

A minimal lesion is one in which there is a single focal infiltrate of one anterior horn or one nucleus of the brainstem.

We feel that this descriptive grading system has advantages in that it conforms to the trained pathologists' impression, is simple to use and does not convey an unwarranted impression of precision. In dealing with a biologic system, numerical quantitation tends to be non-reproducible.

RESULTS

The results obtained are shown in the following tables. The number of plaque-forming units (PFU) actually inoculated intramuscularly are shown so that a comparison of the strains may be made. Table 2 shows the results after inoculation of the Type 1 strains into Rhesus monkeys. As may be seen, monkeys showed clinical signs of polio after inoculation of $10^{7.7}$ PFU, or greater, of the Koprowski or the Lederle Type 1 strains. The first sign was weakness of the leg in which the inoculum had been placed. This occurred on the fifth or sixth day after inoculation. Weakness gradually progressed to partial or complete paralysis of the affected leg, to partial or complete paralysis of the other (left) leg as well. In this, as in the initial study of these strains [1], many more animals showed histologic evidence of polio than the clinical signs would lead one to suppose. With the Sabin Type 1 strain, no clinical signs of poliomyelitis occurred; however, histologic lesions of poliomyelitis were seen. Again, quantitative differences are evident with the Sabin strain; the lesions, in most of the animals, were

TABLE 3. NEUROVIRULENCE FOLLOWING INTRAMUSCULAR INOCULATION OF RHESUS MONKEYS WITH TYPE 2 ATTENUATED POLIOVIRUS

Inoculum-P.F.U.	Clinical Signs	Histopathology		
		Lumbar	Cervical	B.S.
		Koprowski Type II		
$10^{7.8}$-$10^{8.0}$	-	-	-	-
$10^{7.0}$-$10^{7.3}$	0/3*	0/3	0/3	0/3
$10^{6.0}$-$10^{6.5}$	0/4	0/4	0/4	0/4
		Lederle Type II		
$10^{7.8}$-$10^{8.0}$	-	-	-	-
$10^{7.0}$-$10^{7.3}$	0/4	1/4	1/4	1/4
$10^{6.0}$-$10^{6.5}$	0/6	0/6	0/6	0/6
		Sabin Type II		
$10^{7.8}$-$10^{8.0}$	0/4	0/4	1/4	0/4
$10^{7.0}$-$10^{7.3}$	0/6	2/6	0/6	0/6
$10^{6.0}$-$10^{6.5}$	0/4	0/4	0/4	0/4

* No. of monkeys having findings/No. inoculated.

confined to the lumbar cord whereas with the other two strains lesions spread to the cervical cord and brainstem.

Table 3 shows the results with the Type 2 strains. Here, the results are quite different from those produced with the Type 1 strain. No animals became paralyzed and indeed only occasional animals showed histologic evidence of poliomyelitis. Certainly, none of the Type 2 strains appear to have much neurovirulence when inoculated intramuscularly. Table 4 shows the results with the Type 3 strains and again none of the strains appear to have much virulence.

In general, the lesions seen in the paralyzed animals were as severe as those previously described after direct inoculation into the CNS. In some of the non-paralyzed animals, extensive lesions were also seen. In many of these, the lesions were only present in a single level, either lumbar or cervical. It is of interest to note that, in one monkey, at least, localized lesions of poliomyelitis were present in the lumbar spinal cord after inoculation of the vaccine into the upper arm muscles.

How does invasion of the CNS occur after intramuscular inoculation? Two possible routes are: spread along peripheral nerves, or viremia, leading to invasion of the CNS.

Investigations were undertaken to answer this question. The Type 1 strains were chosen for initial study since these appeared to exhibit the highest degree of neurovirulence. Monkeys that had been inoculated with Lederle and Sabin strains intramuscularly and intravenously, were bled on days 1, 2, 3, 5, 7, and 9. Viremia oc-

TABLE 4. NEUROVIRULENCE FOLLOWING INTRAMUSCULAR INOCULATION OF RHESUS MONKEYS WITH TYPE 3 ATTENUATED POLIOVIRUS

Inoculum-P.F.U.	Clinical Signs	Histopathology		
		Lumbar	Cervical	B.S.
		Koprowski Type III		
$10^{8.4}$-$10^{8.6}$	-	-	-	-
$10^{7.7}$-$10^{8.1}$	1/4*	2/4	1/4	1/4
$10^{6.9}$-$10^{7.1}$	0/4	0/4	0/4	0/4
		Lederle Type III		
$10^{8.4}$-$10^{8.6}$	-	-	-	-
$10^{7.7}$-$10^{8.1}$	0/4	1/4	0/4	0/4
$10^{6.9}$-$10^{7.1}$	0/6	1/6	0/6	0/6
		Sabin Type III		
$10^{8.4}$-$10^{8.6}$	0/4	0/4	0/4	0/4
$10^{7.7}$-$10^{8.1}$	0/6	0/6	0/6	0/6
$10^{6.9}$-$10^{7.1}$	0/4	0/4	0/4	0/4

* No. of monkeys having findings/No. inoculated.

curred in many of the intravenously inoculated monkeys on days 1, 2, and 3. Two of the intramuscularly inoculated monkeys (receiving $10^{7.0}$ PFU of virus) also had viremia on days 1, 2, and 3. Two monkeys which received $10^{4.9}$ PFU of virus intramuscularly (a dose insufficient to cause histologic evidence of poliomyelitis) also had viremia on days 5 and 7. Viremia occurring on days 1, 2, and 3 after intramuscular inoculation may not be indicative of virus multiplication and secondary appearance in the blood but rather of immediate spill-over into the bloodstream. However, that viremia which occurred on days 5 and 7 may indicate secondary entrance of the virus into the blood.

In order further to study the invasive properties of these strains, a group of monkeys were anesthetized using intraperitoneal or intravenous sodium pentobarbital. Then, the right sciatic nerve was either surgically separated and the ends tied off or it was frozen with dry ice according to the method of Bodian and Howe [6]. Animals were then inoculated in the thigh muscles or in the gastrocnemius of the leg innervated by the nerve that had been injured. Table 5 indicates the results. The animals having lesions of poliomyelitis are all in the group having surgical section of the sciatic nerve and inoculation in the area of inflammation resulting from surgery. In animals inoculated at a great distance from the surgery no lesions occurred.

As a further investigation of the route of invasion of the CNS, a group of Cynomolgus monkeys were given human poliomyelitis immune globulin into the gastrocnemius muscle in a dosage of 1 or 2 ml. per Kg. of body weight intramuscularly 24 hours before being given Lederle Type 1 or Sabin Type 1 intramuscularly in the opposite leg. The methods used were those of Nathanson and Bodian [7]. Table 6 shows the results of this study. As can be seen, the paralytic rates and histologic findings were essentially the same with or without the globulin, suggesting that the antibody did not protect these monkeys in any way.

Finally, two monkeys were subjected to the following: After anesthesia, the sciatic nerve was injured by freezing and immune globulin was administered, as previously, in the other leg.

Twenty-four hours later, Lederle Type 1 virus pool was inoculated into the gastrocnemius muscle of the leg that had had the nerve surgery. These animals showed weakness of the leg due to the nerve injury. Histologic examination of the spinal cord and brainstem showed no evidence of poliomyelitis.

Thus it appears likely that the virus has the capacity to spread to the CNS along peripheral nerves. Certain facts, however, such as the de-

TABLE 5. VIRULENCE OF ATTENUATED POLIOVIRUS STRAINS FOLLOWING INTRAMUSCULAR INOCULATION AND SCIATIC NERVE INJURY

TYPE 1

Virus strain	Inoc. PFU	Inoculation site	Type of Nerve injury	Histologic Findings		
				Lumbar	Cervical	Brainstem
Lederle	$10^{8.9}$	Thigh muscles	surgical section	2/4*	0/4	1/4
Lederle	$10^{8.2}$	Thigh muscles	surgical section	2/4	0/4	0/4
Sabin	$10^{8.7}$	Thigh muscles	surgical section	2/4	0/4	0/4
Sabin	$10^{8.0}$	Thigh muscles	surgical section	1/4	0/4	0/4
Lederle	$10^{8.2}$	Gastrocnemius	freezing	0/2	0/2	0/2

* No. of monkeys having findings/No. inoculated.

TABLE 6. EFFECT OF GAMMA GLOBULIN ON NEUROVIRULENCE AFTER INTRAMUSCULAR INOCULATION

VIRUS STRAIN	DOSAGE IN PFU	GAMMA GLOBULIN	CLINICAL SIGNS	HISTOLOGIC FINDINGS		
				LUMBAR	CERVICAL	BRAINSTEM
Lederle Type I	$10^{8.5}$	–	2/2‡	2/2	2/2	2/2
	$10^{8.5}$	*	2/3	3/3	2/3	3/3
	$10^{7.7}$	†	1/3	3/3	1/3	3/3
	$10^{7.7}$	–	1/2	1/2	1/2	1/2
Sabin Type I	$10^{8.7}$	–	–	–	–	–
	$10^{8.7}$	*	0/3	3/3	2/3	2/3
	$10^{7.9}$	–	0/3	3/3	1/3	2/3
	$10^{7.9}$	†	0/2	1/2	0/2	1/2

* 2 ml/Kg. body weight.
† 1 ml/Kg. body weight.
‡ No. of monkeys having findings/No. inoculated.

velopment of a late viremia occasionally, the localization of lesions, on occasion, in the cervical enlargement after inoculation in the leg, or in the lumbar enlargement after inoculation in the arm, lead to the conclusion that other routes of spread are possible.

It seems clear that the manifestation of neurovirulence seen following intramuscular is different from that seen following CNS inoculation. This technique is not a "long-distance intraspinal inoculation." Certainly the Lederle Types 2 and 3 strains which appear to have a high degree of neurovirulence after intraspinal inoculation exhibit little virulence following intramuscular inoculation. The Type 1 strains seemingly have a greater invasive ability following peripheral inoculation than do the Types 2 and 3 strains.

In the course of this study, we had occasion to again study the properties of these attenuated vaccine strains after direct inoculation into the CNS. Since the results are, in general, in close agreement with those we presented last year, we thought that they should be presented at this time. Tables 7, 8, and 9 show the comparative neurovirulence for Rhesus monkeys as studied last year and this year. Here the inoculum is equated as closely as possible in plaque-forming units, since the titers last year and this year were somewhat different. There are certain small differences, but these are not considered to be significant. This is for Type 1 (Table 7) and, as you can see, with the Lederle and Sabin's strains, the agreement is quite good.

Table 8 will show results for Type 2. Of interest in the Type 2 strain is that the new pool of Koprowski Type 2 has a titer of 1.1 logs higher than last year and animals had histologic evidence of poliomyelitis after inoculation with this increased titer. The remainder of the results show good correlation.

As may be seen in Table 9, the correlation for Type 3 virus is very good.

Since the virus titers, this year, are for the most part, higher than they were last year, the titers of the inocula are equated as nearly as possible. As you can see, there are certain small differences, but these are not considered significant in view of the biologic variation of the animals.

Figure 1 is a graphic summary of our intramuscular results. This summarizes the previous tables of histological lesions of strains inoculated intramuscularly. Here there are certain curves which do not rise above the base line.

Evaluation of attenuated vaccine strains de-

TABLE 7. COMPARATIVE NEUROVIRULENCE OF ATTENUATED POLIOVIRUS STRAINS—1959 AND 1960 TYPE 1

VIRUS STRAIN	ROUTE OF INOC.	INOCULUM PFU/ml.	1959		1960	
			CLIN. SIGNS	HISTOLOGIC FINDINGS	CLIN. SIGNS	HISTOLOGIC FINDINGS
Lederle	I.T.	$10^{4.2}$–$10^{4.5}$	0/5*	1/5	0/3	1/3
Sabin	I.T.	$10^{7.4}$–$10^{7.7}$	0/9	0/9	0/3	0/3
	I.S.	$10^{3.4}$–$10^{3.7}$	0/5	2/5	0/3	0/3

* No. of monkeys having findings/No. inoculated.

TABLE 8. COMPARATIVE NEUROVIRULENCE OF ATTENUATED POLIOVIRUS STRAINS—1959 AND 1960 TYPE 2

Virus Strain	Route of Inoc.	Inoculum PFU/ml.	1959		1960	
			Clin. Signs	Histologic Findings	Clin. Signs	Histologic Findings
Koprowski	I.T.	$10^{6.0}$	-	-	0/3*	3/3
		$10^{4.9}$	0/5	0/5	-	-
	I.S.	$10^{5.0}$	-	-	2/2	2/2
		$10^{3.9}$	3/4	4/4	-	-
Lederle	I.T.	$10^{2.5}$	2/5	3/5	0/3	2/3
Sabin	I.T.	$10^{7.3}$-$10^{7.4}$	0/4	0/4	0/3	1/3

* No. of monkeys having histologic findings/No. inoculated.

TABLE 9. COMPARATIVE NEUROVIRULENCE OF ATTENUATED POLIOVIRUS STRAINS—1959 AND 1960 TYPE 3

Virus Strain	Route of Inoc.	Inoculum PFU/ml.	1959		1960	
			Clin. Signs	Histologic Findings	Clin. Signs	Histologic Findings
Koprowski	I.T.	$10^{4.2}$-$10^{4.5}$	1/5*	2/5	0/3	1/3
Lederle	I.T.	$10^{6.0}$-$10^{6.3}$	0/5	1/5	0/3	1/3
Sabin	I.T.	$10^{7.1}$-$10^{7.5}$	0/10	0/10	0/3	0/3

* No. of monkeys having findings/No. inoculated.

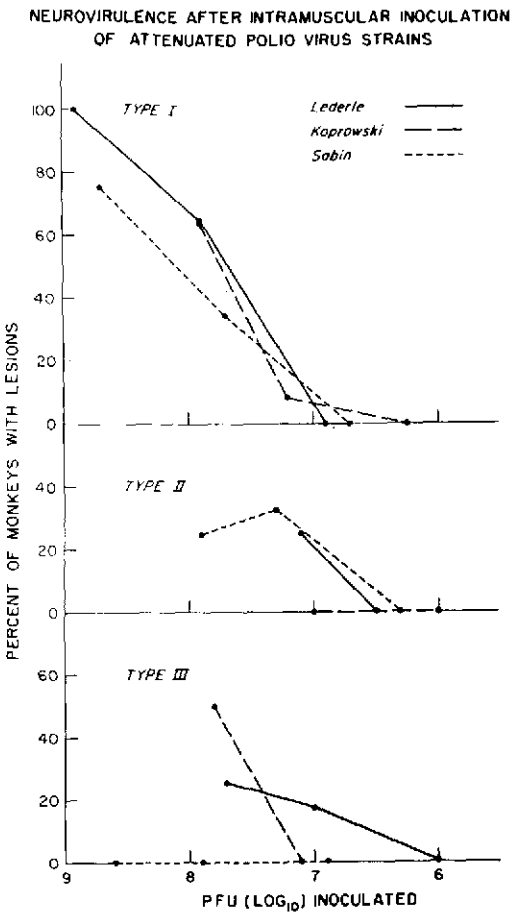


FIG. 1

pends on the use of established reproducible methods and, as we emphasized last year, the proper placement of the inoculum.

The intramuscular test is certainly the simplest one to perform and obviously measures a different property than intraneural inoculation. The intramuscular route in monkeys as will be reported later, becomes important as an added means of studying changing properties of the viruses after oral administration in humans and may be a possible means of distinguishing wild strains in production lots of vaccine.

Moreover, our studies indicate that the Type 1 oral vaccine strains, at least, are capable of reaching motor neurons after peripheral inoculation and of retaining the capacity to damage these neurons, multiply and spread throughout the central nervous system and in some instances, are capable of causing paralysis. The lowest possible neurovirulence following peripheral inoculation may be an important additional criterion for selection of vaccine strains.

REFERENCES

1. Murray, R., Kirschstein, R., Van Hoosier, G. Jr., and Baron, S.: Comparative virulence for Rhesus monkeys of poliovirus strains used for oral administration. In: *Live Poliovirus Vaccines, First International Conference*, Scient. Pub. no. 44 of the Pan American Health Organization, pp. 39-55, 1959.
2. Sabin, A. B.: Properties and behavior of orally administered attenuated poliovirus vaccine. *J. Am. M. Assn.* **164:** 1216-1223, 1957.
3. Kanda, Y. and Melnick, J. L.: *In Vitro* differentiation of virulent and attenuated polioviruses by their growth characteristics on MS cells, *J. Exp. M.* **109:**9-23, 1959.
4. Jungherr, E.: Personal communication.
5. Melnick, J. and Brennan, J. C.: Monkey neurovirulence of attenuated poliovirus vaccines being used in field trials. In: *Live Poliovirus Vaccines, First International Conference*, Scient. Pub. no. 44 of the Pan American Health Organization, pp. 65-101, 1959.
6. Bodian, D. and Howe, H. A.: The rate of progression of poliomyelitis virus in nerves: *Bull. Johns Hopkins Hosp.* **69:**79-85, 1941.
7. Nathanson, N.: Experimental poliomyelitis following intramuscular injection, *Fed. Proc.* **19:**139, 1960.

ACKNOWLEDGMENT

The authors wish to acknowledge the excellent technical assistance of Mr. George Rustin, Mr. Marshall Ford, Mrs. Dorothy Hughes and Mr. Richard Low.

DISCUSSION

CHAIRMAN BURNET: Thank you, Dr. Kirschstein. This paper is now open for discussion. Dr. Bodian.

DR. BODIAN: I would like to point out, first of all, that some years ago we also attempted to use gamma globulin to block intramuscular inoculation. We found quite satisfactory blocking effects, which seem to be in contradiction to the results that Dr. Kirschstein has reported.

I wish to emphasize, however, that at the National Institutes of Health, they were using a minimum of 7.5 logs of virus, whereas in those days we were lucky if we had spinal cord pools that reached 5.5 logs. Therefore, I think one should be careful in assessing the lack of an inhibiting effect of the gamma globulin to realize that the relationship of virus dose and antibody is fundamental. This is a point we keep coming back to year after year, irrespective of the route of inoculation.

As far as the interpretation of the rest of Dr. Kirschstein's story is concerned, I feel a bit uncomfortable about assuming that the intramuscular route is illustrating a different property of the virus as compared with intraneural inoculation, although the evidence certainly suggests it. I believe that Types 2 and 3 viruses were represented by rather fewer monkeys than were represented with Type 1, and it is known that infection rates by the intramuscular route are quite variable.

It seems surprising, certainly, that the Type 1 virus of all three workers should have the same apparent ability to do better by the intramuscular route. I should not have expected it, but perhaps I am wrong.

One distinctive pathogenic property which I believe seems to be clearly established by Dr. Kirschstein's work, and reported by others today, is the property of spreading of virus within the CNS.

After beginning multiplication in the central nervous system, it appears that some of these strains are more capable of spreading throughout the nervous system than others, and it seems to me that this characteristic is fairly well established. The difference between spreading in the nervous system and spreading from the muscles to the spinal cord is something I am not entirely clear about in my own mind.

DR. MOYER: I should like to present two tables which, though slightly different, confirm essentially Dr. Kirschstein's results.

Table 1 shows a number of Type 2 just mentioned by Dr. Bodian. These were not injected intramuscularly, but intravenously, and we attempted to induce trauma by injecting saline intracerebrally. All of these are Type 2 batches and paralysis did not occur in any of them, although in several cases lesions did appear.

TABLE 1. NEUROVIRULENCE IN MONKEYS INOCULATED INTRAVENOUSLY WITH POLIOVIRUS VACCINE

TYPE II

LOT NO.	VIRUS		SALINE		PARAL.	PATHOL: SIGNIF.
TYPE I	ML	LOG*	ML	ROUTE	RATIO	LESIONS
287 P	1	7.5	2x.5	IC	0/4	1+1?/5
296 P	10	7.5	"	"	0/5	5/5
298 P	-	-	"	"	0/4	0/4
"	2	6.8	"	"	0/5	1/5
"	10	7.5	"	"	0/4	2/5
"	2	6.8	-	-	0/5	1/5
"	10	7.5	-	-	0/5	2/5

* To base 10.

TABLE 2. NEUROVIRULENCE IN MONKEYS INOCULATED INTRAVENOUSLY WITH POLIOVIRUS VACCINE

TYPES I AND III

LOT NO	DOSE				PARAL.	PATHOL: SIGNIF.
	VIRUS		ML.	ROUTE		
TYPE I	ML	LOG*	TRI-IMMUNOL		RATIO	LESIONS
166	10	8.6	1	1 IM**	0/5	0/5
			SALINE			
163P	1	7.3	2x.5	IC	0/4	0/6
184P	10	7.9	"	"	0/5	2/5
TYPE III						
368	10	8.3	"	"	0/5	1/5

* To base 10. ** In gluteus.

In the case of Table 2, instead of gamma globulin we injected intramuscularly into the gluteus a commercial preparation of Tri-immunol, a vaccine containing pertussis, diphtheria and tetanus toxoids, and 8.6 logs of the Type 1 virus intravenously. Neither paralysis nor lesions occurred.

In another experiment, Type 1 was injected intravenously and saline intracerebrally: the lesions were few and paralysis did not occur. One batch of Type 3 shown in the table gave essentially the same results.

Perhaps the major point to be made is, as Dr. Kirschstein has already said, that this is not a case of a virus growing and spreading by the blood, since a vast dose intravenously and trauma intracerebrally or intramuscularly did not cause any paralysis.

DR. MELNICK: Dr. Kirschstein mentioned the higher titers of virus found in 1960. I would like to know whether this represents new material, or 1959 material which was retitrated?

DR. KIRSCHSTEIN: New material.

DR. MELNICK: Prepared between 1959 and 1960?

DR. KIRSCHSTEIN: The material that Drs. Cox and Koprowski sent us were new lots from the same seed pools.

DR. MELNICK: Could we have it for the record from those who submitted the new vaccines?

DR. COX: I would like to point out that some years ago we did inoculate monkeys in the deltoid and also with 5 cc. and 10 cc.'s intravenously, and we found nothing. I think that this discovery of the behavior of Type 1, by the intramuscular route in the buttocks, was made somewhat by accident. We had never inoculated monkeys by this route. Actually we did not know about this until last December after we had fed nearly one million people. We did not know that our Type 1 had this property.

Of course, the question was then asked: What do you do in a case like this? Which are the more significant, 20 monkeys or one million people? Naturally we had to make a decision. Since the intramuscular activity was found we have confirmed it. We still have not been able to produce clinical reactions in monkeys by the intravenous route as Dr. Moyer has shown you, even when we inoculated monkeys intravenously with 10 cc.'s, and then tried to break their resistance by intracerebral injections with saline or other agents.

This is a peculiar property. It has been found to exist at present in all Type 1 strains, to a greater extent in some than in others.

You should keep in mind that we are not injecting man intramuscularly, but are feeding man orally. You should also keep in mind that these vaccines are intended to be used in a certain dosage and by a certain route. I should like to remind you that you never vaccinate people on the eyeball with smallpox. We know that they would be blinded under those conditions.

Like everything else, further studies reveal new or unknown properties. This is not limited to polio research. This has been found with every virus that we have ever worked with.

DR. KOPROWSKI: I would like to make a statement concerning gamma globulin. I strongly suspect that two factors are involved: One is the very high concentration of virus used and the other is the titer of antibodies in gamma globulin. Perhaps before Dr. Kirschstein can make a definite statement that gamma globulin administration has no protective effect, a titration of the virus in monkeys injected with high concentration of gamma globulin should be undertaken and compared with the results in controls.

Now a short comment on viremia. I believe the problem of spillover versus multiplication can be settled if titration of the virus in blood can be performed at frequent intervals. If the concentration of circulating virus should drop in 24 hours or 48 hours after infection and later on rise again, we should consider that the virus multiplied in an organ communicating directly with the blood.

DR. KIRSCHSTEIN: The dosage of gamma globulin given to these monkeys is that which Dr. Nathanson had used to prevent monkeys from becoming paralyzed and developing lesions, using Mahoney virus present in some partially inactivated poliovirus vaccine. It had been effective

in his hands and we were using this as he had used it.

The viremia that occurred in the animals on days 1, 2, and 3 was of a titer of $10^{2.0}$ logs, or perhaps a little bit higher, and about the same on days 5 and 7.

One of the reasons why we think that this may be spillover is that we performed a similar experiment in guinea pigs. We gave guinea pigs, into the thigh muscles, five milliliters of Type 1 attenuated virus, which is a rather large amount for the guinea pigs, and bled them for days 1, 2, 3, and 4, and found that they had viremia for that period of time.

DR. BODIAN: I have one more word to say about "spillover." I might remind Dr. Kirschstein that we published data some years ago indicating that with intracardiac inoculation of large amounts of virus it is difficult, if not impossible, to recover the virus from the blood within half an hour. So I doubt very much that the viremia under the circumstances you mentioned is due to "spillover."

CHAIRMAN BURNET: If there are no further comments, we will go on to Dr. Sabin's paper, "Behavior of Cold Mutants of Poliovirus in Human Beings."

8. BEHAVIOR OF COLD MUTANTS OF POLIOVIRUS IN HUMAN BEINGS

DR. ALBERT B. SABIN

The Children's Hospital Research Foundation
Cincinnati College of Medicine
Cincinnati, Ohio

DR. SABIN: The work I am about to describe was carried out in association with Doctors Barnes, Michaels, and Vignec.

The tests in human beings on the 25° C. mutants are still in progress, but I think the results already are of sufficient interest to warrant this report.

The purpose of these studies was to select poliovirus mutants which would be able to multiply extensively at relatively low temperatures —by that I mean about 25° C.—and to determine how this affected their capacity to reproduce at higher temperatures, that is, body temperature of man, for example, in tissue cultures, as well as in the spinal cord of monkeys and in the alimentary tract of human beings.

Accordingly, only strains which had already been tested extensively in monkeys and human beings were selected for this study, so that any changes that would occur might be related to the manipulations that we carried out, because if you start with a strain of virus whose capacity for multiplication in the human alimentary tract you do not know, you could not interpret the results.

We worked with six strains of virus: the three current vaccines that I used, two new Type 1 strains, and one new Type 3 strain.

The two Type 1 strains were derived from stools of healthy non-contact children during a non-epidemic period in Louisiana, and were originally of low thalamic virulence for monkeys.

The Type 3 strain which we used was similarly obtained from a healthy child in Cincinnati. The original stool culture in monkey-kidney cells was *rct*/40—.

I want to explain this expression (*rct*/40—) because it is different from what people have been using to designate the reproductive capacity of a virus at a given temperature.

In a manuscript which has been distributed to some of you, I indicated my preference for expressing the capacity of a poliovirus to multiply at a certain temperature as reproductive capacity at the temperature indicated, because the *T* marker has been used very extensively to indicate sensitivity to inactivation by certain temperatures.

I do not ask you to accept this, but I want you to know what I mean when I speak of *rct*/40+, *rct*/40—, *rct*/25+, or *rct*/25—. This is to represent the capacity of polioviruses to multiply at these different temperatures.

I repeat that this Type 3 strain that we started with, the naturally occurring one, was *rct*/40— originally, and this is particularly important in view of what I shall state later.

All six strains were first propagated for seven passages at 33° C., and when this was found to have little effect on the ability of the resulting populations to propagate at 25° C., propagation at 30° C. was begun.

After the first five passages at 30° C., an increase in activity at 25° C., was noted with all strains, an increase which, however, was not significantly enhanced by further five passages at 30° C. At this stage, propagation was shifted to 25° C. and continued for 20 consecutive passages.

Table 1 shows the gradual transformation in the properties of the virus, by illustrating the effects obtained with just one strain. It illustrates the results that were obtained in the selective enrichment of the 25° C. variant by serial propagation at progressively lower temperatures for the Type 1 current vaccine strain. The original material which had been cultivated at 36° C., when tested in monkey-kidney culture tubes at the indicated temperatures, gave about the same titer when the test was carried

TABLE 1. SELECTIVE ENRICHMENT OF 25° C. VARIANT BY SERIAL PROPAGATION AT PROGRESSIVELY LOWER TEMPERATURES

Type 1 Poliovirus—Strain LSc, 2ab

TEMPERATURE OF PROPAGATION	PASSAGE NO.	CPE TITER (LOG 10 TCD_{50}/ML) AT INDICATED TEMPERATURE					
		36°C	30°C	25°C			
		8 DAYS	8 DAYS	8 DAYS		14 DAYS	
				COMPLETE	PARTIAL	COMPLETE	PARTIAL
36°	VACCINE + 1	8.2	8.2	0	0	0	2.2 slight
33°	7	8.2		0	1.2	3.0	3.7
30°	5	8.1	7.9	0	1.4	5.0	5.3
	10	7.7	7.3	0	4.1	5.4	5.7
25°	5	6.2		3.2	4.2	6.2	6.2
	10	7.4		4.2	6.4	7.7	7.7
	20	7.3		5.2	7.3	7.7	7.7

Selective Enrichment of 25°C Variant by Serial Propagation at Progressively Lower Temperatures
Type 1 Attenuated Polioviruses

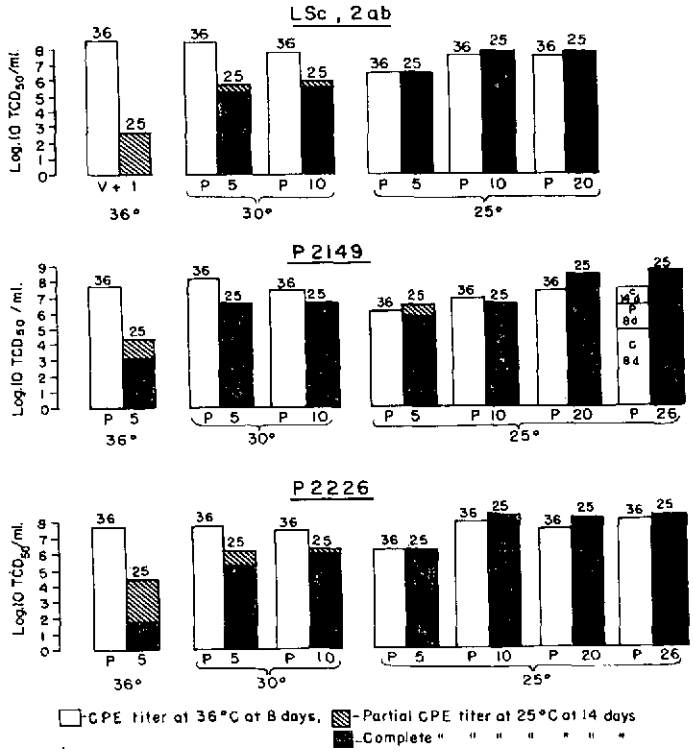


FIG. 1

out at 36° C. and at 30° C. However, for a period of eight days at 25° C., there was no effect even though the multiplicity of virus to cells was almost one thousand.

At 14 days there was still no complete cytopathogenic effect. Only a few cells were affected at the highest multiplicity, with no progression.

Seven passages at 33° C. altered the situation very slightly, and the results obtained after five and 10 passages at 30° C. can be seen. There was still no complete cytopathogenic effect during a period of eight days at 25° C., but there was gradually increased activity at this lower temperature.

This is a very slow process. After the first five passages at 25° C., the yield of virus, when all the cells were destroyed, was approximately 100 times less than that obtained at the higher temperatures.

Incidentally, I should point out that, whenever we switched from one temperature to another, the virus was adsorbed and the unadsorbed virus was washed out; only the progeny coming out at the new temperature was used.

The main point here is to show that after these consecutive selections we obtained a virus which now still multiplied very well at 36° C., but also as extensively at 25° C.

Essentially, Fig. 1 shows what happened with the Type 1 strains.

Figure 2 shows that the initial virus had very low capacity for multiplying at 25° C., and gradually it increased. Initially there is a dip in the yield of virus when multiplying at 25° C., but finally it comes up to a point where it multiplies equally well at 25° C. and at 36° C.

After selection of special plaque progeny, even higher propagation was obtained at 25° C. than at 36° C.

The same thing occurred for Type 3. Although the Type 3 Leon strain had much more

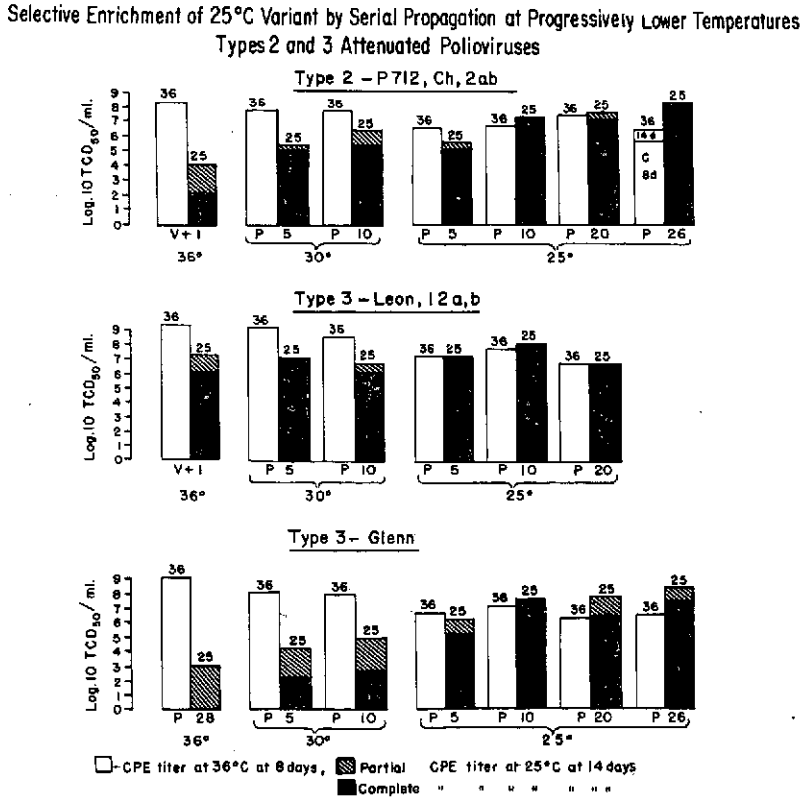


FIG. 2

TABLE 2. DIFFERENCE IN RATE OF PROPAGATION AND EFFICIENCY OF INFECTION AT 36° C AND 25° C OF DIFFERENT TYPE 1 PLAQUE PURIFIED 25° C MUTANT POLIOVIRUSES

DILUTION INOCULATED 0.2 ML	DAY OF FIRST APPEARANCE OF CPE IN EACH MK CULTURE TUBE			
	P 2226, 2ab		P 2149, $1a_2b$	
	25°C	36°C	25°C	36°C
UNDILUTED	1,1	1,1	1,1	1,1
10^{-1}	2,2	1,1	1,1	1,1
10^{-2}	3,3	3,3	2,2	4,4
10^{-3}	3,3	3,3	2,4	4,5
10^{-4}	3,4	3,4 (2)*	5,5	7,7 (1-2)*
10^{-5}	5,5,5,6,6 7,7,7,7,7	4,4,4,4,4 (2) 4,4,4,4,4	5,5,5,5,5 6,6,6,6,7	7,7,8,8,8 (1-2) 8,8,8,8,8
10^{-6}	7,7,7,7,7 7,8,8,8,8	4,4,4,4,4, (2-3) 5,5,5,5,7	7,7,7,8,8 8,8,8,8,8	8,9,9,9,9 (2-3) 9,9,9,9,10
10^{-7}	7,7,7,8,8 8,10,12,0,0	4,4,5,5,5 (3-4-0) 6,6,7,0,0	8,9,9,9,9 9,10,10,10,10	10,15,0,0,0 (2-3-0) 0,0,0,0,0
10^{-8}	9,14,14,0,0 0,0,0,0,0	0,0,0,0,0 (0) 0,0,0,0,0	8,10,12,13,0 0,0,0,0,0	0,0,0,0,0 (0) 0,0,0,0,0
LOG 10 TCD_{50}/ML	8.3	8.1	8.5	7.3

* Figures in parenthesis indicate days of first appearance of CPE at 36° C of original parent virus propagated at 36° C.

capacity originally to multiply at 25° C., it increased further, but the total yield finally was lower than with the other strains.

The Type 3 Glenn strain originally did not produce a complete cytopathogenic effect at 25° C., but there was this gradual alteration in the viral population, so that with the final plaque purified material it multiplied somewhat better at 25° C. than at 36°.

Table 2 shows the difference in the rate of propagation and efficiency of infection at 36° C. and 25° C. of different Type 1 plaque-purified 25° C. polioviruses.

This particular strain (P 2226, 2ab) infected the monkey-kidney cells in as small a dose at 25° C. as at 36° C. But the rapidity of action is still greater at 36° C. than at 25° C.

On the other hand, with strain P 2149, $1a_2b$, which had been submitted to the same procedure, multiplication is much slower at 36° C. than at 25° C., and with approximately 10 to 20 infective units of virus it is not possible to initiate a cytopathogenic effect at 36° C.

It is important to point out that in children this strain P 2149, $1a_2b$ did not multiply at all, even with a dose of about five million to ten million tissue-culture infective doses. Strain P 2226, 2ab multiplied in a few, though still rather poorly, despite its good capacity for multiplication at 36° C. tissue culture.

Table 3 shows that the Type 3 strain (Glenn, $3a_2b$) which multiplies more slowly at 25° C. than at 36° C. but can initiate CPE more readily at 25° C.—a difference of almost two logs—multiplied fairly well in the children. The Type 2, which multiplies more slowly at 36° C. than at 25° C, multiplied poorly or not at all in children.

I should point out here that these modified

TABLE 3. RATE OF PROPAGATION AND EFFICIENCY OF INFECTION AT 36° C AND 25° C OF TYPE 2 AND TYPE 3 PLAQUE PURIFIED 25° C MUTANT POLIOVIRUSES

DILUTION INOCULATED 0.2 ML	DAY OF FIRST APPEARANCE OF CPE IN EACH MK CULTURE TUBE			
	TYPE 2 - P 712, $2a_2b$		TYPE 3 - GLENN, $3a_2b$	
	25°C	36°C	25°C	36°C
UNDILUTED	1,1	1,1	1,1	1,1
10^{-1}	1,1	1,1	1,1	1,1
10^{-2}	2,2	1,1	2,2	2,2
10^{-3}	2,2	4,4	2,4	3,3
10^{-4}	4,4	6,6 (2)*	6,6	4,4 (1)*
10^{-5}	4,4,4,4,4	6,6,7,8,10 (2)	6,8,8,8,8	4,4,5,5,6 (2)
10^{-6}	4,4,4,4,4 5,5,5,5,5	10,10,0,0,0 (2-3) 0,0,0,0,0	8,8,8,9,9 9,9,9,10,10	6,6,6,9,0 (2) 0,0,0,0,0
10^{-7}	6,6,6,6,6 7,7,7,8,8	9,0,0,0,0 (3-6-0) 0,0,0,0,0	9,10,11,12,12 14,15,16,16,18	0,0,0,0,0 (2-5) 0,0,0,0,0
10^{-8}	8,8,11,12,14 0,0,0,0,0	0,0,0,0,0 (4-0) 0,0,0,0,0	13,0,0,0,0 0,0,0,0,0	0,0,0,0,0 (3-5-0) 0,0,0,0,0
10^{-9}	0,0,0,0,0	0,0,0,0,0	0,0,0,0,0	0,0,0,0,0 (0)
LOG 10 TCD_{50}/ML	8.7	6.4	8.3	6.5

* Figures in parenthesis indicate days of first appearance of CPE at 36° C. of original parent virus propagated at 36° C.

viruses do not behave like the usual polioviruses because the cytopathogenic effect may start at six days and it may take four days before it goes to completion.

As for Table 4, I should like to point out that the two Type 1 strains, P 2149 and P 2226, were originally eliminated from consideration for practical use because we could not get rid of the spinal neurovirulence, even though on intracerebral inoculation they were avirulent. However, after these selective procedures, it was possible to inoculate huge amounts intraspinally without getting any paralytic effect at all, even using a somewhat more sensitive method with a smaller needle. In most instances, the lesions were limited to the site of the scar without any spread beyond it.

On the other hand, with the Type 1 vaccine strain (LSc, 2ab) it was not possible, by passages at 30° C., to influence the residual spinal neurovirulence, and even after 20 passages at 25° C., there was still some residual greater action than in the other strains.

With the Type 2 strain shown in Table 5, it was possible to obtain virus which, on intraspinal inoculation, was virtually without effect. On the other hand, the 25° C. variant of the current Type 3 vaccine strain (Leon $12a_1b$) retained the ability to produce mutants in the spinal cord of an occasional monkey. These occasional monkeys that exhibited paralysis after inoculation with the 25° C. virus showed a completely altered virus in the spinal cord from that originally inoculated, in that it also possessed the capacity to multiply at 40° C.

However, a totally different result was ob-

TABLE 4. SPINAL NEUROVIRULENCE IN MONKEYS OF ORIGINAL ATTENUATED TYPE 1 POLIOVIRUSES AND OF 25° C MUTANTS DERIVED FROM THEM

STRAIN	TEMP. OF PROPAGAT. °C	PASSAGE NO.	PROGENY OF SINGLE PLAQUE	LOG 10 TCD_{50} PER ML.	SPINAL TEST IN MONKEYS		
					METHOD NEEDLE	PARALYTOGENIC EFFECT OF 0.1 ML. OF INDICATED DILUTION	
						UNDILUTED	10^{-1}
P 2149	36	8	12 separate plaques	7.3 - 8.1	20 g.	50/60	51/60
	25	20	-	8.2	20 g. 26 g.	0/5 0/5	
		26	$1a_2b$	8.5	27 g.	0/10	0/5
P 2226	36	8	2ab 4ab	7.5 7.5	20 g. "	5/5 5/5	4/5 5/5
	25	20	-	8.2	" 26 g.	0/5 0/5	
		26	2ab	8.3	27 g.	0/10	0/5
L Sc	36	Many	2ab	8.0	20 g. 27 g.	7/30 8/10	5/20 4/6
	30	10	-	7.7	27 g.	8/10	
	25	20	-	7.7	26 g. 20 g.	1/5 0/5	

TABLE 5. SPINAL NEUROVIRULENCE IN MONKEYS OF ORIGINAL ATTENUATED TYPES 2 AND 3 POLIOVIRUSES AND OF 25° C MUTANTS DERIVED FROM THEM

TYPE	STRAIN	TEMP. OF PROPAGAT. °C	PASSAGE NO.	PROGENY OF SINGLE PLAQUE	LOG 10 TCD_{50} PER ML.	SPINAL TEST IN MONKEYS		
						METHOD NEEDLE	PARALYTOGENIC EFFECT OF 0.1 ML. OF INDICATED DILUTION	
							UNDILUTED	10^{-1}
2	P 712	36	15	2ab	7.7	20 g. 27 g.	1/20 1 + 4 sl/10	0/5 1 + 3 sl/10
		25	20		7.7	20 g. 26 g.	0/5 0/5	
			26	$2a_2b$	8.7	27 g.	0/10	0/5
3	LEON	36	40	$12a_1b$	7.9	20 g. 27 g.	4/20 9/10	0/5 3/6
		25	20		6.7	20 g. 26 g.	1 sl/5 1/5	
	GLENN	36	27		7.0	20 g.		3/4
		25	20		7.7	20 g. 26 g.	0/5 0/5	
			26	$3a_2b$	8.3	27 g.	0/10	0/5

tained with the "Glenn" strain of Type 3 virus.

With the 25° C. variant of the "Glenn" strain it was possible to inoculate as many as one hundred million tissue-culture infective doses intraspinally without producing either paralysis or significant lesions. With the exception of a few perivascular infiltrations around the site of inoculation, there was usually no evidence of any histologic change that was different from that found in monkeys inoculated only with control, uninfected fluid.

I should like to describe now what happened on tests in human beings. These tests were carried out at a foundling hospital in New York City in February. The interesting thing is that even though it was wintertime, and even though this was one of the most sanitary institutions I have ever been in, about 16 of the first group of 23 children had their own viruses in the stool before we started. Actually, most of them were very slow in growing; most of them were adenoviruses.

To summarize, with the first Type 1 strain mentioned previously, i.e., P 2149, in a dose of $10^{6.5}TCD_{50}$ in five children, there was neither antibody formation nor multiplication even in the absence of any interfering agent.

With the second strain, "P 2226", which multiplied a little faster at 36° C. in tissue culture we have two groups of children by now. In one group of 5, that received $10^{6.5}TCD_{50}$ in 0.01 ml. antibody formation occurred in only two, one very late. With the larger dose of $10^{7.5}TCD_{50}$; only one out of four had multiplication and antibody formation. It is evident that both of these Type 1 strains lost their capacity for adequate multiplication in the human intestinal tract. The Type 2 strain was tested in a dose of $10^{6.7}$ and $10^{7.7}TCD_{50}$ in a total of eight children without homotypic antibody. It multiplied a little in one or two children, with development of a low antibody titer, and in the others it did not multiply at all.

However, the Type 3 "Glenn" strain, although it was quite similar to the Type 1, P 2226 strain in its behavior in tissue culture, multiplied very well in the children despite the presence of interfering agents. This particular mutant multiplies extensively at 25° C. We obtained culture fluids $10^{9}TCD_{50}$ per ml.

On the first test with 0.01 ml., multiplication and antibody formation were found in only five out of seven children, but when we re-fed one of the negative children with the same dose, the virus multiplied and antibody developed.

We performed another test with 0.01 ml. in three additional children and it multiplied in all, with antibody forming in all. With the larger dose of 0.1 ml., i.e., $10^{8}TCD_{50}$, in six children, virus multiplication and antibody formation occurred in all. With 0.001 ml., i.e., $10^{6}TCD_{50}$ we seem to have reached a probable 50 per cent end-point: it was three out of five.

The level of virus multiplication was very often in the range of 500,000 to 1,000,000 tissue-culture doses per gram of feces. Now comes the interesting thing of what happens to this virus after multiplication in the gastrointestinal tract. We have a very interesting marker here, the 25° C. marker, which is possessed by very few viruses. What we found, in brief, is that within a few days after multiplication in the intestinal tract, the capacity to multiply at 25° C. was not lost, but there appeared virus particles with the capacity to multiply at 40° C. The original stool may have only 1 per cent or less of the total virus that can multiply at 40° C. but after one passage in tissue culture at 36° C. one can obtain cultures that yield about the same titer at 40° C., 36° C., and 25° C. Thus, the cultures have not lost their capacity to grow well at 25° C., but have gained the capacity to grow at 40° C. This is remarkable because we have all found this with Type 3 viruses and it happens with all strains. The capacity for multiplication at 40° C. is something that is quickly acquired in a part of the population when it multiplies in the intestinal tract. As stated previously, it does not necessarily bear any relationship to neurovirulence as tested in monkeys. We have not yet had time to test the 25° C. excreted viruses in monkeys.

To summarize, it is quite clear that the Type 1 and Type 2 25° C. viruses, which retained the capacity to multiply in tissue culture at 36° C. multiplied so poorly in the intestinal tract of human beings that they would be quite worthless as immunizing agents by the oral route.

The Type 3 "Glenn" virus does seem to multiply well. It undergoes changes, at least as regards its reproductive capacity at 40° C., which are similar to those observed with the present Type

3 vaccine strains that have been studied. Future tests will show whether or not the monkey neurovirulence tests on excreted virus will be very different from those that we obtained with the present strain.

Accordingly, at the present time I do not know whether this Type 3 strain would have any superior qualifications over the others, but I think it has thrown a great deal of light on the relationship between reproductive capacity in tissue cultures and reproductive capacity in the intestinal tract.

DISCUSSION

CHAIRMAN BURNET: Thank you, Dr. Sabin. Dr. Dulbecco.

DR. DULBECCO: I would like to ask Dr. Sabin one question. He said that the culture as a whole had both properties, multiplied at 25 and at 40. Does this mean that individual plaque lines were able to do so, or was the culture a mixture of the two types, of which half were able to do one thing and half the other, and therefore the culture as a whole appeared to do both things?

DR. SABIN: The data that I reported involved tests on the first-passage monkey-kidney culture fluid. If you suggest the possibility that in those culture fluids half of the virus particles were made up of those that could multiply well at 25° C. and not at 40° C., while the other half of particles that multipled well at 40° C. but not at 25° C., one can only say that that may be possible. Actually, in order to prove that a single virus particle has both properties, the tests would have to be carried out on purified single plaque progeny.

CHAIRMAN BURNET: Are there any further questions or comments?

DR. BODIAN: I think one should re-emphasize Dr. Dulbecco's suggestion in interpreting his very interesting data, namely, if there really are a large number of particles which are capable of growing at each temperature, we are saying that it is possible with human passage to quickly select very large proportions of new virus. This would be another interpretation of Dr. Sabin's data to add to the possibility he has suggested.

DR. SABIN: Selection can occur predominantly in the first-passage tissue culture. The original stool may contain only 1 per cent or less of virus particles capable of multiplying at 40° C., and then a single passage at 36° C. gives these particular ones a selective advantage in the final population. We always have to think of this in quantitative terms. I think that a basis for such a selective advantage was provided by studies that Dr. Lwoff carried out. He showed that virus particles that can multiply at 40° C., when grown at 36° C. to 37° C. produce progeny more rapidly than viruses which cannot grow well at 40° C.

So that starting off equally, the *rct*/40+ particles can end up in the majority after a single passage in tissue culture at 36° C.

I should like to add that we also found the reverse here, just as we found with Type 3 viruses before, namely, that after a long continued propagation in the same child, one may end up with a virus population that is again *rct*/40—.

DR. STUART-HARRIS: I should like to make two brief comments. It seems to me that in comparison with the data which we presented last year, this year we have a good deal more information concerning the properties of the strains used as vaccines and also of the properties of the strains recovered from children immunized with vaccines. All of this information seems to me to point in the same direction, namely, that whatever particular property we study in relation to these strains, we come up with something which can only be described as a graded characteristic.

I think that this is brought out in regard to the *T* marker, of which we have heard so much, that one has strains which vary in property over quite a wide range, a point which I am certain was emphasized before by Dr. Sabin when, in the earlier studies on neurovirulence, he noted that this, in fact, was a graded characteristic. One did not just have virulent strains or nonvirulent strains; one had a spectrum of this particular property.

And it does make one feel, I think, that in this particular virus, the poliovirus, one is dealing with something which has infinite capacity for variation. What the interpretation of that may be I would not like to say. At any rate, it does seem to me to be an extremely variable virus in all these various ways.

My second comment is in regard to Professor Zhdanov's remarks at the first session, when he

said that the real practical significance of these variations can only be apparent if one studies the ecological situation. I take it that he would agree that the ecological situation is not just the epidemiological one, but also includes the properties of the hosts.

I am once more reminded of the fact that whatever studies are carried out on children in any particular part of the world, it is necessary to be cautious about translating the results to children in other parts of the world, namely, that one has, it seems to me, to carry out studies on the children in any particular area where the vaccine is being used and find out exactly how the viruses behave in these hosts.

DR. DULBECCO: With reference to the first remark Dr. Stuart-Harris made on the variability of poliovirus, I must say that we have a considerable amount of experimental evidence which concurs with what he said. That is, the mutation frequencies that we could determine are actually quite large, since they fall into the range of about 10^{-4}, which is a considerably high value for a mutation frequency.

Whether this is due to the fact that the virus is an RNA virus, and therefore is for some reason more unstable, we do not know. There is an additional factor which appeared when we tried to isolate the mutants that I discussed before. Actually, probably for every mutant listed there, we found a number of different steps so that there are many intermediate degrees of expressions of the same characters, and not simply a few well-defined types. This presents a problem which is not clearly understandable, at least for the origin of all these intermediate types of mutation.

One can theorize and propose a reasonable explanation. But certainly it is a fact which very greatly disturbs the worker with the mutants and also hampers the interpretation of the facts.

DR. KOPROWSKI: To comment on Dr. Dulbecco's and Dr. Stuart-Harris' statements concerning "infinite capacity for variation" of poliovirus, I doubt whether poliovirus occupies in this context a unique position among animal viruses, even though it has been studied more extensively for this property than other viruses.

Influenza viruses, for one, have certainly a "capacity for variation" equal to, if not surpassing, that of poliovirus. The pox viruses with all their markers, so extensively studied by Fenner, probably are subject to as extensive variations as poliovirus.

CHAIRMAN BURNET: I should like to comment on that myself. The question I should like to put to Dr. Dulbecco particularly is this: Has any positive evidence of recombination in poliovirus yet been obtained? For my own satisfaction, I should like to hear what the situation is in regard to that.

DR. DULBECCO: We do not have any evidence. I must say that we have been laboring very hard to try to get this evidence. Of the characters which were listed in the table I presented,* essentially all have been crossed with each other, and a number of pairs of independent mutants of the same type have also been crossed with each other. The fact is that there is no convincing evidence that a combination has been obtained.

DR. KITAOKA: There are many markers on poliovirus, for example, the *M* marker and the *T* marker, and the data on work done in my laboratory have been published. These markers are not so stable, but are sometimes changed by other factors which are not clear.

Some genetic characters of poliovirus are also not so stable, because *M* is influenced by factors, some of which are still unknown, though one is known to be an agar extract.

My supposition is that poliovirus may contact many factors probably existing in the intestines. Some of them may play a role of factor to produce back mutants, that is, the wild or original strain.

* See p. 48.

THIRD SESSION

TUESDAY, 7 JUNE 1960, 9:00 A.M.

Chairman
PROFESSOR V. M. ZHDANOV
Academic Secretary
Academy of Medical Sciences
Moscow, USSR

TOPIC II. SAFETY. (B) FIELD EVIDENCE OF SAFETY

(1) STABILITY ON HUMAN PASSAGE

Presentation of Papers by:
Dr. Dorothy M. Horstmann

(DISCUSSION)

Dr. Samuel Baron

(DISCUSSION)

Dr. J. D. Verlinde

(DISCUSSION)

(2) SPREAD OF VIRUS IN THE COMMUNITY

Dr. Henry M. Gelfand
for
Dr. John P. Fox

(DISCUSSION)

Dr. Anne C. Kimball
Dr. John R. Paul

TOPIC II. SAFETY. (B) FIELD EVIDENCE OF SAFETY

9. EFFECTIVENESS OF TRIVALENT VACCINE IN IMMUNIZING YOUNG CHILDREN. STUDIES ON THE STABILITY OF THE VACCINE STRAINS AFTER HUMAN PASSAGE*

DOROTHY M. HORSTMANN, JOHN R. PAUL, E. PETER ISACSON, AND JAMES C. NIEDERMAN

Section of Epidemiology and Preventive Medicine, Yale University School of Medicine, and the WHO Regional Poliomyelitis Laboratory for the Americas

DR. HORSTMANN *(presenting the paper)*: The use of trivalent oral poliomyelitis vaccine has great appeal both on theoretical grounds and in terms of simplicity of administration. At the First International Conference on Live Poliovirus Vaccines in June 1959, several papers were presented which suggested that such a composite vaccine might indeed be a practical means of inducing immunity in a satisfactory per cent of vaccinees, particularly if a large dose of each type was used.[1-4]

However, the data raised certain questions as to whether the high antibody conversion rates reported for all three types were in fact a result of the trivalent vaccine ingested, or whether heterotypic antibody responses, or in particular contact infection among vaccinees, played a significant role in the end result. The spread of vaccine strains to contacts is a well documented phenomenon, and conceivably, in a population in which all three strains are fed at once, several children might be infected with Types 1 and 3 virus, while their playmates acquired Types 1 and 2 infection. In the normal course of events, a so-called "ping-pong effect", with exchange of viruses between susceptibles, might account for a significant per cent of the total final immunization achieved. In order to explore this possibility under controlled circumstances, and to evaluate more precisely the efficacy of a trivalent vaccine, a small trial was set up among young susceptible children. An additional object was to test the stability of the vaccine viruses by testing *T* and *d* markers of strains excreted by vaccinees and their contacts.

MATERIALS AND METHODS

Site and outline of project. The study was carried out in Santo Domingo de Heredia, a village with a population of 4810, located 12 Km. north of San José, Costa Rica. Although a mass oral vaccine campaign was in full swing in San José, the capital city, no oral vaccine had been administered in Santo Domingo before the start of our trial.

Forty-eight families each with several young children under five years of age participated. With few exceptions, families living at least two blocks away from one another were chosen, so that the chance of interfamilial contact infection was minimized. In each family only one child, under two years of age, received vaccine, while the others under five years were given a placebo. The ages of the 48 index children and their 77 contacts are given in Table 1. At the termination of the project 2½ months after it began,

* Representing studies planned and carried out by the Yale Poliomyelitis Study Unit under the auspices of the Pan American Sanitary Bureau and in close cooperation with the Ministry of Health of Costa Rica.

the number of participating families had been reduced to 42.

TABLE 1. AGE OF STUDY POPULATION: 48 FAMILIES IN WHICH TRIVALENT VACCINE WAS GIVEN TO ONE CHILD IN EACH FAMILY

	TOTAL NUMBER	AGE	NUMBER
VACCINEES	48	6–12 MOS 13–18 „ 19–22 „	27 19 2
CONTACTS	77	2–12 MOS. 13–23 „ 2–4 YEARS	6 6 65

Vaccine dosage. Trivalent vaccine, kindly supplied by Dr. H. Cox, was used. The titer for each of the three types was approximately 10^5 to $10^{5.5}$ TCD_{50}, and this amount was contained in 2 ml. of cherry flavored syrup. The placebo given to contacts consisted of cherry syrup only. A second similar dose of the same lot of vaccine was administered to the index children one month after the first dose.

Collection of specimens. Pre-vaccinal rectal swabs and blood specimens were obtained from the index children and their contacts. Following vaccine (or placebo) administration, rectal swabs were collected twice weekly from the entire study group. A second blood collection was made one month after the first dose of vaccine and immediately before the second dose. Rectal swab collections continued for another two weeks, and final, third blood specimens were obtained after one month from the index children but not from the contacts.

Laboratory tests. Virus isolation was carried out in monkey kidney (MK) tissue cultures. All rectal swabs were tested in MK bottle cultures, and in addition, the pre-vaccinal specimens were tested in Hep.-2 cells. Identification of isolates was carried out in MK tube cultures, using appropriate antisera, singly or in combination, as indicated.

Neutralization tests on the blood specimens of vaccinees were performed by the CPE (cytopathogenic effect) method in tubes; on sera from contacts, the colorimetric method in plastic panels was used, but some specimens were tested by both methods.

RESULTS

In the course of the study, 1703 rectal swabs were examined, 691 from the index children, and 1012 from their contacts (Table 2). A total of 892 virus strains was isolated, 637 of them being polioviruses. Surprisingly few of the vaccinees were found to be excreting mixtures of different types of polioviruses: only 45 specimens contained more than one type, and in only three were all three types present.

TABLE 2. ISOLATION OF VIRUSES FROM RECTAL SWABS, INDEX CHILDREN AND CONTACTS

	NUMBER OF CHILDREN	RECTAL SWABS		NO. STRAINS ISOLATED	
		NO. TESTED	POSITIVE	POLIOVIRUSES	OTHERS
VACCINEES	48	691	427 (62%)	378	98
CONTACTS	77	1012	408 (41%)	259	157
TOTAL	125	1703	835 (49%)	637	255
				892	

An analysis of the results in terms of the overall patterns of response, the spread of polioviruses to contacts, and the role of enteroviruses other than polioviruses will be given by Dr. Paul. The present discussion will be limited to the effectiveness of the trivalent vaccine in inducing infection and immunity in the index children. A pre-vaccinal virus survey based on tests of two rectal swabs collected 4-5 days before and on the day of vaccine ingestion, indicated that 20 of the 48 vaccinees (42 per cent) were already infected with some agent at the time of vaccination. In the present analysis, these 20 children are not considered, and only data on the 28 index children who were free of possibly interfering viruses will be given. Of the 28, 19 lacked antibody to all three types. None of the rest had antibody to Type 1, 6 were positive for Type 2, 2 for Type 3, and one had both Types 2 and 3 antibody.

The responses of these children to the first and second doses of vaccine in terms of virus excretion and antibody rises are given in Table 3. Antibody conversions one month after the first dose were observed in 42 per cent for Type 1, 10 per cent for Type 2, and 83 per cent for Type 3. Virus excretion of Types 1 and 3 was present in a somewhat higher per cent; subsequent tests indicated that antibodies had developed in most of these children by the third bleeding. By the time one month had elapsed after the second dose, the conversion rates were 75 per cent, 37 per cent, and 91 per cent, respectively, for the three types; the per cent excreting viruses followed closely. An exception was excretion of Type 2: we failed to detect Type 2 virus in three children who showed antibody conversions for this type.

In connection with these results, the family infection patterns were reviewed carefully. As will be reported by Dr. Paul, the spread of Type 1 and Type 3 viruses was considerable, but in no instance was there evidence that the index child acquired infection from a contact. In no family did contacts excrete a virus type which did not infect the index child. Among the total 48 families, there were five in which the index child was not infected after ingesting the first dose of vaccine, and in three of these, the second dose also failed to establish any poliovirus in the vaccinee. In none of these families was poliovirus isolated at any time from any of the contacts. We feel reasonably certain, therefore, that the results given for the vaccinees, represent the immunizing effect of the trivalent vaccine ingested by them, and not infection derived from some other source.

Table 3. Antibody Conversions and Virus Excretion in Homotypic Negative Children Free of Enterovirus Infection When Vaccinated

TYPE	NUMBER	FIRST DOSE PERCENT $\bar{C}$		SECOND DOSE PERCENT $\bar{C}$	
		ANTIBODY CONVERSION	VIRUS EXCRETION	ANTIBODY CONVERSION	VIRUS EXCRETION
I	28	42	62	75	71
II	21	10	10	35	19
III	25	83	84	88	88

Responses of children with pre-vaccinal antibody. Because of the small numbers, all index children with pre-vaccinal antibodies to any type are included in this analysis. There were 11 who possessed Type 2 antibody prior to vaccination; none of these were later demonstrated to excrete Type 2 virus, but all became infected with Type 1 and/or Type 3. The Type 2 antibody responses were, nevertheless, striking: as indicated in Table 4, 8 of the 11 showed four-

fold or greater rises. In the absence of Type 2 excretion, these are interpreted as heterotypic Type 2 responses to infection with Types 1 or 3 virus. The three who failed to show such heterotypic rises all had high Type 2 titers ($\geq$512) to start with; in two instances these may well have already represented heterotypic responses since the children were currently infected with Type 3 virus, prior to vaccine ingestion. Similar heterotypic rises were observed in index children with pre-vaccinal Type 3 antibodies who became infected with other types.

DISCUSSION

The results presented indicate that in the vaccine used, the infectiousness of the three types of virus was quite different, and the use of a relatively large dose (10^6TCD_{50}) did not completely overcome interference between the three types. The Type 3 component behaved as the dominant one, and produced a considerably higher number of infections than did Type 1, while Type 2, under these circumstances, was greatly suppressed. The impact of the second dose of vaccine, one month after the first, was not great. Probably if the interval between the two doses had been longer, the second might have been more effective.

In interpreting the results of this study, considerable stress has been laid on controlling for such factors as contact spread and heterotypic antibody responses, which may be misleading and may give false impressions of the actual effectiveness of the trivalent vaccine per se. If these qualifications are taken into consideration in comparing our results with those of others using the same trivalent vaccine, the differences are actually not great.[1, 2, 5] However, in older children [1] and adults [2, 6] higher conversion rates for Type 1, and particularly for Type 2 have been reported. A factor which might well be involved here is the immune status

TABLE 4. HETEROTYPIC TYPE 2 ANTIBODY RESPONSES TO INFECTION WITH POLIOVIRUSES TYPES 1 AND 3

NUMBER OF CHILD	SERUM	NEUTRALIZING ANTIBODIES 1	2	3	POLIOVIRUS EXCRETION
11–1	pre	<4	64	<4	
	post	64	>1024	256	1, 3
23–1	pre	<4	256	<4	
	post	64	1024	128	1, 3
36–1	pre	<4	256	<4	
	post	64	>512	64	1, 3
6–1	pre	<4	256	32	
	post	64	1024	128	1
29–1	pre	<4	256	<4	
	post	<4	1024	>1024	3
26–1	pre	<4	64	<4	
	post	8	1024	1024	3
47–1	pre	<4	>512	<4	
	post	<4	>512	64	3
28–1*	pre	<4	512	8	
	post	<4	>512	128	3
5–1*	pre	<4	1024	256	
	post	<4	1024	1024	3
27–1*	pre	<4	256	<4	
	post	<4	>512	256	3
3–1	pre	<4	64	<4	
	post	4	1024	1024	3

* Excreted Type 3 at the time of vaccine ingestion.

with respect to heterotypic poliovirus types. In our study, in which younger children, few of whom had antibody to any type, were fed trivalent vaccine, the highly infectious Type 3 seemed to take over the susceptible sites in the intestinal tract, almost completely crowding out the less infectious Type 2 and suppressing also to some extent Type 1. In older individuals, however, a sizeable per cent may be expected already to possess Type 3 (and/or Type 1) antibodies, so that Type 2 has considerably less competition and can establish itself and produce infection and immunity in a higher per cent of individuals. Actually, this is borne out in our study, in which 3 of 6 children already possessing Type 3 antibody became infected with Type 2 virus on the first feeding of trivalent vaccine. In contrast, Type 2 infections occurred in only 2 of 29 triple negatives on first exposure.

In a serological study, largely in adults, who were fed trivalent vaccine containing $10^{6.2}TCD_{50}$ of each of the three types, Cox *et al.*[2] have reported that fourfold antibody rises occurred in a very high per cent of individuals: >90 per cent for Types 1 and 3, and 72 per cent for Type 2. These are considered to be either primary or "booster" effects due to vaccine takes with the three types. However, if in this study only the homotypic negatives for each type are considered, and only conversions which can reasonably be attributed to infection with the vaccine strains (i.e. rises of from <4 to at least 8 or 16 in the pH neutralization test) are counted, the results are not strikingly different from those of others testing the same trivalent vaccine. The most likely explanation of the high per cent with prevaccinal antibody who showed "booster" effects in Cox's study is that many experienced heterotypic responses to Type 2 as a result of Type 1 and/or Type 3 infection. Such heterotypic responses, controlled by virus excretion studies, were well demonstrated in our trial, and have also been observed by Oker-Blom using the same strains [7] and by us using Sabin's strains.[8]

One of the main objects of our Costa Rica study was to attempt to answer the question as to whether trivalent vaccine can be considered an effective immunizing agent per se, resulting in infection and significant antibody responses to all three types in young susceptibles. In reviewing the results on the selected group of 28 who were free of enterovirus infection at the time of vaccination, it seems clear that within the special framework under which this trial was conducted, this particular vaccine was quite effective in producing immunity to Type 3 (88 per cent), less effective for Type 1 (75 per cent) and poor for Type 2 (35 per cent). It should be emphasized that these results apply only to susceptible, largely triple-negative children aged six months to two years, in whom the possibility of contact infection and heterotypic antibody responses have been ruled out. In a sense, the results represent a minimum response, which may be modified by such factors as age, degree of exposure, pre-existing enterovirus infection, and contact infection from other vaccinees. The data suggest that in preparing a trivalent vaccine, consideration should be given to the degree of infectiousness of each of the three types, and to the tendency of one type to be dominant. Simply increasing the dosage of all three types probably will not overcome the problem of dominance of one type and Krugman *et al.*[9] have demonstrated that even if the dose for each type is raised to >30,000,000 TCD_{50}, Type 2 remains dominant in the Sabin vaccine. It would seem logical, therefore, that rather than using equally large doses of the three types in a trivalent vaccine, attention should be directed to adjusting the amount of each type in line with its known degree of infectiousness. Until this combination has been worked out, the administration of the three types separately, with perhaps a follow-up dose of trivalent vaccine after some months as has been recommended by Chumakov *et al.*[10] would seem to be the most effective means of producing solid, trivalent immunity in susceptible, largely triple-negative individuals.

MARKER TESTS ON VIRUS STRAINS EXCRETED BY VACCINEES AND THEIR CONTACTS

A number of strains were tested to see if the *T* and *d* characters had changed in the course of first or second human passage. Work with the *d* marker is incomplete and only the results with the thermal sensitivity test will be given at this time.

In testing for the *T* marker, duplicate titrations of first or second MK passages were set up in tubes, using four monkey kidney tubes per log

TABLE 5. RESULTS OF TESTS FOR THE THERMAL SENSITIVITY (*T* MARKER) OF 100 POLIOVIRUS STRAINS EXCRETED BY VACCINEES AND THEIR CONTACTS

TYPE	SOURCE	WEEKS TO VACCINATION						TOTAL STRAINS TESTED
		1–2 NO.		3–4 NO.		5–7 NO.		
		T−	T+	T−	T+	T−	T+	
I	15 VACCINEES	9	0	11	0	5	0	27
	15 CONTACTS	4	0	7	0	8	0	24
II	3 VACCINEES	2	0	3	0			5
	1 CONTACT			1	0			1
III	16 VACCINEES	13	0	7	0	4	0	24
	12 CONTACTS	13	0	5	0	2	0	20

dilution. One set of tubes was incubated at 36° and one at 40° C. The results were read at four and seven days, and on the basis of the differences in titer at the two temperatures, the strains were designated as *T*—, *T* intermediate, or *T*+.[11] In each test, a known *T*+ (Type 1 Mahoney) and known *T*— (Type 1 LSc) strain were included as controls.

The results for 101 strains, isolated at different times after the vaccine was given, are shown in Table 5. To our surprise, all isolates of all three types behaved as *T* negative strains, with differences of 5 logs or more between the titer at 36° and 40° C.

In addition to the postvaccinal strains tested, seven Type 3 poliovirus strains isolated from the study group before the vaccine was administered have all been shown to possess the *T* negative character. It is entirely possible that these strains represent a spread of Type 3 vaccine strain from the nearby city of San José, where this type had been used on a large scale for the few weeks preceding our study.

Table 6 shows the results on the *d* markers of 40 of the same strains which were tested for *T* markers. The three vaccine strains themselves, as present in the preparation fed, were tested in our laboratory, and all were classified as *d* intermediate. Of the 40 strains excreted by vaccinees and contacts, three had the *d*+ character, two of these being Type 1 and one Type 3. A number of other strains were like the vaccine strains, *d* intermediate, and some were *d*—.

The results of tests with the two markers *T* and *d* suggest so far that among susceptible young Costa Rican children the vaccine strains used were quite stable on human passage. The results with Type 3, particularly with the *T* marker, are at variance with the usual experience with Type 3 vaccine strains and with Koprowski's experience reported yesterday with the same Type 3 Fox's strain. At present we do not have an explanation of this discrepancy. As has been shown [11] the *T* marker does not always correlate with monkey neurovirulence, nor with the *d* marker.[12] Further tests, in order to compare the *T* and *d* markers with monkey neurovirulence of excreted strains, are now in progress.

ACKNOWLEDGMENTS

Grateful acknowledgment is expressed by the authors to the following: Dr. José Manuel Quirce, Minister of Health, and Dr. Oscar Vargas-Méndez, Director General of Health, Costa Rica;

TABLE 6. *d* MARKERS OF 40 STRAINS FROM VACCINEES AND CONTACTS

TYPE	SOURCE	WEEKS P VACCINATION									TOTAL STRAINS TESTED
		1-2			3-4			6-7			
		D-	I	D+	D-	I	D+	D-	I	D+	
I	12 VACCINEES	1	0	0	3	4	0	4	3	0	15
	11 CONTACTS				1	4	1	0	6	1	13
II	3 VACCINEES	2	1	0							3
	1 CONTACT	0	1	0							1
III	5 VACCINEES				4	0	0	1	1	1	7
	1 CONTACT				1	0	0				1

Dr. Juan A. Montoya, Pan American Sanitary Bureau Epidemiology Consultant, and Dr. Joaquín Nuñez, Technical Director, National Campaign against Poliomyelitis, Costa Rica; also to Mrs. María José Ramírez Vargas and Miss Jael Chacón Zamora, Santo Domingo de Heredia Health Center, and Mrs. Mary Morales, Ministry of Health, San José, Costa Rica. Without the cooperation of these individuals this study would not have been possible.

REFERENCES

1. Prem, K. A., and McKelvey, J. L.: Antibody response in adults and children following simultaneous oral administration of three type strains of live attenuated poliomyelitis virus. In: *Live Poliovirus Vaccines, First International Conference.* Scient. Pub. no. 44 of the Pan American Health Organization, pp. 249-253, 1959.
2. Cox, H., Cabasso, V. J., *et al.:* Immunologic response to trivalent oral poliomyelitis vaccine. In: *Live Poliovirus Vaccines, First International Conference.* Scient. Pub. no. 44 of the Pan American Health Organization, pp. 229-248, 1959.
3. Chumakov, M. P., Voroshilova, M. K., *et al.:* Preliminary report on mass oral immunization of population against poliomyelitis with live virus vaccine from A. B. Sabin's attenuated strains. In: *Live Poliovirus Vaccines, First International Conference.* Scient. Pub. no. 44 of the Pan American Health Organization, pp. 517-529, 1959.
4. Smorodintsev, A. A., Ilyenko, V. I., *et al.:* Part 2. Virological and immunological characteristics of vaccinal infection in children inoculated *per os* with a live poliomyelitis vaccine made from the Sabin strains. In: *Live Poliovirus Vaccines, First International Conference.* Scient. Pub. no. 44 of the Pan American Health Organization, pp. 312-323, 1959.
5. Prem, K. A., McKelvey, J. L., and Fergus, J.: Immunologic response of infants under six months of age to oral trivalent poliomyelitis vaccine. In: *Live Poliovirus Vaccines, First International Conference.* Scient. Pub. no. 44 of the Pan American Health Organization, pp. 254-259, 1959.
6. ———, ———, ———: Immunologic response of pregnant women to oral trivalent poliomyelitis vaccine. In: *Live Poliovirus Vaccines, First International Conference.* Scient. Pub. no. 44 of the Pan American Health Organization, pp. 260-269, 1959.

7. Oker-Blom, N.: Personal communication.
8. Yale Poliomyelitis Study Unit. Unpublished observations.
9. Krugman, S., *et al.:* Immunization of newborn infants with live attenuated poliovirus vaccine. Paper presented at the meeting of the American Pediatric Society, Swampscott, Mass., 5 May 1960.
10. Chumakov, M. P., *et al.:* Report No. 2 on mass oral immunization of population in the Soviet Union against poliomyelitis with live vaccine from A. B. Sabin's attenuated strains. Academy of Medical Sciences of the USSR, Institute for Poliomyelitis Research, Moscow, 1960.
11. Sabin, A. B.: Reproductive capacity of polioviruses of diverse origins at various temperatures. The Gustav Stern Symposium on Perspectives in Virology, II. In press.
12. Benyesh-Melnick, M., and Melnick, Joseph L.: The use of *in vitro* markers and monkey neurovirulence tests to follow genetic changes in attenuated poliovirus multiplying in the human alimentary tract. In: *Live Poliovirus Vaccines, First International Conference.* Scient. Pub. no. 44 of the Pan American Health Organization, pp. 179-198, 1959.

DISCUSSION

CHAIRMAN ZHDANOV: Thank you, Dr. Horstmann. This paper is now open for discussion.

DR. GEAR: I should like to ask Dr. Horstmann if she considers that her results are a recommendation for feeding trivalent vaccine, rather than monovalent vaccine? Feeding trivalent vaccine more than once gives an opportunity for the intestinal tract to become infected with the particular type for which antibodies are not present, and I would be grateful to have Dr. Horstmann tell us whether she thinks that her results are in favor of feeding triple vaccines, rather than monovalent vaccine.

DR. HORSTMANN: I think the results have to be interpreted in relation to the environment, the age, and immune status of those to be vaccinated. On the basis of our findings, I would not recommend trivalent vaccine if one expects to accomplish rapid immunization of a triple-negative child with one dose of vaccine. Perhaps when we know more about optimum dosage level combinations for any particular preparation a single dose of trivalent vaccine will be more effective than is currently the case.

In the meantime, my feeling is that if one wishes to use the trivalent vaccine, it should be given repeatedly, and at somewhat longer intervals than we used.

DR. COX: These results confirmed what we found out about our Type 2 strain. The Type 2 strain of course has been the most modified by laboratory manipulations of any of the strains we have. Actually, our Type 3 strain is the only one of our strains that has never been adapted to a foreign host. We think this may be partially responsible for its greater ability to infect the human gut.

Furthermore, this particular batch of vaccine was made up in a considerable hurry and did not contain as much virus as we had intended.

Data to be presented later will show results obtained with a vaccine containing more virus, namely, 6.1 logs of virus per strain. Actually, in the Florida trials, where we apparently obtained our best results, we compensated somewhat for the Type 2 deficiency by actually putting in a bit more than 6.1 logs per dose.

We have found, by asking people who have worked with monovalent versus trivalent, that it is much better to feed trivalent twice, and certainly at a longer interval than one month, than to carry out monovalent feedings.

We believe that there is still resistance in the gut which may interfere with feeding trivalent vaccine at a one-month interval. But when it comes to practicality and expense, it is much easier to feed trivalent twice than it is to feed the monovalents separately.

We have been considerably encouraged by what we have seen with the trivalent vaccine because I think the results Dr. Horstmann obtained are probably the poorest we have seen and certainly somewhat inferior to our other data. This may be due to the quantity of virus fed, and perhaps to some other factors we do not understand as yet. To be sure, they are sufficiently encouraging so that we feel it worthwhile to go ahead and solve this problem. There is no doubt in our minds that this can be done.

DR. FLIPSE: I should like to ask Dr. Horstmann if she has, and if so could make available for comparison, the conversion data at the 1:4 level.

There is considerable disagreement, obviously, as to its significance, but it would make it possible for those of us who are reporting data in terms of both 1:4 and 1:16 conversions to compare her data with other data.

DR. HORSTMANN: As I indicated in our study, all of the homotypic negative children who became infected and converted, with one or two possible exceptions, developed antibody titers considerably greater than 1:16. Therefore, the conversion rates at 1:4 are virtually the same as at 1:16. Furthermore, we used the CPE tube neutralization method in testing the sera. This method measures high avidity antibody and gives lower titers than the pH colorimetric test which

I assume was used in Dr. Flipse's work. The results are therefore not directly comparable because of the differences in technique. Titers of 4 or 8 by the pH method (which measures low avidity antibody) are apt to be <4 in the CPE test.

DR. BARON: I should like to ask Dr. Horstmann a question concerning the *d* marker. In Dr. Cabasso's paper of yesterday he indicated that the Lederle Types 1 and 3 strains were *d* positive, and I notice that most of the excreted virus studied by Dr. Horstmann were *d* negative. I was wondering if she had done *d* tests on the original vaccine virus, and what the *d* marker was.

DR. HORSTMANN: We tested aliquots of the three strains of the original vaccine which was fed. All were found to have the $d\pm$ marker, although Dr. Cabasso reported that the Types 1 and 3 were $d+$ in his laboratory.

DR. CABASSO: All this points out one fact that I would like to emphasize: the method for establishing these markers certainly will have to be made uniform if we are to obtain comparable values in all laboratories.

We have taken pains to count every single plaque or seeming plaque which occurs in our bottles or plates, even the so-called pinpoint plaques, and it may very well be that these few pinpoint plaques, which may not be counted in other laboratories, can account for the differences between laboratories.

That is why I should like to make a plea for getting techniques as uniform as possible and for making them available to all the laboratories concerned, in order to obtain results that can be compared.

DR. BODIAN: I should like to ask one question and to comment on the heterotypic responses. The question is whether Dr. Horstmann could give us poliomyelitis virus conversion rates or infection rates in those who were infected with enteric viruses in that population, as compared with those who had no enteric virus infections.

DR. HORSTMANN: I can answer that question simply, by saying that the rates were really not very different with the exception of responses to Type 1 in which, on the first dose, were just about half as great in those with pre-existing enterovirus infection as in the children who were not excreting enteroviruses. No significant interference with Type 3 occurred, and Type 2 responses were equally poor in both groups of children.

DR. BODIAN: In connection with heterotypic responses, we had an unusual opportunity to examine this question in analyzing a group of sera collected by Dr. Paffenbarger from a small village in Greenland, a village of about 400 inhabitants, samples of serum having been taken from alternate individuals of all age groups. This was a population which had recently had a Type 3 poliomyelitis infection, with no clinical cases but involving about 90 per cent or more of all children and adults.

We were very much impressed by the heterotypic Type 2 responses following the Type 3 exposure, and this was readily apparent because the Type 2 virus obviously had not been present in this community for 14 years. The situation was very much like the Alaskan situation reported by Dr. Paul.

In the group below the age of 14, all antibody levels to Type 2 were of the order of 1:4 to 1:16, whereas in older age groups higher levels characteristic of previous infection were found.

With the Type 1, there were practically no heterotypic responses, although the Type 1 virus had apparently been absent for the previous 25 years. No individuals below the age of 25 had significant Type 1 antibody levels.

I should like to add to this the plea that when conversion rates are presented, we are told the level at which the tests were done, so that we can assess the possibility of the heterotypic response, particularly the Type 2 antibody response.

DR. SABIN: The heterotypic responses are nothing new. I reported them a number of years ago in a study we carried out on young adult volunteers, and found that heterotypic neutralizing antibody responses occurred with each of the three types, following feedings of Type 1, Type 2, and Type 3.

Whether or not a heterotypic antibody response was obtained depended somewhat on the level of antibody that the individual had. In individuals

who had no antibody at all, as demonstrable by the tube neutralization test, the heterotypic response was of a very low order, below 1:16.

If the tests were carried out beyond four weeks, persistence of the low level response was rare.

I should like to ask Dr. Horstmann a question for the record, and that is whether the search for other enteric viruses in these children was carried out only in monkey kidney or also in a human cell line and newborn mice?

DR. HORSTMANN: All of the pre-vaccinal specimens were tested in monkey-kidney cells and Hep.-2 cells. We did not use infant mice.

DR. SABIN: I should like to point out that in our own experience the infant mice contributed very little to the total. Only about 2 per cent of the viruses from children in Mexico that we could not isolate in monkey kidney and Hep.-2 cells, were recovered in infant mice; therefore, my reaction to the question I just asked is that probably most of the enteric viruses that are detectable by available methods were detected here.

DR. HORSTMANN: Yes.

DR. PLOTKIN: I wonder if Dr. Horstmann, or anyone else, has reinfection data after trivalent vaccine. The question in my mind, of course, is whether the relative resistance which has been observed after feeding monovalent vaccine would be equally observed after trivalent vaccine.

DR. HORSTMANN: Later Dr. Paul will give results on revaccination with trivalent vaccine.

CHAIRMAN ZHDANOV: We shall now proceed with Dr. Baron's paper on the "Laboratory Investigations of the Attenuated Poliovirus Vaccine Strains."

10. LABORATORY INVESTIGATIONS OF THE ATTENUATED POLIOVIRUS VACCINE STRAINS. II. TISSUE CULTURE CHARACTERISTICS BEFORE AND AFTER GASTROINTESTINAL PASSAGE

SAMUEL BARON, ROBERT M. FRIEDMAN, RUTH L. KIRSCHSTEIN, GERALD L. BORMAN, RODERICK MURRAY, AND GEORGE A. HOTTLE

Division of Biologics Standards
National Institutes of Health, Bethesda, Maryland

DR. BARON *(presenting the paper)*: Low neurovirulence for primates is considered a prerequisite for virus strains to be used in live poliovirus vaccines and indeed, the presently proposed strains were selected on this basis. An additional requirement is stability of the low neurovirulence characteristic during vaccine production and human passage. Studies of the neurovirulence of vaccine strains and of stability of their neurovirulence characteristics have been reported by many workers.[1] These studies, however, have often given rise to quite different conclusions. Studies have been reported from our laboratory in which an effort was made to obtain under standardized conditions, comparative information concerning the neurovirulence for monkeys of the various vaccine strains presently proposed.[2, 3] The present report concerns current studies of stability of the neurovirulence characteristics during laboratory and human passage of vaccine strains.

Material and Methods. Vaccine strains were obtained from Drs. Koprowski, Cox, and Sabin and are listed with their titers as determined in our laboratory in Table 1.

Stool samples were obtained either as whole stool or suspensions. Stools from contacts of persons fed Koprowski Type 1 virus were obtained from Dr. M. Shär in Switzerland; Sabin Type 3 stools, from Dr. C. H. Stuart-Harris in England; and stools containing Lederle Types 1, 2, and 3 strains from Dr. Flipse and Dr. Erickson in Miami, Florida. Ten per cent stool suspensions were passed through a Swinney filter containing a Seitz pad which had been pre-treated with tryptose phosphate broth. Stools from per-

TABLE 1. STRAINS OF POLIOVIRUS STUDIED

KOPROWSKI GROUP		
TYPE I	WISTAR-CHAT POOL 13	$10^{7.2}$ PFU/ML
TYPE I	WISTAR WIP-1 POOL 1	$10^{7.4}$ PFU/ML
TYPE II	TN 19 POOL 1	$10^{6.0}$ PFU/ML
TYPE III	WFX POOL WY 13	$10^{7.1}$ PFU/ML
LEDERLE GROUP		
TYPE I	LEDERLE-SM, # 7-1231-166	$10^{7.9}$ PFU/ML
TYPE I	LEDERLE-SM, # 7-1231-114	$10^{6.4}$ PFU/ML
TYPE II	LEDERLE-MEF-1, # 7-1232-243	$10^{6.5}$ PFU/ML
TYPE III	LEDERLE-FOX, # 7-1233-344	$10^{7.0}$ PFU/ML
SABIN GROUP		
TYPE I	L SC, 2 AB	$10^{7.7}$ PFU/ML
TYPE II	P 72, CH, 2 AB	$10^{7.3}$ PFU/ML
TYPE III	LEON, 12 A, B	$10^{7.9}$ PFU/ML

sons fed trivalent Lederle vaccine were incubated for 1 hour at 37° C. with divalent rabbit, anti-poliovirus serum (50 MIT units of each antibody per 0.1 ml.) so that single poliovirus types could be studied.

Viral titrations were performed in Rhesus kidney-cell culture tubes containing Earle's balanced salt solution (0.22 per cent sodium bicarbonate) and 0.5 per cent lactalbumin hydrolysate. Plaque assays were done in 2 oz. bottles using the same medium containing 20 per cent skim milk.[4]

Temperature (*T*) characteristics were determined by incubating half the inoculated culture tubes or plaque bottles in a 36.0° ± 0.3 incubator and half at an incubator temperature of 39.5° C. or 40.0° C. A final reading was done on the sixth or seventh day. Tests included the appropriate controls with seed virus strain as well as the virulent Mahoney Type 1 strain. *T* character is expressed as $\log_{10}$ titer at 36° C. minus $\log_{10}$ titer at 39.5° C. or 40° C. Plaque diameter *T* character is a ratio of the diameter at 39.5° C. over diameter at 36° C. expressed as per cent.

Neurovirulence of virus preparations for monkeys was determined by inoculating amounts of virus calculated to be at the end-point of infectivity for the homotypic seed virus. Three Rhesus monkeys were inoculated by each route, intrathalamically (I TH), intraspinally (IS), and intramuscularly (IM)—a total of nine monkeys—observed for paralysis, sacrificed 14 to 21 days after inoculation and portions of the CNS were examined histopathologically by methods previously described.[2, 3] Monkey neurovirulence results are expressed as the maximum plaque-forming units (PFU) of virus causing no lesions (threshold).[2]

RESULTS

Comparison of T Character and Monkey Neurovirulence. The *T* character may be measured by several methods, including monkey-kidney cell culture tube titration, plaque count assay and the measurement of plaque diameter as determined at 36° C. and 39.5° C. To determine which of these techniques best correlates with neurovirulence for monkeys, a comparison was made with the results of monkey studies previously reported from this laboratory.[2, 3] In the results presented in Fig. 1 each *T* characteristic is the average of three separate determinations. A shorter line for *T* character indicates increased ability to grow at 39.5° C. and a shorter line for monkey neurovirulence indicates increased virulence. No determination is indicated by absence of a line. The plaque count determination of *T* character of Types 1 and 2 appears to be correlated better with monkey neurovirulence following CNS inoculation than do the tube titration or plaque diameter methods. This correlation is especially striking with the Lederle Type 2 strain which shows higher neurovirulence following CNS inoculation; however, this strain shows a tube titration *T* characteristic suggestive of low virulence, whereas the plaque count *T* character indicates greater virulence. There was poor correlation between all *T* determinations and monkey virulence in the case of each Type 3 strain. In addition, correlation is lacking between the *T* characteristic and neurovirulence following IM inoculation. The latter observations are consistent with other data indicating that neurovirulence following IM inoculation appears to measure different properties than does direct central nervous system (CNS) inoculation.[5, 8]

During the course of this study it was noted (Fig. 2) that there was a considerable differential of virus growth when temperature of incubation was raised from 39.5° C. to 40° C. ± 0.3. While the indicated temperature difference was 0.5° C., we could not exclude a somewhat greater difference due to a temperature variation of ±0.3° C. within the incubators and due to a variation of ±0.2° C. between thermometers. The higher temperature permitted better differentiation of virus strains but the overall ranking of virulence by *T* character remained the same. In addition the ranking by *T* character of the Type 3 strains appeared better correlated with neurovirulence for monkeys following CNS inoculation when the higher temperature of incubation was 40° C. rather than 39.5° C.

As a result of these experiments, plaque count assay was adopted as a reasonable screening test for the detection of changes in neurovirulence of monkeys inoculated I TH or IS.

Effect of Laboratory Manipulation on the T Character and Monkey Neurovirulence. Alteration of the *T* character and monkey neurovirulence has been reported to be associated with laboratory manipulation of attenuated viruses.[6]

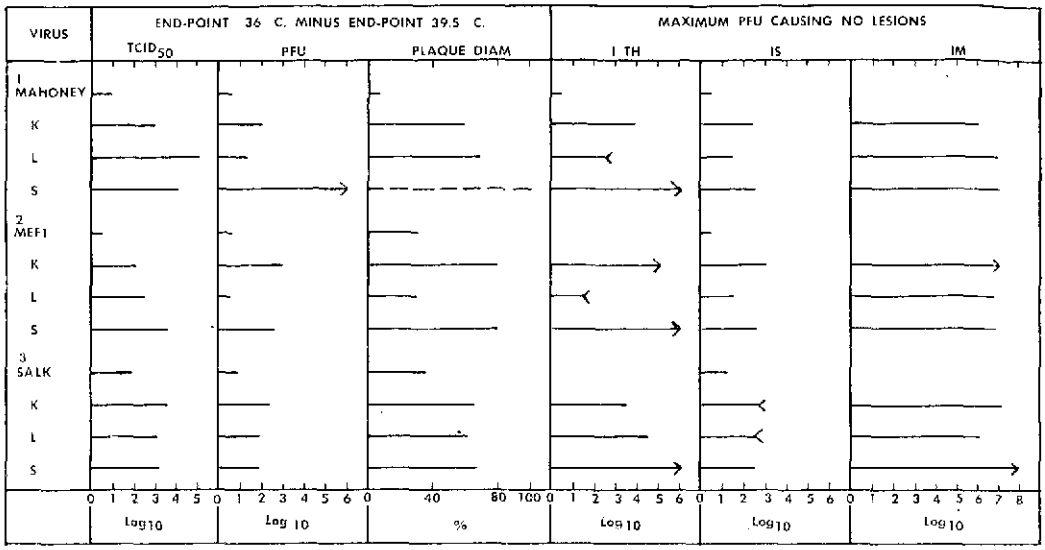


FIG. 1. *T* character and monkey neurovirulence.

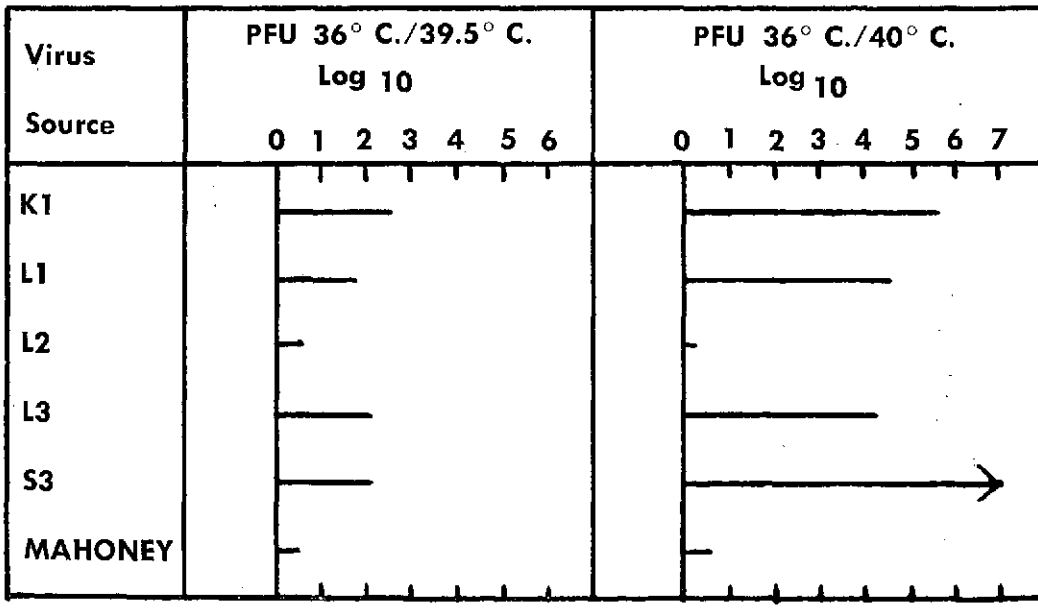


FIG. 2. *T* character 36°C./39.5°C. and 40.0°C.

To better understand the effect of various growth conditions on these two characteristics, attenuated virus pools which, in our laboratory, had been propagated in different environments, were studied for *T* character and in some instances for neurovirulence in monkeys. As may be noted in Fig. 3, one or two passages at 36° C. of an experimental Koprowski Type 1 attenuated virus and a Sabin Type 3 vaccine virus resulted in no apparent change in *T* character, whereas two passages of the same Koprowski Type 1 virus at 40° C. resulted in rapid adaptation to growth at that temperature and some de-adaptation to growth at 36° C. This virus, when inoculated into monkeys by the I TH route demonstrated marked increase in neurovirulence as evidenced by a decrease in the maximum number of plaque-forming units (PFU) which caused no lesions (threshold). In addition to further demonstrating some correlation of plaque *T* character and monkey neurovirulence, these results illustrate how rapidly virulence may change during laboratory propagation.

Another change in *T* character of virus during laboratory manipulation is illustrated in Fig. 4. As may be seen, one passage of Sabin Type 3 virus in a plaque system at 36° C. resulted in increased ability of the virus extracted from the agar to grow at 40° C. No marked increase in neurovirulence was observed although some increase could not be excluded. These results indicate that the *T* character and monkey neurovirulence are most reliably determined in the original virus specimen with a minimum of laboratory manipulation.

T Character and Monkey Neurovirulence of Excreted Virus. Stools of persons fed Sabin Type 3, Koprowski Type 1, and trivalent Lederle vaccines were studied for *T* character and monkey neurovirulence.

The plaque count *T* character of Sabin Type 3 virus, determined directly on sequential stools

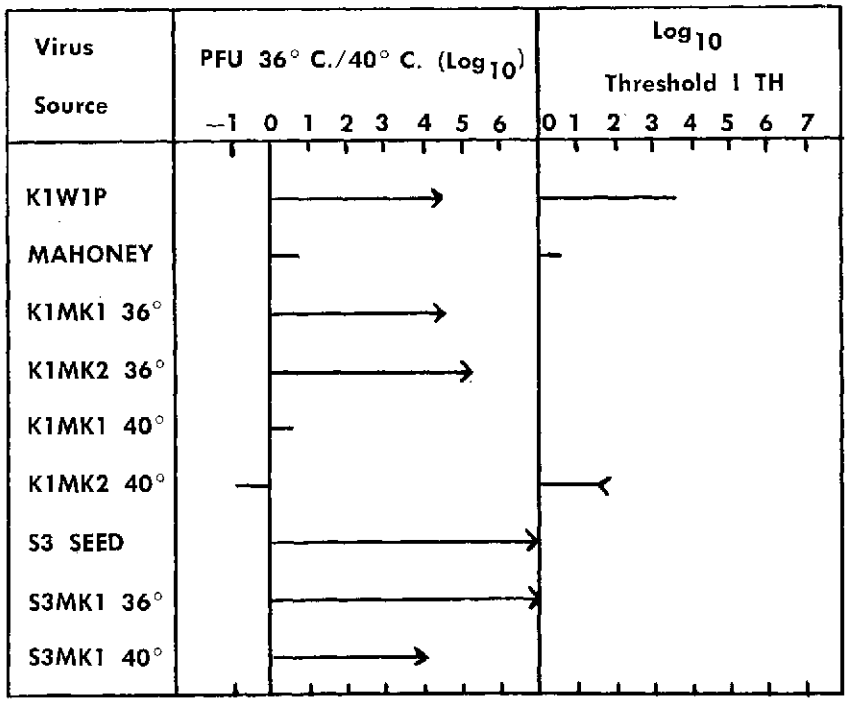


FIG. 3. Effect of laboratory procedures on *T* character and monkey neurovirulence.

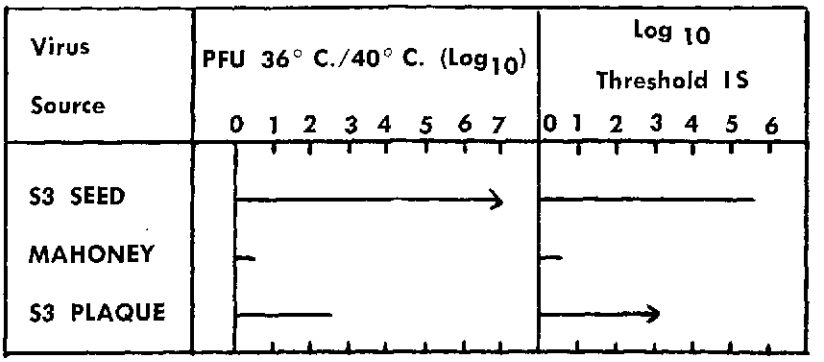


FIG. 4. Effect of laboratory procedures on *T* character and monkey neurovirulence.

from subject 1202, is shown in Fig. 5 and compared with the original seed virus and the virulent Mahoney Type 1 strain. In confirmation of the work of Stuart-Harris *et al.*, on these same stool specimens,[7, 8] the *T* character of the excreted virus changed toward the value of the Mahoney strain. As may be seen in Fig. 6, the virus in stools of patient 1203 shows a similar change of *T* character. Figure 7 illustrates similar results from patient 1205. In order to obtain sufficient virus for monkey virulence tests, the virus in the 20 day stool specimen was passaged once in monkey-kidney cell cultures and the resulting fluid was inoculated I TH, IS, and IM into monkeys. This single passage resulted in some loss of ability of the virus to propagate at 39.5° C. and was, thus, intermediate in *T* character. As illustrated, a moderate increase in neurovirulence following I TH and IS inoculation was observed.

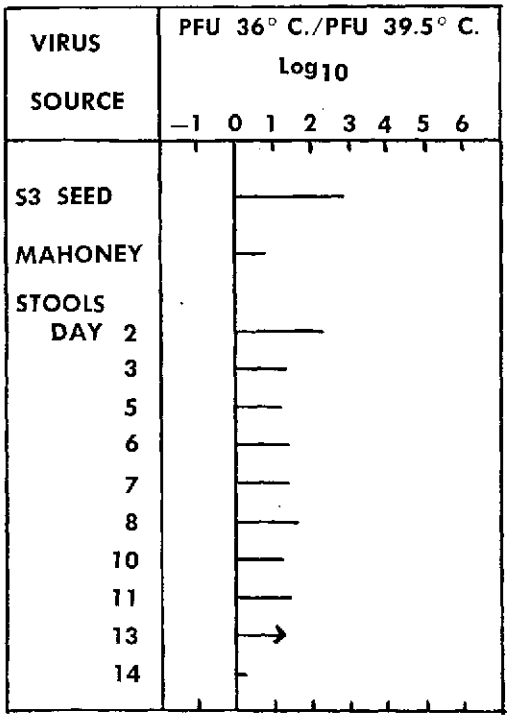


FIG. 5. *T* character of excreted virus (1202).

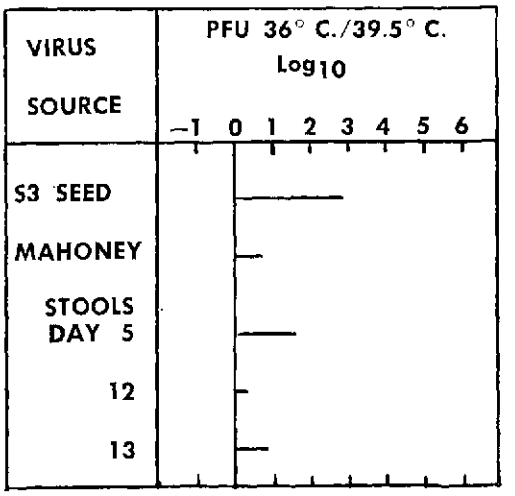


FIG. 6. *T* character of excreted virus (1203).

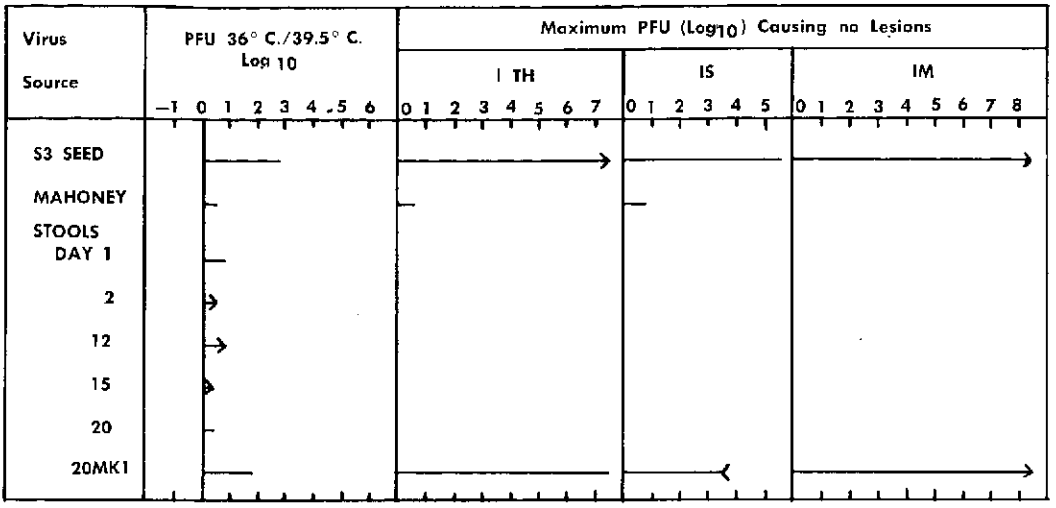


FIG. 7. *T* character and monkey neurovirulence of excreted virus (1205).

The *T* character of Koprowski Type 1 virus, determined directly on two stool specimens from contacts of persons fed vaccine virus, is shown in Fig. 8. An increase of ability of excreted virus to propagate at 39.5° C. was observed and one monkey-kidney cell culture passage at 36° C. again altered the *T* character, but this time toward greater ability to grow at 39.5° C. Correlated with the changed *T* character after passage in cell culture, was a definite increase of monkey neurovirulence following CNS inoculation as demonstrated by the lower threshold value and higher rate of occurrence of paralysis.

The *T* character of the Lederle Type 3 virus in the stool of subject J.S. who was fed trivalent vaccine is shown in Fig. 9. The virus in the stool demonstrated marked ability to propagate at 40° C. and this ability was retained after one passage in monkey-kidney cell cultures at 36° C., but was partially lost after a second passage in

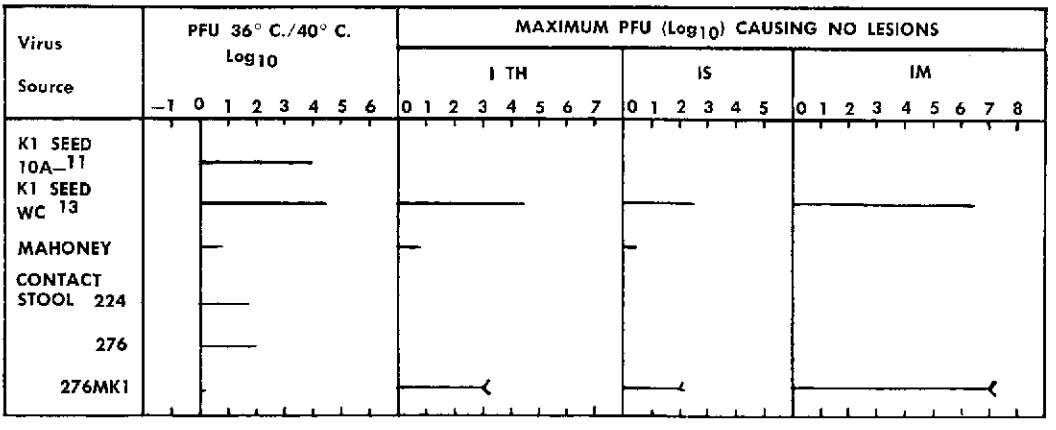


FIG. 8. *T* character and monkey neurovirulence of excreted virus.

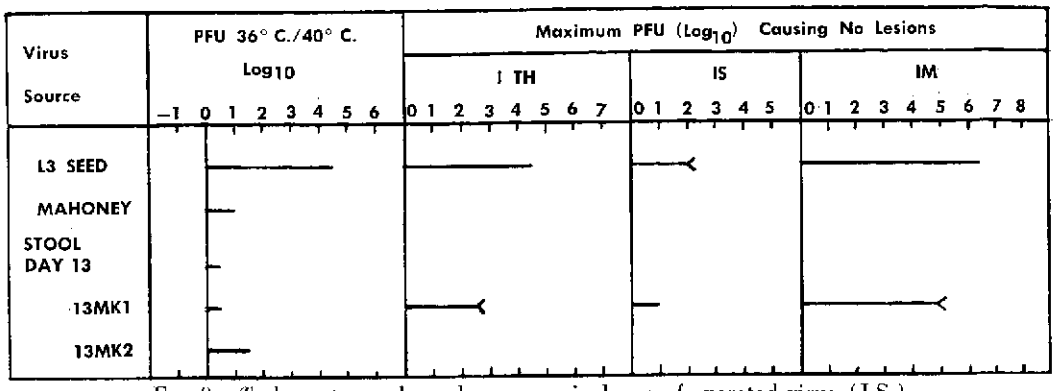


FIG. 9. *T* character and monkey neurovirulence of excreted virus (J.S.).

cell culture. The virus from the first monkey-kidney passage of the stool showed a corresponding increase in neurovirulence when injected into monkeys by any of the three routes.

As shown in Fig. 10, Lederle Type 1 virus in the stool of subject J.S. was studied for *T* character and monkey neurovirulence. As may be seen, virus from a single monkey-kidney cell culture passage had increased ability to grow at 40° C., but did not exhibit increased neurovirulence for monkeys.

The previous findings demonstrated that even

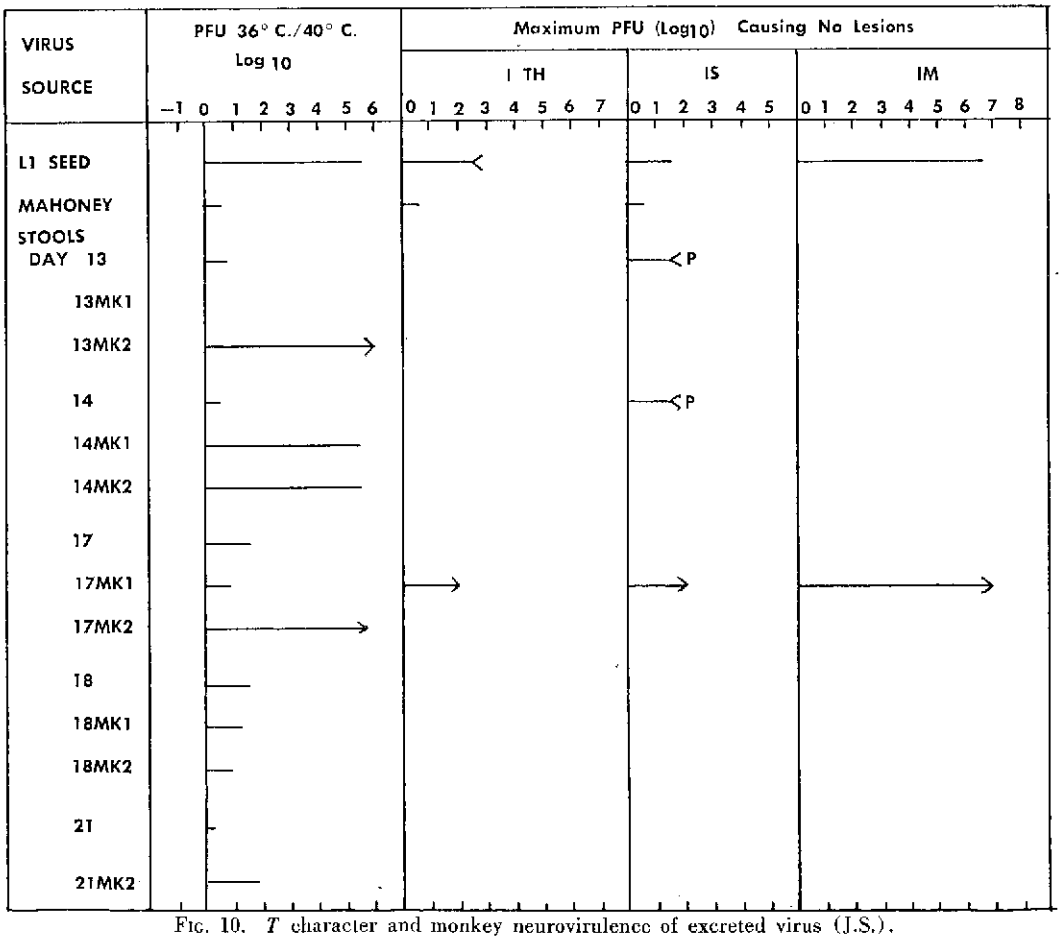


FIG. 10. *T* character and monkey neurovirulence of excreted virus (J.S.).

a single monkey-kidney cell culture passage may radically alter the *T* character of a virus strain. With this in mind two original filtered 10 per cent stool suspensions of Lederle Type 1 virus from subject J.S. were inoculated directly into the spinal cord of Rhesus monkeys. As is illustrated in Fig. 10, the virus in the 13 and 14 day stools manifested a markedly increased ability to grow at 40° C., and also exhibited a markedly increased paralytic rate and a decreased histopathologic threshold value.

The prime objective of these continuing studies is to determine the stability of the monkey neurovirulence character of vaccine strains in the course of human passage. The results to date indicate that: (a) the *T* character, as determined in a plaque system, is very often, but not invariably correlated with monkey neurovirulence; (b) laboratory manipulation of vaccine virus or excreted virus frequently results in rapid alteration of *T* character and monkey neurovirulence, indicating the importance of studying the virus directly in vaccine or stool specimens; (c) virus in, or derived from, stools of persons fed vaccine virus exhibits a high frequency of increased ability to propagate at 40° C., and increased monkey neurovirulence.

ACKNOWLEDGMENTS

The authors wish to acknowledge the excellent technical assistance of Mr. Marshall Ford, Mrs. Dorothy S. Hughes, Mr. Richard J. Low, and Mr. George Rustin.

REFERENCES

1. Pan American Health Organization: In: *Live Poliovirus Vaccines, First International Conference.* Scient. Pub. no. 44, 1959.
2. Murray, R., Kirschstein, R. L., Van Hoosier, G. L., and Baron, Samuel: Comparative virulence for Rhesus monkeys of poliovirus strains used for oral administration. In: *Live Poliovirus Vaccines, First International Conference.* Scient. Pub. no. 44 of the Pan American Health Organization, pp. 39-64, 1959.
3. Kirschstein, R. L., Borman, G. S., Baron, Samuel, Friedman, R., Murray, R., and Hottle, G. A. (See this volume, pp. 90-97).
4. Baron, Samuel, and Low, R. J.: New maintenance medium for cell culture. *Science* **128:** 89-90, 1958.
5. Sabin, A. B., Hennessen, W. A., and Winsser, J.: Studies on variants of poliomyelitis virus. I. Experimental segregation and properties of avirulent variants of three immunologic types. *J. Exp. M.* **99:** 551-576, 1954.
6. Sabin, A. B.: Paper presented at the Gustav Stern Symposium on Perspectives in Virology. To be published in 1960.
7. Clarke, S. K. R., Goffe, A. P., Stuart-Harris, C. H., and Herzog, E. G.: A small-scale trial of Type 3 attenuated living poliovirus vaccine. *Brit. M. J.* **2:** 1188-1193, 1958.
8. Stuart-Harris, C. H., and Goffe, A. P.: Personal communication.

DISCUSSION

CHAIRMAN ZHDANOV: This paper is now open for discussion. Dr. Koprowski.

DR. KOPROWSKI: Many of the speakers, including Dr. Baron, have not specified the type of equipment they used to control exactly the temperature of their incubators. The equipment currently available on the American market has failed, in our hands, to control small differences in the temperature of incubation to guarantee reproducibility of the results even if adequate control titrations are included in the test. This is particularly true when the temperature range 39°-40° C. is explored. An incubator where fluctuations of 0.5° are encountered, has proven useless in the study of behavior of viruses at the upper temperature range and frequently we had to discard our results as not valid because of poor reproducibility.

Only when following Dr. Lwoff's advice, of installing Mergatherm termal regulators in our water-baths, guaranteeing fluctuations of temperature not exceeding 0.05°, have we been able to reproduce the results satisfactorily.

DR. SABIN: The incubators that we used for our temperature studies were incubators with large fan systems, to provide good circulation of air, and while the variation in temperature can be within about half a degree, we have never found any difficulty whatever in the tests with Type 1 and Type 2 viruses. I reported this yesterday, and this very great consistency for Types 1 and 2 viruses was also apparent in Dr. Melnick's report.

With Type 3, however, smaller alterations in temperature can give rise to considerable difficulties.

For that reason, we consider no test valid unless the original seed virus, run at the same time in the incubator, gives the required difference in titer. With that as a control, the results are fairly reproducible.

In our conclusions, we should never speak of polioviruses in general, as Dr. Baron has just spoken; rather, we must speak separately for the different types. The great stability for Type 1 and Type 2, which was reported yesterday, must be distinguished from the special properties demonstrated by Type 3, except those indicated earlier by Dr. Horstmann.

This brings me to a question: whether there may be certain environmental or dietetic factors in the intestinal tract, which may be conducive to increasing the population of virus particles in Type 3, that can multiply at 40° C. Costa Rica seems to offer a particularly good environment to obtain some data on this particular question.

DR. BARON: I should like to second Dr. Sabin's comment that the seed virus be run in parallel to any *T* determination.

The variability extends into the Type 2 and some of the Type 1 strains, depending on which strain one is dealing with; the Mahoney and LSc strains may not be suitable as controls for the *T* determinations because of their stability of *T* character which is, unfortunately, not found in many of the other seed strains.

DR. MELNICK: I should like to support Dr. Baron's work relating to the use of the stools themselves in laboratory tests, rather than tissue-culture passages, because, as his work has pointed out so well, the viruses may change on passage in the laboratory.

This leads me to the question about other agents which may be present in the intestines of the vaccinated children, and one of these might well be the virus which Dr. Hillemann talked about at the second session, the so-called vacuolating virus. I wonder whether Dr. Baron has looked for this virus in some of the specimens from vaccinated children that he has been working with in the last few months.

DR. BARON: We are in the process of doing those studies now, but the results are not complete.

DR. MELNICK: Are there any preliminary results?

DR. BARON: They are too preliminary to report as yet.

DR. MELNICK: Are they negative?

DR. BARON: They are plus-minus.

DR. SABIN: I should like to point out that in the course of work with excreted attenuated viruses, which goes back many years, all the initial work was always done with the original stools. We went on from work with the original stools to first tissue-culture passages only when it was found that original stools exhibiting no change nevertheless exhibited altered behavior when first tissue-culture passage was used, for the simple reason that larger amounts of virus were needed for the test.

This is particularly true when the intrathalamic inoculation of monkeys is used. With original stools inoculated intrathalamically, with the amounts of virus that are present there, and with the strains most recently used, it has not ever been possible to get anything on intrathalamic inoculation. The reason for going on to first monkey-kidney tissue-culture passage was to obtain the larger quantities of virus in which changes could then be demonstrated.

Testing original stools is, therefore, not new. It was the original procedure used many years ago. There is in the literature very extensive data reported on what has been found, using original stools.

Culture passages, to repeat, are used to test the large populations of virus particles.

DR. BARON: When we come to the problem of getting enough virus for stools to do intrathalamic and intramuscular tests on monkeys, I am reminded of some of Dr. Logrippo's work a number of years back. He described the use of barrels of stools, passaged through resin columns, in order to concentrate virus efficiently in attempts to obtain poliovirus CF antigens.

DR. BODIAN: I think Dr. Sabin satisfactorily explained the reasons for his going to passage material, but I should like to emphasize, from Dr. Baron's very meticulous study, the demonstration that where titers of virus in stools are at a satisfactory level, it becomes important to use Dr. Sabin's original method of using stool suspension rather than subcultured virus.

DR. SABIN: This is never-ending, but I believe that the best thing to do, for anyone who is interested, is to analyze quantitatively the data that are amply reported in the literature. There is quite a difference between using the intraspinal technique, where we know that one can have virus producing paralytic effects in rather small doses, while a million tissue-culture infective doses of the same virus given intrathalamically may be without paralytic effect.

This is not the place to go over work that has been covered in the last five or six years; it is already recorded in literature, and anyone who wants to follow up should cover the literature which is on record.

DR. BODIAN: I should like to remind Dr. Sabin that information on all of the strains which are before us for consideration is not available on this point.

CHAIRMAN ZHDANOV: We shall now proceed with the paper by Dr. Verlinde on "Epidemiological and Virological Survey Following Oral Administration of Live Poliovirus Vaccine."

11. EPIDEMIOLOGICAL AND VIROLOGICAL SURVEY FOLLOWING ORAL ADMINISTRATION OF LIVE POLIOVIRUS VACCINE

J. D. VERLINDE AND J. B. WILTERDINK

Laboratory of Microbiology, Netherlands Institute for Preventive Medicine and State University, Leiden

DR. VERLINDE *(presenting the paper)*: From May 1957, through 1959, four small groups of individuals have been fed all three types of Sabin's live poliovirus vaccine. Details on the development and duration of alimentary infection, the amount of virus excreted, the development of antibody, and the determination of monkey neurovirulence of the excreted viruses have been presented at the preceding Conference.[1]

The epidemiological conditions in The Netherlands during these three years have been extremely favorable for the study of the epidemiological safety of the vaccine. The last extensive epidemic of poliomyelities, which was mainly due to Type 1, occurred in 1956. During the next three years both the incidence of clinical poliomyelitis and the relative incidence of Type 1 virus decreased progressively, and the years 1958 and 1959 showed an exceptionally low incidence of poliomyelitis indeed (Table 1 and Fig. 1).

The population is relatively susceptible, since more than 50 per cent of the children under the age of 13 lack antibody against at least one type.[2]

With a few exceptions, all vaccinated individuals were residents of the city of Leiden or its immediate vicinity, in the province of South-Holland. In this study a comparison is made of the incidence of reported cases of poliomyelitis, the vast majority of them being paralytic, and the incidence of poliovirus infection as determined by virological examination of materials from patients suffering from various illnesses known to be potentially associated with either polioviruses or other enteroviruses in five different regions of the country (Figure 2), i.e.:

Region I: Leiden and vicinity, with a population of 150,000.

Region II: the coastal area from approximately 40 Km. north to approximately 40 Km. south of Leiden, with a population of 1,200,000.

Region III: the rest of the province of South-Holland and the larger part of North-Holland, with a population of 2,500,000.

Region IV: the southern area of The Netherlands, with a population of 2,600,000.

Region V: the rest of The Netherlands, with a population of 4,600,000.

The incidence of clinical poliomyelitis during three-month periods as reported to the Chief Medical Officer of Health is demonstrated in Fig. 3, which shows that the incidence in the post-epidemic year 1957 is still relatively high, whereas that in the next two years is extremely low. In 1957, all regions show the usual seasonal increase during the third three-month period. There is no evidence of an increase of the number of reported cases during and after vaccina-

TABLE 1. POLIOMYELITIS IN THE NETHERLANDS, 1956-1959

	1956	1957	1958	1959
Number of notified cases (paralytic and non-paralytic)	2185	216	37	12
Number of patients examined virologically	3500	1300	1200	700
Number of polioviruses isolated: Type 1	649	40	11	1
Type 2	59	21	2	2
Type 3	10	60	9	—

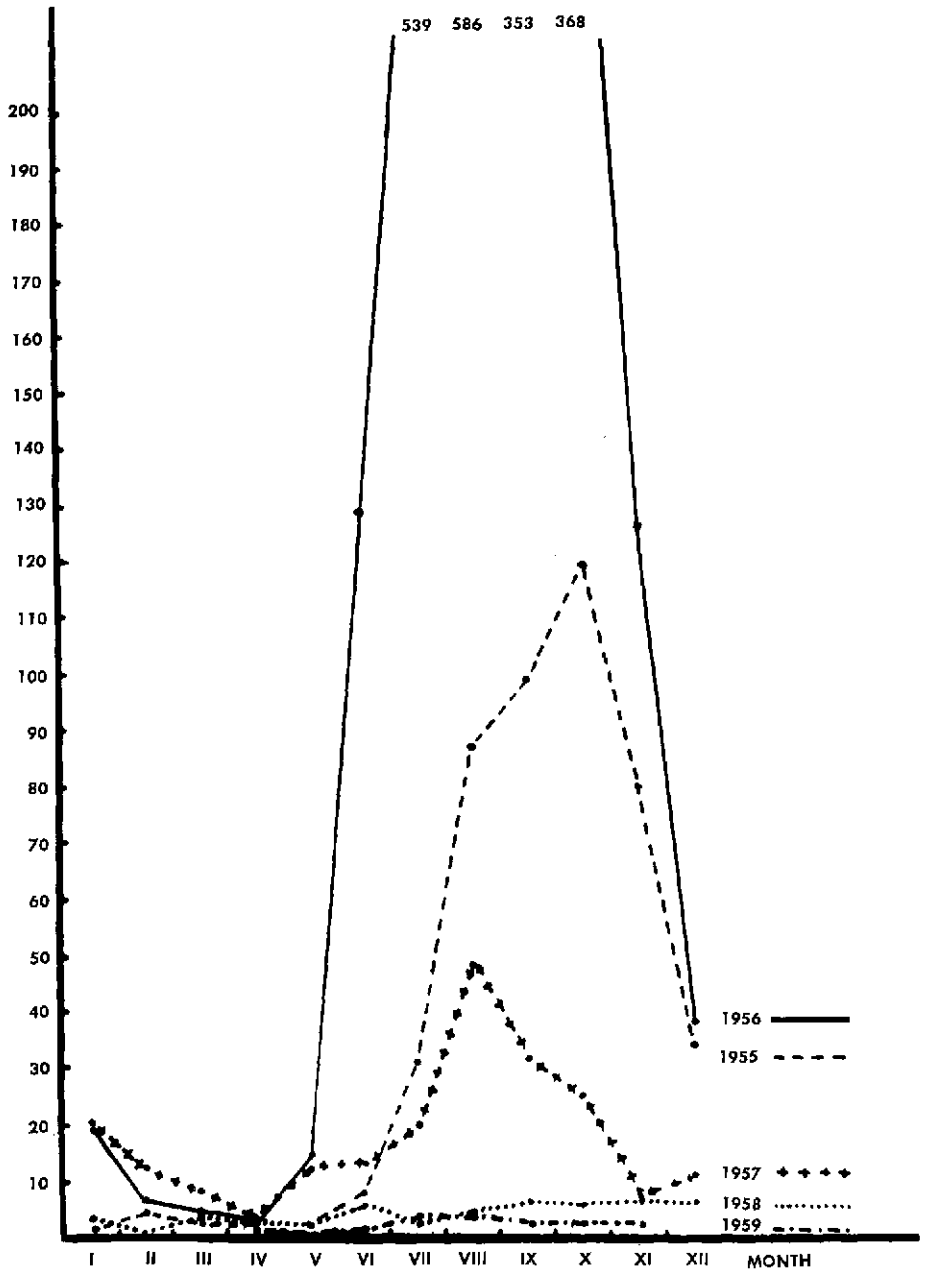


FIG. 1. Number of reported cases of poliomyelitis in The Netherlands, 1955-1959.

tion trials, neither in Region I, nor in the other regions.

This is also evident from the morbidity rates as recorded in Fig. 4, with the exception, however, of the fourth three-month period of 1959 in Region I. During this period, four cases have been reported in Region I, which are reflected in a relatively high morbidity rate in an area with a population of only 150,000. Poliovirus Type 2 has been isolated from two of these cases, but the other two were probably not due to poliovirus infection, since we failed to isolate virus

from several stool samples from both patients and no serological evidence of poliovirus infection was found. Hence, the morbidity rate is probably half of that recorded in Fig. 4.

With regard to the remarks made by Professor Dick[3] at the first Conference, we hesitate to put forward our opinion that the two Type 2 cases are definitely not due to the introduction of the vaccine into the population. The types of vaccine have been administered in the order Type 1—Type 3—Type 2, the Type 2 vaccine having been fed in December. Both cases, however, occurred in October, two months prior to the administration of the Type 2 vaccine!

Hence, all trials, particularly the three made during the years 1958 and 1959 with an exceptionally low incidence of poliomyelitis, clearly show that the introduction of the vaccine into the population has not resulted in the occurrence of clinical poliomyelitis. You will note that there has not been a single case of poliomyelitis in the Leiden region for two years, from September 1957 to October 1959.

The incidence of poliovirus infection in the different regions as determined by virological examination of patients, irrespective of the character of the clinical syndrome, is presented in Fig. 5, which shows that in none of the years in which oral vaccine has been administered there has been a significant increase in the incidence of poliovirus infections in the Leiden region as compared with the other regions. On the contrary,

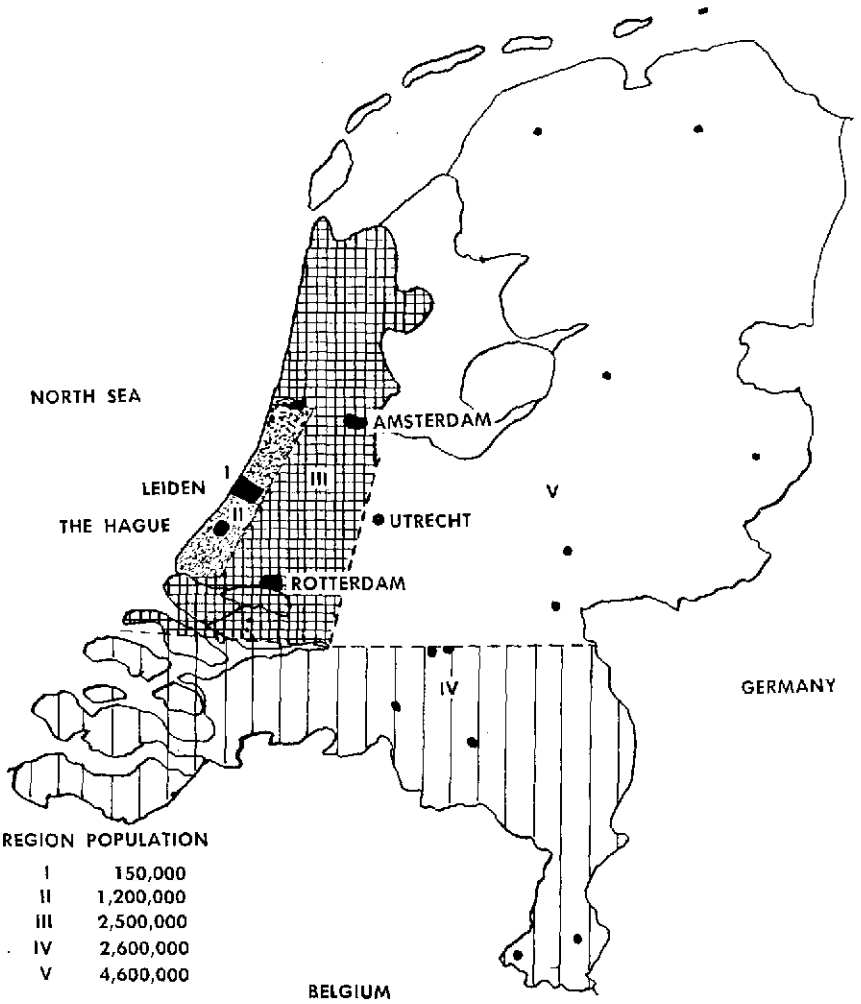


FIG. 2. Map of The Netherlands, with indication of the study areas.

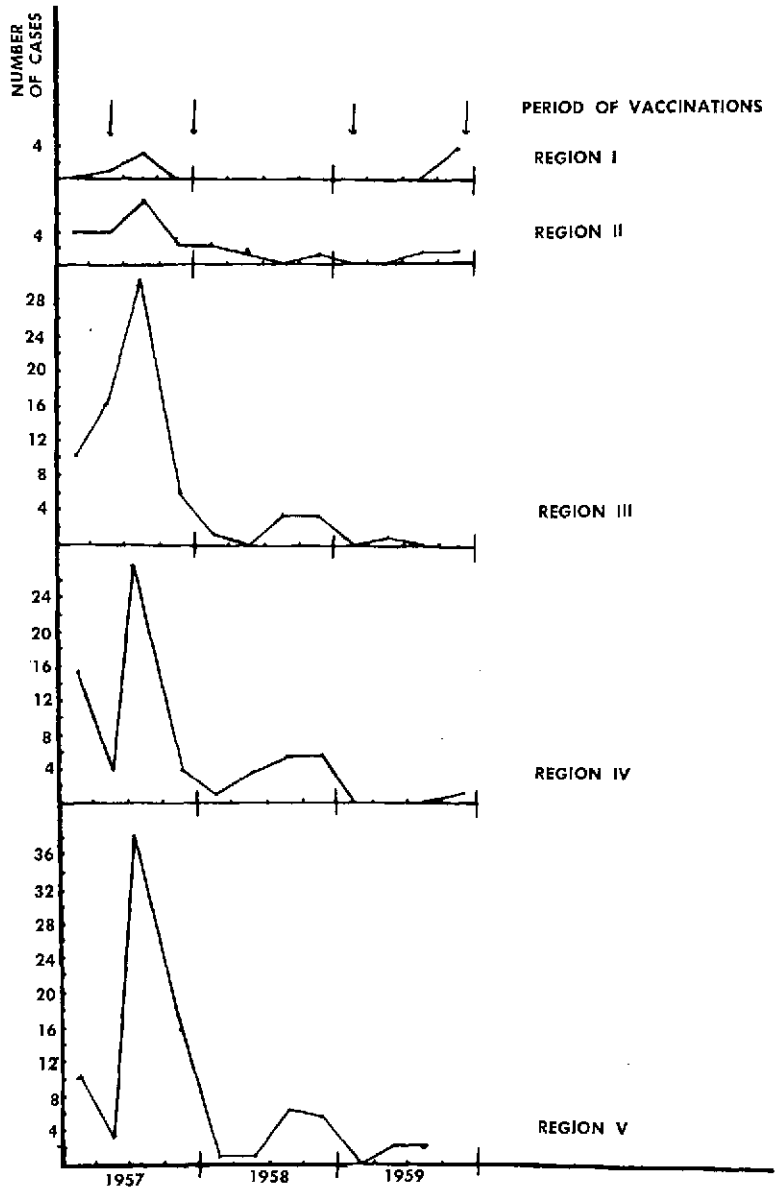


FIG. 3. Number of reported cases of poliomyelitis (irrespective of virological confirmation) during 3-month periods.

the incidence in the Leiden region has usually been lower than elsewhere, with the exception of the two cases of non-paralytic Type 2 poliomyelitis in 1959, which have already been discussed.

Several poliovirus strains isolated from patients and several strains excreted by vaccinated individuals during the same period have been examined for *T* marker and neurovirulence in Cynomolgus monkeys following intracerebral inoculation into the right thalamic region of 1 ml. of fluid of the first or second monkey-kidney culture passage.

The *T* marker has been examined by inoculation of ten-fold dilutions of monkey-kidney culture virus into eight monolayers of trypsinized

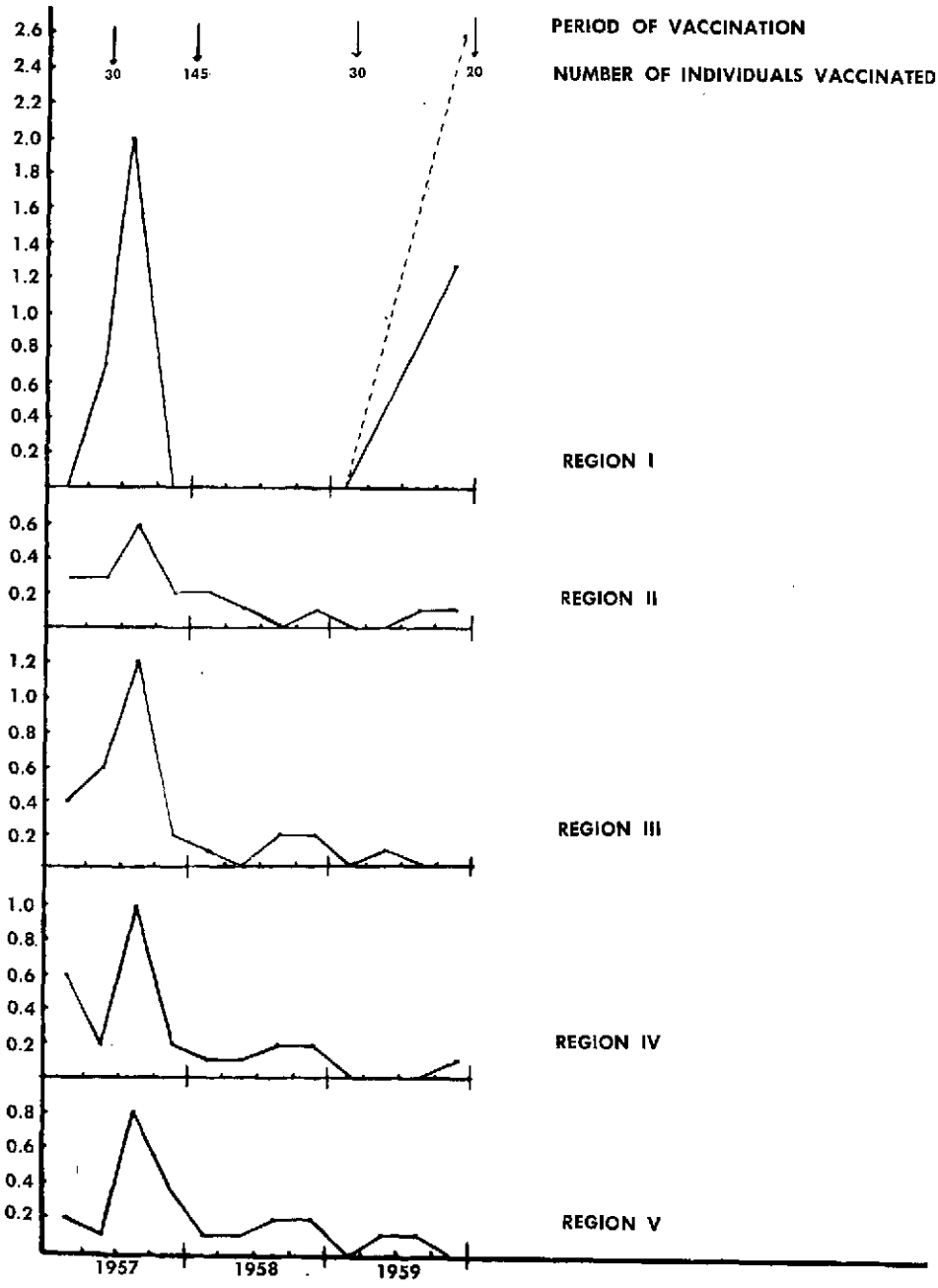


FIG. 4. Morbidity per 100,000 of population of reported cases of poliomyelitis during 3-month periods.

monkey kidney-cells in a medium consisting of Hanks' solution with 0.5 per cent lactalbumin hydrolysate, 5 per cent horse serum, and the usual antibiotics. Four of the monolayers were incubated at 37° C. and four at 40° C. The final reading was made at the eighth day and the titers were calculated according to the method of Reed and Muench. It was found in preliminary titrations that the difference in titer at both temperatures was usually less than two

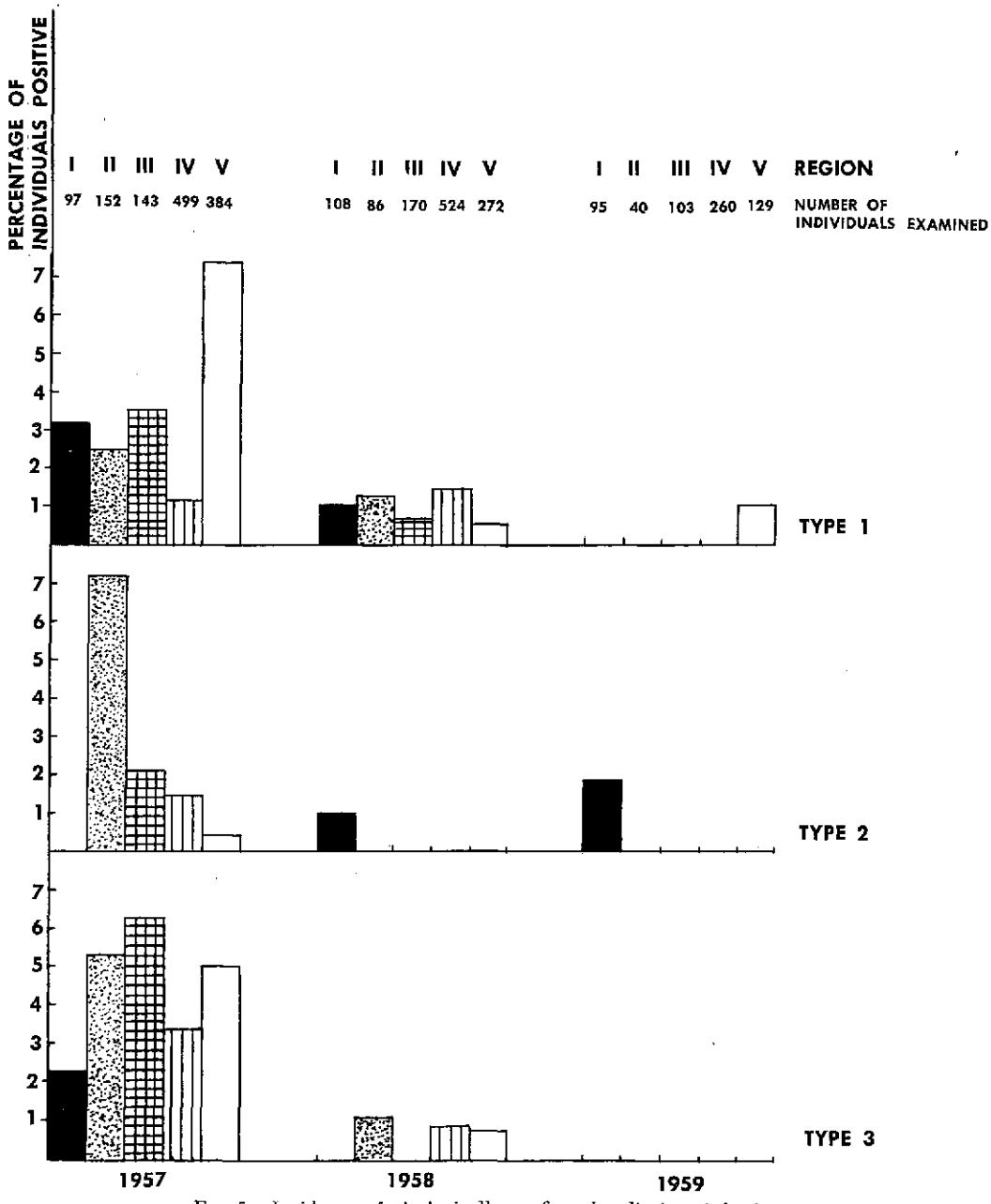


FIG. 5. Incidence of virologically confirmed poliovirus infections.

logs for highly virulent strains and more than four logs for highly attenuated strains, so that, in agreement with Yoshioka, Riordan, and Horstmann[4], the strains were classified as:

T+: difference less than 2 logs.

I: difference 2 to 3.9 logs

T: difference 4 logs or more

Table 2A shows that all Type 1 strains excreted by vaccinated individuals, with the exception of one intermediate strain, possess the *T* character. This character is correlated with absence of neurovirulence following intracerebral inoculation of amounts of virus varying from $10^{5.7}$ to $10^{8.2}$ TCD_{50}, with the exception, however,

TABLE 2A. *T* MARKER AND INTRACEREBRAL NEUROVIRULENCE OF TYPE 1 STRAINS EXCRETED BY VACCINATED INDIVIDUALS

VACCINEE NUMBER	DAY AFTER VACCINE FEEDING	MONKEY NEUROVIRULENCE		DIFFERENCE LOG TITER AT 40° C. AND 37° C.	*T* MARKER
			TCD_{50} LOG 10 INOCULATED		
3	8	−	7.2	5.0	T
14/1	23	−	8.1	5.3	T
14/2	33	−	8.0	6.0	T
22	15	−	7.7	5.3	T
23	6	−	8.2	5.5	T
29/1	9	−	7.6	4.5	T
29/2	20	−	7.0	5.8	T
29/3	22	−	7.1	4.5	T
52/1	4	−	7.7	4.0	T
52/2	19	−	8.0	5.8	T
63	15	−	7.0	6.0	T
73	14	−	8.0	4.0	T
125	16	−	6.2	2.5	I
191	31	−	5.8	4.2	T
200	12	−	6.0	4.8	T
202/1	12	−	5.7	4.0	T
202/2	18	−	6.4	4.3	T
202/3	25	+	6.8	5.0	T

TABLE 2B. *T* MARKER AND INTRACEREBRAL NEUROVIRULENCE OF TYPE 2 STRAINS EXCRETED BY VACCINATED INDIVIDUALS

VACCINEE NUMBER	DAY AFTER VACCINE FEEDING	MONKEY NEUROVIRULENCE		DIFFERENCE LOG TITER AT 40° C. AND 37° C.	*T* MARKER
			TCD_{50} LOG 10 INOCULATED		
23	17	−	8.4	5.2	T
30/1	15	−	7.8	4.0	T
30/2	23	−	8.4	4.7	T
43	5	−	7.0	4.2	T
44	7	−	6.4	3.0	I
73	13	−	6.5	4.0	T
106	6	−	6.3	4.0	T
185/1	7	−	5.7	5.0	T
185/2	21	−	5.2	4.2	T
187	15	−	5.4	4.2	T
188	22	−	4.8	3.5	I
190	7	−	5.0	3.0	I
196/1	5	−	4.0	4.0	T
196/2	14	−	5.7	4.0	T
196/3	19	−	5.3	4.0	T
202	12	−	5.0	2.0	I

TABLE 2C. *T* MARKER AND INTRACEREBRAL NEUROVIRULENCE OF TYPE 3 STRAINS EXCRETED BY VACCINATED INDIVIDUALS

VACCINEE NUMBER	DAY AFTER VACCINE FEEDING	MONKEY NEUROVIRULENCE		DIFFERENCE LOG TITER AT 40° C. AND 37° C.	*T* MARKER
			TCD_{50} LOG 10 INOCULATED		
6	5	−	8.0	3.0	I
10	14	+	8.2	0	T+
14	15	+	7.9	4.2	T
23	8	−	7.6	2.3	I
26	8	−	8.4	4.8	T
28	29	−	8.0	4.4	T
29/1	36	−	7.8	1.0	T+
29/2	44	−	7.2	1.2	T+
114	19	−	7.1	5.5	T
185/1	9	+	6.3	1.0	T+
185/2	23	+	7.0	3.5	I
188/1	9	+	7.2	1.0	T+
188/2	29	+	6.2	1.5	T+
191	13	−	6.2	3.2	I
196/1	12	+	6.3	1.0	T+
196/2	21	−	5.5	1.0	T+
196/3	27	−	6.0	1.0	T+
202/1	12	+	7.4	3.0	I
202/2	19	+	6.5	3.3	I
202/3	26	+	6.7	0.2	T+

of one strain. Three strains excreted by vaccinee 202 possessed the *T* character, which was correlated with absence of neurovirulence in those excreted on the 12th and 18th day, whereas that excreted on the 25th day exhibited intracerebral activity following inoculation of $10^{6.8}$ TCD_{50}.

Twelve of 16 Type 2 strains excreted by vaccinated individuals possessed the *T* character and four were intermediate, as recorded in Table 2B. None of them showed evidence of neurovirulence following intracerebral inoculation of amounts of virus as high as $10^{4.0}$ to $10^{8.4}$ TCD_{50}.

A less regular pattern shows the Type 3 strains (Table 2C). Ten of 20 strains excreted by vaccinated individuals possess the *T*+ character, and six of them exhibited intracerebral activity. Three of six intermediate strains showed evidence of increased neurovirulence. Moreover, one of four *T* strains produced paralysis after intracerebral inoculation of $10^{7.9}$ TCD_{50}.

When summarizing these observations, regardless of the types, intracerebral activity has been shown in 2 of 33 (6 per cent) of the *T* strains, in 3 of 11 (27 per cent) of the intermediate strains and in 6 of 10 (60 per cent) of the *T*+ strains excreted by vaccinated individuals.

In conclusion, the genetic stability of the Type 3 vaccine strain is less than that of Types 1 and 2. Nevertheless, there has been no evidence of any harmful effect either to the vaccinated individuals or to the community in which all three

types have been allowed to spread and to multiply.

REFERENCES

1. Verlinde, J. D. and Wilterdink, J. B.: A small-scale trial on vaccination and revaccination with live attenuated polioviruses in the Netherlands. In: *Live Poliovirus Vaccines, First International Conference.* Scient. Pub. no. 44 of the Pan American Health Organization, pp. 355-366, 1959.
2. Hofman, B. and Wilterdink, J. B.: To be published.
3. Dick, G. W. A. and Dane, D. S.: The evaluation of live poliovirus vaccines. In: *Live Poliovirus Vaccines, First International Conference.* Scient. Pub. no. 44 of the Pan American Health Organization, pp. 6-13, 1959.
4. Yoshioka, I., Riordan, J. T., and Horstmann, D. M.: Thermal sensitivity of polioviruses isolated during oral vaccine field trial: Comparison with monkey neurovirulence. *Proc. Soc. Exp. Biol.* **102**: 342-347, 1959.

DISCUSSION

CHAIRMAN ZHDANOV: This paper is now open for discussion.

DR. SABIN: I wonder if Dr. Verlinde would indicate the number of families in Leiden in which the vaccine strains were fed. I think his data are of importance to supplement other available epidemiological data in which the feeding was done on a much larger scale. I know that he fed only a small number of families in the city of Leiden, and that therefore it represents a situation in which a small number are fed, and a larger number are exposed. This supplements the tests in the Soviet Union last year, where approximately 50 to 60 per cent of the population were fed in some regions, and also more recent studies in other areas where 80 to 90 per cent of susceptible age groups were fed. I wonder if Dr. Verlinde could, for the record, indicate how many families were actually involved in the Leiden feedings over the two-year period.

DR. VERLINDE: Approximately 40 families have received vaccine strains in the Leiden region.

DR. BODIAN: I should like to ask Dr. Verlinde whether he has any measure of the spread of virus from those families into the community, because we have here a very excellent situation for assessing the epidemiological significance, if any, of an increase in neurovirulence. Just as we have data from Professor Smorodintsev's study in regions where there were large numbers of unvaccinated individuals, here we have even a larger group of contacts who may have been exposed to excreted virus. Is there any measure of the spread of the strains?

DR. VERLINDE: Unfortunately we do not have that measure. It appeared to be very difficult to obtain fecal specimens, for instance, from the neighbors of the families, and especially from the school children with whom the vaccinated children had been in contact.

The only measure we have is the virological examination of stools from children who exhibited some kind of illness during the three years. But in the normal population we have, unfortunately, no evidence of spread of the vaccine virus as could have been determined by examination of a large number of stools from healthy contacts.

DR. MELNICK: We are going to hear a number of papers about vaccine effectiveness as the Conference proceeds. In this regard it is of interest to call attention to the record of poliomyelitis that Dr. Verlinde showed us for The Netherlands.

If we look at Table 1 of his report,* we find that the number of polio cases reported for 1956 to 1959 varied from over 2,000 in 1956, to 216 in 1957, then fell sharply to 37 in 1958, and to only 12 in 1959.

Had Dr. Verlinde carried out a mass vaccination trial instead of feeding virus to a few families, we would have here an excellent example of what might well have been considered to be vaccine effectiveness.

DR. VERLINDE: Salk vaccination started in The Netherlands in 1957, and in the subsequent years an increasing number of children has been vaccinated. I do not know exactly up to what age, but I think up to 9 or 10 years. This year children up to 14 or 15 years of age will be vaccinated.

During the first two years, 1957 and 1958, only children of one and two, perhaps three, years of age have been vaccinated.

CHAIRMAN ZHDANOV: If there are no further comments, we shall proceed with the next paper on the "Spread of a Vaccine Strain of Poliovirus in Southern Louisiana Communities." The presentation will be made by Dr. Gelfand.

* See p. 134.

12. SPREAD OF A VACCINE STRAIN OF POLIOVIRUS IN SOUTHERN LOUISIANA COMMUNITIES*

JOHN P. FOX, DOROTHY R. LEBLANC, HENRY M. GELFAND, DOROTHY J. CLEMMER, AND LOUIS POTASH

Division of Epidemiology, Department of Tropical Medicine and Public Health, Tulane University School of Medicine, New Orleans, Louisiana, and the Division of Epidemiology, Public Health Research Institute of the City of New York, Inc.

DR. GELFAND *(presenting the paper)*: With any new vaccine, safety and effectiveness with respect to the immediate recipients, are of paramount concern. In the case of live poliovirus vaccines, their demonstrated spread to unvaccinated persons is the source of an additional important concern. Such ability to spread may be considered to enhance materially the risk of vaccination, if the observed tendency for the vaccine strains to reacquire neurovirulence during human passage is judged to be significant for man. Alternatively, to the extent that contact infections safely augment the immunity of the population, spread may prove to be a beneficial phenomenon. In either event, the circumstances which favor or impede spread and the nature of contact-acquired infections are worthy of study.

A year ago at the First International Conference on Live Poliovirus Vaccines, we reported on the spread of the Sabin vaccine strains within individual Louisiana households.[1] Our observations clearly indicated that most extensive spread was related to poor environmental hygiene and to the use of the Type 3 vaccine strain. They also suggested that pharyngeal excretion of virus, which commonly follows administration of maximal doses of vaccine, may favor virus spread, although very considerable spread was observed in the well documented absence of pharyngeal excretion. We also reported that, unlike primary infections, infections resulting from contact often were abortive with but one or two days of fecal virus excretion. We can now add that such abortive infections rarely induced immune response. An additional factor greatly favoring virus spread, the very young age of the vaccinee (under 18 months), first became evident in Gard's Swedish household study[2] and was again noted in the New Jersey study of Plotkin *et al.*[3]

Overall, perhaps the most significant fact to emerge from studies so far reported, and particularly well demonstrated in our own household study, is the relatively limited capacity of the vaccine strains to spread, as compared with that of the obviously more infectious wild poliovirus strains. Episodes of infection with vaccine viruses in our study households involved only 8 per cent of the susceptible contacts in upper economic families and 51 per cent in the lower economic groups; whereas, during 1953-1957, in the total group of households from which were derived our vaccine virus study families, episodes of wild poliovirus infection involved 86 and 93 per cent, respectively, of upper and lower economic susceptible children.[1-4]

The study of interhousehold or community spread under widely divergent conditions was also reported a year ago. In an Arizona Indian community characterized by very primitive sanitation, Sabin's Type 1 strain failed to spread, probably because of the combination of minimal seeding of the community, a highly immune population, and an abundant flora of other enteroviruses.[7] Within a highly susceptible group of married student families in Minnesota, extensive sequential introduction of all three Lederle strains was followed by a mean spread to only 3 per cent of adults and 7 per cent of children.[8] Finally, the wintertime introduction of the Lederle Type 1 strain into an isolated island community in Finland, by feeding about 20 per cent of the population was followed by more extensive spread, 34 per cent overall, and 45 per cent of the nonimmunes.[9]

* Aided by grants from The National Foundation and the Division of Research Grants, National Institutes of Health, U. S. Public Health Service.

Our own exploration of interhousehold spread began in relation to the previous household studies when we demonstrated spread following deliberately arranged, brief play exposure of children. This led us to the study herein reported which was designed specifically to explore the dissemination of vaccine virus under optimal conditions in the unmanipulated community. Although preliminary results have been reported elsewhere,[10] substantial additional information is now available.

METHODS AND MATERIALS

General plan of the study. Several small, well-defined, lower economic neighborhood communities in the Evangeline area were chosen as candidates for study on the basis of our knowledge of their physical and social characteristics and our estimates as to probable sero-immunity patterns as derived from previous investigations.[4-6] Within each community approximately 25 households were recruited on a voluntary basis, and from these were obtained pertinent data, including a Salk vaccination history (later confirmed through official health records), and blood specimens from each member. Review of the vaccination history and the results of testing the sera for neutralizing antibody permitted us to select two communities containing a high proportion of children susceptible to Type 3 poliovirus.

Within the chosen communities the participating families were paired, one to receive vaccine and the other an identical appearing placebo, only on the basis of the number of probably susceptible children under 15 years of age and address to insure that the vaccinated families were widely scattered. Feeding was begun early in June, just after school was dismissed. All children in a household received the same material, vaccine or placebo, the specific nature of which was not divulged until the end of the observation period.

Base-line blood and stool specimens were obtained from each child on or just before the day of feeding. During the next 12 weeks, with occasional exceptions, additional fecal specimens were contributed twice weekly. These were collected during weekly visits made by the nurse epidemiologist (D.R.L.) to obtain information as to illness and significant social activities of the children. At the end of August, terminal bloods were collected and, as a promised reward, all children were fed trivalent vaccine.

Vaccine strains and administration. Because of the susceptibility status of the chosen communities, only the Type 3 (Leon $12a_1b$) strain was used. This was provided by Dr. Sabin from the large pool that had fulfilled his rigid criteria for purity and attenuation.[11] The stock vaccine was distributed in amounts appropriate to the needs of the individual families, the family-identified containers then being held at —20° C. until just before use. Disposable medicine droppers were employed to squirt 1 ml. amounts (containing 7.3 logs of virus) into the oral pharynx of each child. Placebo salt solution with phenol red was packaged and handled in the same manner.

Laboratory procedures. The laboratory methods employed were standard and have been described in detail elsewhere.[6,12] Monolayer cultures of monkey-kidney cells were employed throughout and visual detection of cytopathic effect was used as the index for virus isolation and identification and for serum neutralization. Serum antibody titers are expressed as 50 per cent end-point values in terms of the final dilution of serum and were based on the use of two cultures per two-fold serum dilution mixed with aliquots of virus calculated to contain approximately 100 $TCID_{50}$ per culture inoculum.

Characteristics of the study communities. The two communities chosen were small, distinct neighborhoods located in the town of Morgan City and Franklin, Louisiana. Their physical extent and the distribution of the study households are shown in Figures 1 and 2. Although not isolated from their respective towns, each constituted a relatively homogeneous play community. Special features propitious to virus spread include low economic level and associated poor environmental sanitation with many functioning outdoor privies, poor personal hygiene as indicated by the usual unwashed state of the children, large families and intense social intermingling. The pertinent population data are presented in Table 1, which indicates, in particular, the relative overall abundance of the children and the sizeable proportions of the child population seeded by vaccine feeding (17 per cent in Morgan City and 43 per cent in Franklin) or designated

by placebo feeding to serve as indices of virus spread (21 and 39 per cent, respectively).

Analysis of the data. The histories of Salk vaccination were verified from official local health records and can be considered to be quite accurate. However, since the participating households had not been under observation prior to vaccination, certainty as to individual natural immunity status often was not possible. Rather than make arbitrary judgments in the individual case, the children have been assigned to two groups according to their prefeeding titer of neutralizing antibody, one with no antibody or titers below 1:32 (presumed to include chiefly the natural susceptibles) and the other with titers of 1:32 or higher (presumed to include most of those naturally immune).

RESULTS

Although this study was in no sense a safety trial, it may be said at the outset that no significant illnesses possibly attributable to the vaccine virus were observed. The basic data relate to extent of infection with the vaccine strain, the

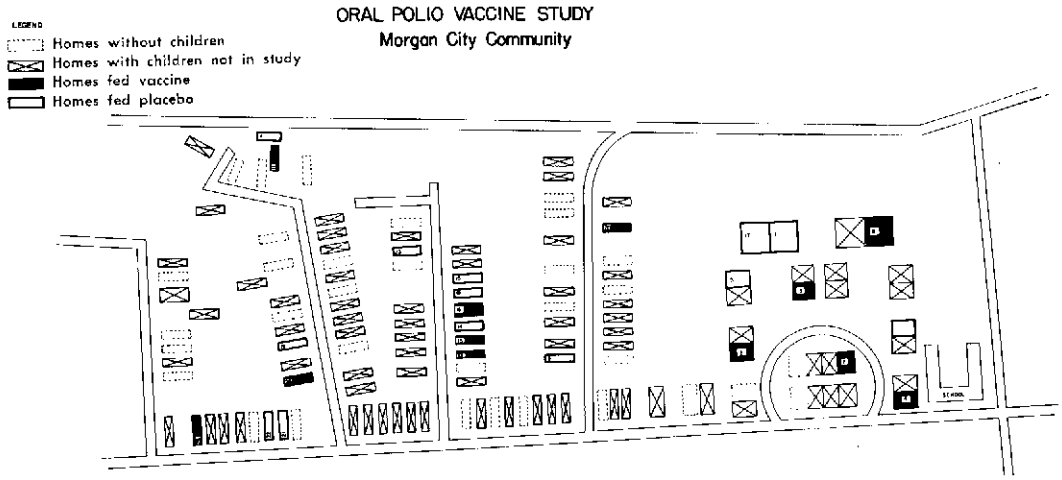


FIG. 1. Map of Morgan City Community showing location of all dwellings and indicating the participating vaccine and placebo-fed households.

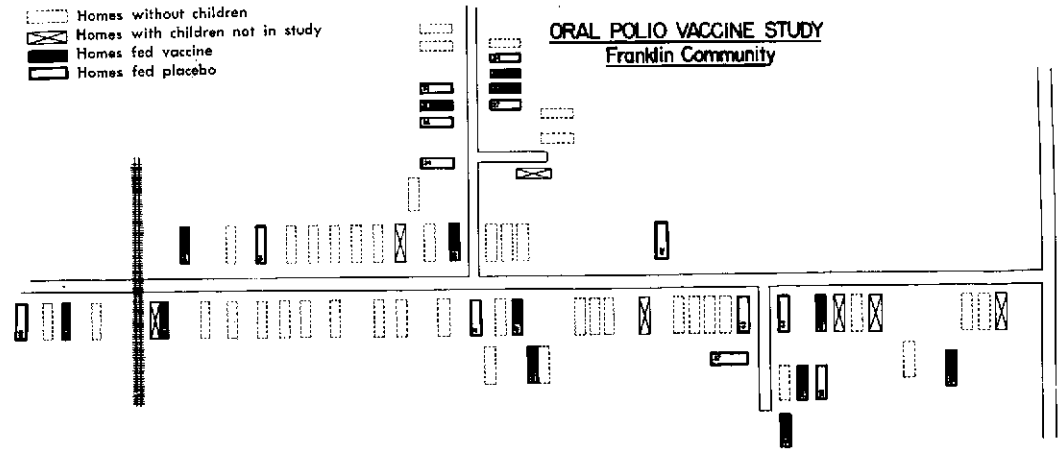


FIG. 2. Map of Franklin Community showing location of all dwellings and indicating the participating vaccine and placebo-fed households.

TABLE 1. POPULATION DATA FOR THE STUDY COMMUNITIES IN MORGAN CITY AND FRANKLIN, LOUISIANA

NEIGHBORHOOD	GROUP	NUMBER OF HOUSEHOLDS	CHILDREN	
			NUMBER	% OF TOTAL
In Morgan City	Total	133	332	100
	placebo	13	69	21
	vaccine	12	55	17
In Franklin	Total	75	130	100
	placebo	13	51	39
	vaccine	13	56	43

pattern of spread, the response to infection and the occurrence and possible influence on spread of concurrent wild enterovirus infections.

Extent of infection and homologous antibody response. The infection and sero-response experience of the vaccine-fed children are summarized in Table 2. Of 55 children in the low titer group, 53 (96 per cent) developed immediate alimentary infections. The two exceptions were children in the Franklin community who were excreting Coxsackie B2 virus when the vaccine was administered, and both of these later became infected with P3 virus during a delayed, recurrent household episode. Significant antibody response, defined as four-fold or greater increase in titer, followed immediate infection in all but four instances in which the sera remained without antibody in 1:8 final dilution despite demonstrated fecal virus excretion, abortive in one child, but persisting for 12 to 20 days in the other three. A rather different pattern resulted in the high-titer group. While immediate alimentary infection was demonstrated in 38 (68 per cent) of the 56 children, only seven of these evinced significant antibody response.

In Table 3 are shown the infection and response data for the placebo-fed children. In both communities, contact infections were more fre-

TABLE 2. FECAL EXCRETION OF TYPE 3 POLIOVIRUS AND ANTIBODY RESPONSE IN VACCINE-FED CHILDREN, BY AGE, PRIOR HOMOLOGOUS ANTIBODY TITERS AND RESIDENCE

Neighborhood	Age (years)	Prior titer 1:<32			Prior titer 1:32+		
		No. fed	No. excreting	No. response	No. fed	No. excreting	No. response
In Morgan City	0-5	24	24	24	4	4	1
	6-9	3	3	2	10	5	0
	10-15	4	4	3	10	9	2
	all	31	31	29	24	18	3
In Franklin	0-5	16	14	14	8	4	1
	6-9	6	6	5	12	9	3
	10-15	2	2	1	12	7	0
	all	24	22	20	32	20	4
Both	all	55	53	49	56	38	7

quent in children below 6 years of age and in the low-titer group. Overall, spread was clearly more extensive in the more heavily seeded Franklin community (70 per cent of low and 32 per cent of high-titer children) than in Morgan City (33 and 25 per cent, respectively). All told, 28 low-titer children developed alimentary infection and, of these, all but nine manifested significant antibody response. In the high-titer group, 17 were observed to excrete virus, but only four evinced antibody response.

Wide variations were observed in duration of fecal virus excretion. In the vaccine-fed children, the mean observed excretion was 22 and 9.7 days for the low and high-titer groups, and the range was, respectively, from 1 to 63 days and from 1 to 31 days. Among the placebo-fed children, the corresponding figures were 17 and 6.8 days for the means, and from 1 to 37 days and from 1 to 34 days for the ranges. For the vaccine-fed children, Fig. 3 indicates the relation of the post-feeding antibody titer to that observed prefeeding and also, on a rough qualitative basis, to the fact and duration of fecal excretion of virus. Of those actually observed to shed virus in the feces, abortive excretion for only one or two days, indicated by the circle with a central dot, was observed in 5 of 53 low and 14 of 40 high-titer children. Similar data for the placebo-fed children are presented in Fig. 4 which indicates the much greater frequency with which contact transmission resulted in abortive infection. Such was observed in 7 of 28 infected low-titer children and in 9 of 17 in the high-titer group. Of special importance is the fact that these abortive infections were commonly associated with failure to develop a significant increase in antibody titer. Finally, in agreement with our previous observations, well established alimentary infection also failed to elicit a significant response in children with very high initial antibody titers.

The pattern of vaccine virus spread. It is difficult to generalize about the pattern of spread within the placebo-fed household units. In one Franklin and four Morgan City families, the entire household episode of P3 virus infection consisted of fecal excretion by only one child detected on only one day (2 in high and 3 in low-titer children), despite the availability of from 5 to 8 other children in each family, including a total of 11 with low and 19 with high titers. At the other extreme, all or all but one child became infected in six families, three in each community. When more than one child became infected, the interval between onsets of viral excretion varied widely; several children might begin excretion within a few days with others beginning excretion two or more weeks later. In one household, in which all of six children became infected, the onsets of excretion were on days 28, 28, 29, 46, 60, and 60 of observation.

What might be termed the week-by-week volume

TABLE 3. FECAL EXCRETION OF TYPE 3 POLIOVIRUS AND ANTIBODY RESPONSE IN PLACEBO-FED CHILDREN, BY AGE, PRIOR HOMOLOGOUS ANTIBODY TITER AND RESIDENCE

Neighborhood	Age (years)	Prior titer 1:<32			Prior titer 1:32+		
		No. fed	No. excreting	No. response	No. fed	No. excreting	No. response
In Morgan City	0-5	23	9	7	7	2	0
	6-9	6	1	0	15	4	1
	10-15	7	2	1	10	2	0
	all	36	12	8	32	8	1
In Franklin	0-5	18	15	11	9	4	2
	6-9	2	0	0	11	4	1
	10-15	3	1	0	8	1	0
	all	23	16	11	28	9	3
Both	all	59	28	19	60	17	4

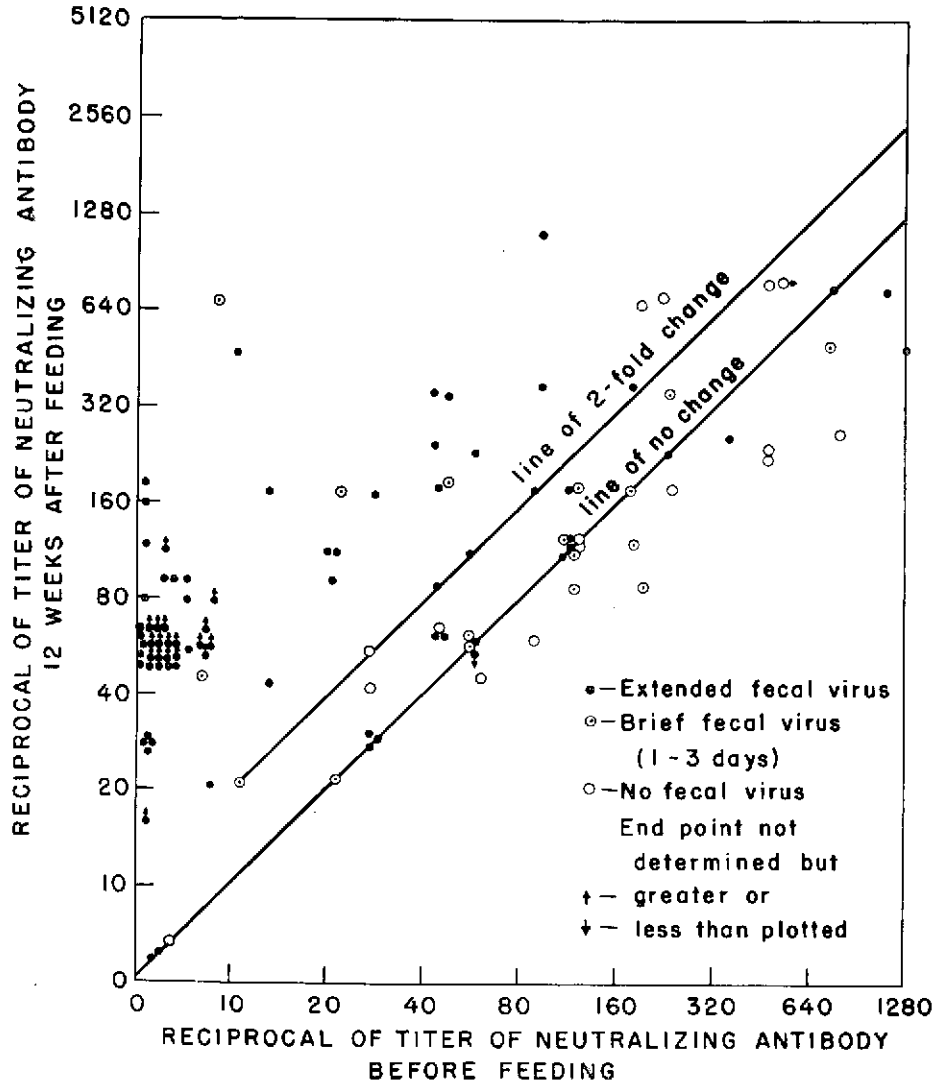


FIG. 3. Titers of homologous neutralizing antibody at the beginning and end of observation in vaccine-fed children in Morgan City and Franklin.

of excretion in the Morgan City neighborhood is indicated in the upper half of Fig. 5 which shows how many children were found excreting virus in each week of observation. Excretion in the vaccine-fed children was maximal immediately after feeding, and declined abruptly after the third week. Overall, it was more frequent and persisted longer in the low-titer group. Among the placebo-fed children, the peak frequency of virus shedding was in weeks 4 and 5 and again the overall frequency was greater in the low-titer group. In the lower half of the figure, comparable data are presented for Franklin. These differ from those for Morgan City chiefly in that infection began earlier in the placebo-fed children with the peak of shedding in the third week in the low-titer group.

The pattern of viral spread among Morgan City families is indicated in Table 4 in which episodes of infection with P3 or other enterovirus in each household are indicated by week of onset. Understandably, P3 virus appeared immedi-

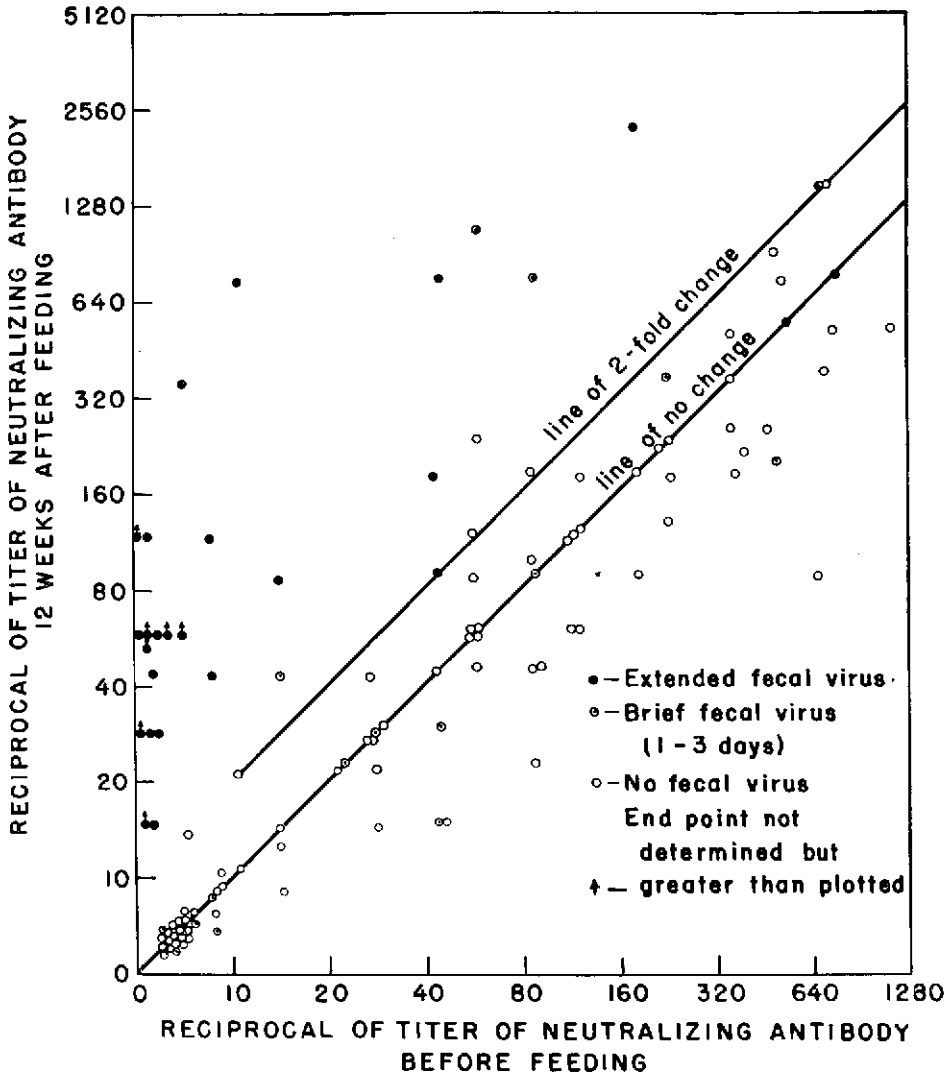


FIG. 4. Titers of homologous neutralizing antibody at the beginning and end of observation in placebo-fed children in Morgan City and Franklin.

ately after feeding in all of the vaccine-fed families. In the placebo-fed group, a total of eight episodes of P3 infection were observed with three in the second, two each in the third and fourth, and one in the fifth week. In the more heavily seeded Franklin community (Table 5) spread to households was no more extensive, but it began earlier and continued longer with five episodes in the first, two in the third, and one in the sixth week. A recurrence of P3 spread, possibly representing the invasion of a wild strain, began in the eighth week in vaccine-fed household 230, reinvaded placebo household 232 in the ninth week, and reached the heretofore uninfected household 237 in the tenth week. Both reinvaded families had been free of P3 virus for at least four weeks, and in both one or two previously infected members were reinfected.

The occurrence of wild enterovirus infections. In Tables 4 and 5 are recorded an impressive total of 86 episodes of wild enterovirus infection which were detected during the 12 weeks of com-

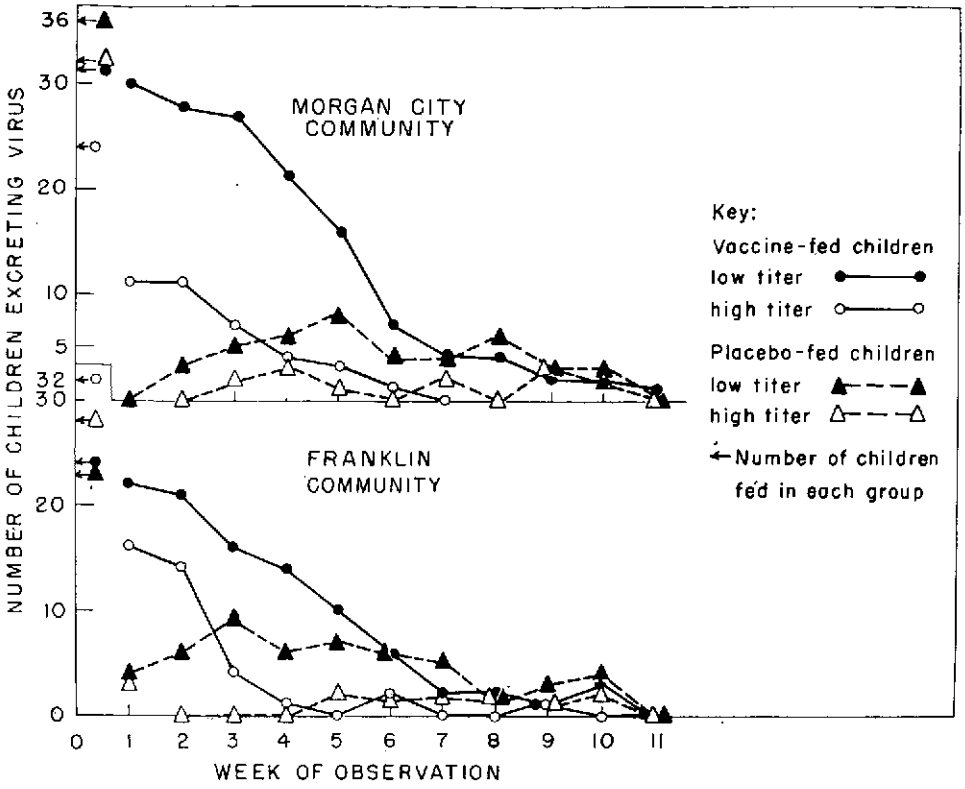


FIG. 5. Vaccine virus excretion by week of observation.

munity observation. Since, in at least two studies, pre-existing infection with such viruses has been observed to block infection with orally administered vaccine strains,[13,14] one might anticipate that this abundant occurrence of wild enteroviruses would provide additional examples of interference. Although numerous children in Morgan City and Franklin households were undergoing infection with P1, B2, or B3 viruses when they were fed 7.3 logs of P3 vaccine virus, only two in the low-titer group, both with B2 infections, failed to develop immediate alimentary infection with P3 virus.

Examination of Tables 4 and 5 provides no evidence that other enterovirus infection interfered with spread of the vaccine strain. Not only did several families escape invasion during the period of most active vaccine spread despite freedom from other infection but, in seven instances, a household was invaded in the same week by both the vaccine strain and a wild virus. Further, in Franklin households 227 and 232, the vaccine strain entered during the first observation week and displaced a pre-existing infection with B2 virus.

In Table 6 the episodes of wild enterovirus infection are summarized for the vaccine and placebo families in each community. Of some interest is the fact that the viral flora of these neighboring communities differed significantly. Coxsackie B3, E14, and E19 were virtually restricted to Morgan City as were B5 and E7 to Franklin. Of perhaps greater interest is an observation suggesting that administration of the vaccine virus may have provided brief protection against infection with wild viruses. During the first two weeks after administration of the vaccine, the vaccine-fed households experienced but a single wild virus episode, whereas seven were detected in the placebo-fed families.

TABLE 4. WEEK OF ONSET OF ENTEROVIRUS INFECTION IN MORGAN CITY FAMILIES

Vaccine fed	Household number	Virus first excreted in indicated week											
		-1	1	2	3	4	5	6	7	8	9	10	11
Placebo	201			Ad	P3,B3								
	205				P1	P3		B3					
	206	E10				P3							
	210			P3,Ad			P1	B3			B2		
	214				B3		P3		E19				
	215					B3		B2	E14				
	217			UN					B3				
	218					P3,B3				B2			
	219	B2									E14	B3	
	221							B3					
	222	B2									E14		
	223			P3,B2								B3	
	224			P3					B2				Ad2,B5
Type 3 virus	202		P3			B3							
	203		P3										
	204		P3							P1		B2	
	207		P3								B3		
	208		P3		B2				B3				UN
	211		P3		Ad2			P1		B2			
	212		P3								B2		
	213	P1	P3,B2		B3			E19					
	216		P3					B2,E19					
	220		P3							E19			Ad1
	225		P3						UN				
	226		P3								E7		UN

P = poliovirus; E = ECHO virus; B = Coxsackie group B vrius; Ad = adenovirus; UN = unidentified virus; types designated by numerals, e.g. P1 = poliovirus Type 1.

DISCUSSION AND SUMMARY

The most infective available vaccine strain of poliovirus, Sabin's Type 3, was widely introduced into two small, cohesive communities under conditions considered optimal for its spread. These conditions included, in particular, a high proportion of susceptible young children among an abundant child population, intense interhousehold social contact, very poor environmental and personal hygiene, and heavy seeding of the child population with large doses of the vaccine virus to insure maximal pharyngeal excretion at a time when wild strains normally begin active spread. Despite these conditions, the results observed lend further support to the conclusion suggested by previous observations, including our own study of intrahousehold spread, that the potential of the vaccine strains for spread is relatively limited as compared to that of wild poliovirus strains. Active spread was observed only in the first two or three weeks after vaccination, and it essentially terminated with 51 per cent of the low and 72 per cent of the high-titer children (62 per cent overall) still untouched. These figures take into account infections related to the late recurrence of P3 virus in Franklin which may have represented the entrance of a wild strain. The two important implications of this restricted ability to spread are: (1) that concern as to reversion of vaccine strains to significant virulence may be rather academic; and (2) that spread cannot be relied upon to immunize susceptibles missed during vaccination programs. This latter implication is reinforced by the observation that infections resulting through contact, possibly because of transmission of a minimal infecting dose, are frequently abortive and fail to induce immune response.

The study also provides additional information regarding factors that may influence virus spread.

TABLE 5. WEEK OF ONSET OF ENTEROVIRUS INFECTION IN FRANKLIN FAMILIES

Vaccine fed	Household number	Virus first excreted in indicated week											
		-1	1	2	3	4	5	6	7	8	9	10	11
Placebo	227	B2	P3										
	229		P3,B2										
	232	B2	P3						UN		(P3)		
	234	B2											
	236						UN						
	237							B5				(P3)	
	238						B5					P1	
	241		P3,Ad						E7				
	242		B2		P3								
	243				P3			B5					
	244									E14			
	249	B2						P3,E7					
	251		P3						B2				
Type 3 virus	228	B3	P3							UN	P1		
	230	B2	P3							(P3)			P1
	231	B2	P3									UN	
	233		P3										
	239		P3			B5							
	240		P3		B5								
	245	B2	P3				UN						
	246		P3										
	247		P3						E7				
	248		P3					B2	E7				
	250	B2	P3					E7					
	252		P3						E7				
	254		P3						E7				

P = poliovirus; E = ECHO virus; B = Coxsackie group B virus; Ad = adenovirus; UN = unidentified virus; types designated by numerals, e.g. P1 = poliovirus Type 1.

TABLE 6. SUMMARY OF WILD ENTEROVIRUS INFECTIONS OBSERVED IN MORGAN CITY AND FRANKLIN COMMUNITIES BY TIME OF INITIATION AFTER INTRODUCTION OF SABIN TYPE 3 VACCINE POLIOVIRUS

Community	Wild entero-virus	Number of household episodes of wild enterovirus infection initiated in indicated weeks of observation												
		Placebo-fed families						Vaccine-fed families						All
		-1	1-2	3-5	6-8	9-11	Any	-1	1-2	3-5	6-8	9-11	Any	Any
Morgan City	P1			2			2	1			2		3	5
	B2	2	1*		3	1	7		1	1	3	1	6	13
	B3			4	4	2	10			2	1	2	5	15
	B5					1	1						0	1
	E7						0					1	1	1
	E14				1	2	3						0	3
	E19				1		1				2		2	3
	other*	1	3			1	5			1	1	3	5	10
	All	3	4	6	9	7	29	1	1	4	9	7	22	51
Franklin	P1					1	1					2	2	3
	B2	4	2		1		7	4			1		5	12
	B3						0	1					1	1
	B5			1	2		3			2			2	5
	E7				2		2				5		5	7
	E14				1		1						0	1
	E19						0						0	0
	other*		1	1	1		3			1	1	1	3	6
	All	4	3	2	7	1	17	5	0	3	7	3	18	35
Both	All	7	7	8	16	8	46	6	1	7	16	10	40	86

* Other includes adenoviruses, untyped enteroviruses, and one isolation of E10.

Not too surprisingly, the proportion of unvaccinated who became infected by contact was directly related to the proportion of the population vaccinated. The more extensive spread observed in the more heavily seeded Franklin community also testifies to the relative lack of influence on spread of prior Salk vaccination since the proportion of children so vaccinated in Franklin (54 per cent with three or four shots and 79 per cent with at least one) was much greater than in Morgan City (15 and 40 per cent, respectively). Finally, although the abundant occurrence of wild enteroviruses in the two communities confirmed our judgment that conditions for enterovirus spread were very favorable, we were unable to show that such wild infections interfered with vaccine virus spread. Rather, the reverse may have been true since administration of the vaccine seems to have afforded transient protection against wild enterovirus infection.

REFERENCES

1. Gelfand, H. M., Potash, L., LeBlanc, D. R., and Fox, J. P.: Revised preliminary report on the Louisiana observations of the natural spread within families of living vaccine strains of poliovirus. In: *Live Poliovirus Vaccines, First International Conference.* Scient. Pub. no. 44 of the Pan American Health Organization, pp. 203-217, 1959.
2. Gard, S., Böttiger, M., and Lagercrantz, R.: Vaccination with attenuated poliovirus Type 1, the CHAT strain. In: *Live Poliovirus Vaccines, First International Conference.* Scient. Pub. no. 44 of the Pan American Health Organization, pp. 350-354, 1959.
3. Plotkin, S. A., Koprowski, H., Richardson, S. N., and Stokes, Jr., J.: Vaccination of families against poliomyelitis by feeding and by contact spread of living attenuated virus: including studies of virus properties after human intestinal passage. *Acta paediat.* In press.
4. Fox, J. P., Gelfand, H. M., LeBlanc, D. R., and Conwell, D. P.: Studies on the development of natural immunity to poliomyelitis in Louisiana. I. Overall plan, methods and observations as to patterns of immunity in the study group. *Am. J. Hyg.* **65:** 344-366, 1957.
5. Gelfand, H. M., LeBlanc, D. R., Fox, J. P., and Conwell, D. P.: Studies on the development of natural immunity to poliomyelitis in Louisiana. II. Description and analysis of episodes of infection observed in study group households. *Am. J. Hyg.* **65:** 367-385, 1957.
6. ———, ———, ———, and Potash, L.: Studies on the development of natural immunity to poliomyelitis in Louisiana. IV. Natural infections with polioviruses following immunization with a formalin-inactivated vaccine. *Am. J. Hyg.* **70:** 312-327, 1959.
7. Paul, J. R., Horstmann, D. M., Riordan, J. T., Niederman, J. C., and Yoshioka, I.: The use of Sabin's attenuated Type 1 poliovirus vaccine in different environments, and newer techniques for testing the virulence of recovered strains. In: *Live Poliovirus Vaccines, First International Conference.* Scient. Pub. no. 44 of the Pan American Health Organization, pp. 218-225, 1959.
8. Barr, R. N., Bauer, H., Kleinman, H., Johnson, E. A., Martins da Silva, M., Kimball, A. C., and Cooney, M. K.: The use of orally administered live attenuated polioviruses as a vaccine in a community setting: a controlled study. In: *Live Poliovirus Vaccines, First International Conference.* Scient. Pub. no. 44 of the Pan American Health Organization, pp. 369-407, 1959.
9. Oker-Blom, N., Strandström, H., and Eriksson, A. W.: A small-scale trial with live poliovirus vaccine in an isolated island community. In: *Live Poliovirus Vaccines, First International Conference.* Scient. Pub. no. 44 of the Pan American Health Organization, pp. 580-587, 1959.
10. Gelfand, H. M., LeBlanc, D. R., Potash, L., Clemmer, D. I., and Fox, J. P.: The spread of living attenuated strains of polioviruses in two communities in Southern Louisiana. *Am. J. Pub. Health,* 1960. In press.
11. Sabin, A. B.: Properties of attenuated polioviruses and their behavior in human beings. New York Academy of Sciences, Monograph no. 5, pp. 113-127, 1957.
12. Gelfand, H. M., Potash, L., LeBlanc, D. R., and Fox, J. P.: Intrafamilial and interfamilial spread of living vaccine strains of

polioviruses, *J. Am. M. Assn.* **170:** 2039-2048, 1959.

13. Benyesh-Melnick, M., Melnick, J. L., and Ramos Alvarez, M.: Poliomyelitis infection rate among Mexican children fed attenuated poliovirus vaccines. In: *Live Poliovirus Vaccines, First International Conference.* Scient. Pub. no. 44 of the Pan American Health Organization, pp. 272-285, 1959.

14. Fodor, A. R., Schaeffer, M., and Hall, A.: Inhibiting effect of wild Type 2 poliovirus during immunization with attenuated live virus vaccine. *Bacteriological Proceedings,* Society of American Bacteriologists. (May 1960). In press.

DISCUSSION

CHAIRMAN ZHDANOV: The paper presented by Dr. Gelfand is open for discussion.

DR. PAUL: This very fine paper by Dr. Fox and Dr. Gelfand brings up some points that hardly need discussion. However, as there will be other studies of this kind done in various types of communities, perhaps the point that Dr. Cabasso mentioned earlier deserves re-emphasis, namely, that there should be a certain degree of standardization if we are going to compare results of one study with another. For instance, I note that in Dr. Fox's study, individuals with neutralizing antibody levels of less than 1:32 were considered susceptibles. This is not exactly the level that some others have used.

Also, to be considered is the optimal time for measuring the antibody level after monovalent or trivalent poliovaccine has been given, because the antibody level will go up slighly in the population as time goes on, and in some cases it will also go down.

DR. GELFAND: The use of 1:32 as a break-off point in our study was chosen because this population had received a variable number of Salk vaccine inoculations, and we had to choose some arbitrary point to make a presumption of natural susceptibility, or natural immunity. Based on a mean sort of response to Salk vaccine, this seemed to be a reasonable break-off point. This was not chosen with any biologic knowledge of what is a proper place to decide immunity or lack of it, but because some children had none, some had up to four Salk shots.

DR. DICK: I should like to discuss Dr. Gelfand's observation that infections resulting through contact are frequently abortive, probably because of transmission of a minimum effective dose. I think it is important to remember that this refers to Sabin's Type 3 virus.

With Type 2 virus, the position seems to be quite different. We have been titrating in babies a fecal filtrate from a child fed with Sabin Type 2 virus. The amount of virus excreted after a feeding of a fecal filtrate of 10 TCD_{50} is in the region of 6 or 7 logs per gram of stool, which can go on over a period of up to at least 21 days.

Now, of course, there are two differences here: first, the type of virus, and second, the age of the children whom we studied, who were between 6 and 16 months.

DR. HORSTMANN: In support of Dr. Gelfand's comment that dosage is probably important in determining the character of contact infection with the Sabin Type 3 strain, I might mention experiments with this strain in a different type of population, that is, in an institutionalized population of retarded individuals, an environment in which fecal contamination was very great. Here, the contact infection rate was 100 per cent and the contacts in this situation, presumably having been exposed to large doses, when infected excreted virus over a long period of time, in the same manner as did the vaccinees themselves.

So, using the same strain under different epidemiological circumstances, in which maximum dosage was acquired by the contacts, there was no difference between the duration of virus excretion by vaccinees or contacts.

DR. MELNICK: Dr. Gelfand has commented on the lack of interference between the natural enteroviruses and the Type 3 vaccine which was fed. It should be pointed out that he used a dose about 100 times that used in previous studies, in which interference had been found. Dr. Gelfand also told me about some observations in families, which indicated that interference might well have played a role in their study. I wonder if he would care to comment on this at this time?

DR. GELFAND: Dr. Melnick's question is somewhat embarrassing. As frequently happens, I am sure, collaborators can sometimes disagree slightly in interpretation. I was reading the manuscript prepared by Dr. Fox and we disagree on some minor points, one of which is the possible role of interference in our study.

One of the points I made from the text was that the lack of interference was manifested in one way by the simultaneous entrance into a household of the vaccine strain and a wild virus strain. Dr. Fox and I have spoken about this, and in each instance, of course, the vaccine virus and the wild virus invaded a different individual in the same household.

Without more detailed serologic information on these people, I believe that this frequent happening may actually be a circumstantial indication of interference, because there was in these instances often no continued intrafamilial dissemination of either the vaccine strain or the wild virus. Therefore, this might be interpreted as the manifestation of interference, rather than of the absence of interference.

We have a number of household examples in which the children who are actually excreting the wild virus at the time of vaccine administration did not become infected, whereas other children in the household did become infected. To give an example, in one vaccine-fed household consisting of seven children, whose ages and immunity status to polio 3 are shown below, stools were collected on the day before vaccine feeding, and five of them were found to be excreting Cocksackie B^2 as follows:

Age of Child	P3 Immunity	Virus Isolations on Indicated Day			
		0	4	8	12
12	I	0	0	0	0
11	S	B^2	0	3	3
9	S	B^2	3	3	3
6	S	B^2	B^2	B^2	0
5	S	B^2	B^2	B^2	0
4	S	B^2	B^2	0	3
3	S	0	3	3	3

The serial records of virus isolations following feeding of vaccine on the fourth day, the eighth day, and the twelfth day following vaccine feeding are briefly summarized.

Unfortunately, we do not have Coxsackie B^2 serology, so it is difficult to know what the immune status of these individuals was to Coxsackie B^2. I do not know, for example, whether the youngest child had not yet had an opportunity to become infected with Coxsackie B^2, or whether the child had naturally terminated his Coxsackie infection. At any rate, this child immediately became infected with the vaccine virus. Some of the others gradually terminated their B^2 virus and became infected. Two of them never became infected with poliovirus Type 3. We have a number of such family episodes that suggest to me the role of interference.

Dr. Gear: I should like to ask Dr. Gelfand whether these enterovirus infections caused any clinical manifestations, and if so, whether these in any way embarrassed him or those responsible for the feeding?

Dr. Gelfand: There were no symptoms whatsoever from any of the children in the study which could be possibly related to enterovirus infection during the entire three months. We were fortunate.

Dr. Bodian: This admirable study reported by Dr. Gelfand raises interesting questions. I should like to comment on two.

First of all, I recall that a few weeks ago Dr. Sabin took me severely to task for suggesting that placebo studies were feasible in relation to the evaluation of live virus vaccine.

It seems to me that the results of this study suggest that this possibility should be examined again. Certainly, the evidence of spread is not such as to suggest that spread alone would eliminate any possibility of carrying on a controlled evaluation of the effectiveness of live poliovirus vaccines.

I wish to point out that either placebo studies or matched control studies have the further advantage that one is able to control the laboratory methods and the results of laboratory methods. Therefore, I merely wish to put in my bid again for consideration of the continued use of this type of study wherever possible.

The second point I should like to refer to is this: In the studies just reported, we have rates of reinfection with the Type 3 virus, due either to the vaccine virus or to wild viruses, which I calculated very roughly to be about 14 per cent.

This is reminiscent of the animal work that was being done in the late 1940's at Michigan

and Yale Universities and at Johns Hopkins. In chimpanzees, the reinfection rates we obtained were approximately 10 or 12 per cent, several months after the primary infection with same type.

We therefore have some evidence here, and I am sure there will be more, that the susceptibility to reinfection is not an insignificant factor in connection with immunization with live polioviruses. How this affects the concept of eradication of wild polioviruses remains to be seen. It is interesting to note how closely the results reported by Dr. Gelfand compare with the results obtained in chimpanzees, both in Dr. Melnick's and Dr. Horstmann's studies and in our studies in Baltimore.

DR. SMADEL: Before the discussion on Dr. Gelfand's excellent paper terminates, I should like to draw attention to the information he presented on abortive infection and the failure of individuals with such infections to develop an immunological response. This could be discussed at great length, and I see no point in going into it now. However, I present the matter for consideration in order that this not be accepted without further thought.

Frankly, I find it very difficult to believe that an appreciable amount of multiplication of virus can occur in tissue without having some sort of immunological response on the part of the host. In the absence of such a response one would expect to have a continuing infection, such as sometimes occurs in children who, following inoculation with smallpox vaccine, fail to develop antibodies and continue to have lesions.

Before we accept the idea that no antibody response occurred in certain of these individuals who excreted virus for an appreciable length of time, we must be careful to define what we mean by an antibody response. Moreover, if we find no evidence of antibody response, using our presently employed methods, we must explore various other techniques, including the most delicate available before we say that the immunologic mechanism failed to be stimulated. If failure does indeed occur, then why does the vaccinee cease to excrete virus?

DR. LANGMUIR: I am taken by the comments in Dr. Gelfand's paper where he points out the marked difference in spread by social class, that is, 8 per cent of the susceptible contacts in the upper economic group, and 51 per cent in the lower economic group.*

This is exceedingly reminiscent of the poliomyelitis pattern as it is occurring in this country now, where we have very limited spread of polio in our upper economic groups, but sharp and severe epidemics in our lower groups.

While I admit that it is highly controversial, I am slowly coming to the conclusion that there is a very marked difference in the rate of spread of polioviruses in this country, by social economic group, and I am forced to conclude that this is being influenced by the status of Salk vaccine.

I therefore should like to suggest the possibility that, perhaps in this study, the very low level of spread, i.e., 8 per cent in the upper economic group, may be influenced by a Salk vaccination in this group in Louisiana. I also wonder if we could have more information as to the distribution of the Salk vaccine by economic groups in the Louisiana study.

DR. GELFAND: The percentages referred to by Dr. Langmuir were derived from the study of intrahousehold virus dissemination, presented last year.

In the study carried out last year, we ourselves vaccinated equally both the upper and lower economic households. Therefore, the difference in spread by economic status could not be related to Salk vaccination, since all of those individuals had been Salk-vaccinated three times, by us, and with the same material.

DR. BODIAN: I am surprised that Dr. Langmuir has proposed a new interpretation of a well-known phenomenon, because I am sure he is well aware of the fact that there is also a quite different interpretation of this difference in spread.

The evidence for changing age selection in poliomyelitis, which has been discussed in the last decade or two, and its relationship to economic status, is indicative of factors which play a role without the effects of formalized vaccine immunization. It is conceivable that formalized vaccine immunization could reinforce the differ-

* See p. 144.

ence in spread related to economic status, and its related ecologic concomitants, such as crowding and family size.

DR. GEAR: One brief comment on this question. Similar change in incidence of poliomeylitis in the different economic groups has been noticed recently in communities in South Africa, in the absence of Salk vaccination.

DR. SABIN: I should like to comment on the subject of abortive infections, particularly in the light of the comments made by Dr. Dick and Dr. Smadel.

In selecting the arbitrary titer of 32, which was necessitated by the circumstance of not knowing the status of natural immunity before Salk vaccine, we must realize that there were some naturally immune persons among those with the low titers, as well as some without naturally acquired immunity among those with the high titers; because some children do develop higher titers than 32 after three or four doses of Salk vaccine.

Accordingly, some of the abortive infections could easily be accounted for on the basis of reinfection of previously naturally immune persons.

Secondly, the failure of antibody response in some of these abortive infections might be accounted for on the basis of a high level of pre-existing antibody. In our own studies of reinfection with Type 3 virus, we found that this particular type produced reinfection in 50 per cent of naturally immune adults and it may be higher in children. We found that if the pre-existing antibody titers were not very high, then even an abortive infection of one or two days' duration gave rise to an increase in antibody. But if the antibody levels were high to begin with, excretion of virus for a short period of time did not further boost the antibody level.

I should like to ask Dr. Gelfand whether there were any children without any demonstrable antibody prior to oral vaccination, in whom an abortive infection occurred, and in whom there was no antibody response.

I should also like to comment on the remarks made by Dr. Dick. He worked with my Type 2 virus strain, and my own titrations with this Type 2 virus showed that it was capable of initiating infection with very small doses of virus, which was not true of the Type 1 and Type 3 strains that I selected.

With Type 1, I found that even a dose of 10,000 TCD_{50} given to young adults produced infection irregularly and when it occurred it was associated with virus excretion for only about seven to 10 days; antibody could be demonstrated at four weeks, but not at six or eight weeks.

This finding adds emphasis to the point raised by Dr. Paul, that is, that you cannot examine antibody just at one time after infection and have a complete picture.

We also have evidence that some people can have infection for three to four weeks with Type 3 virus and when the antibody is determined at a given point, six or eight weeks later, they have no demonstrable antibody by methods we now have available; however, when tested for resistance of the intestinal tract two years later, demonstrated a very marked resistance.

Would Dr. Gelfand please answer the question I posed to him?

DR. GELFAND: The answer to the first question is very simple. We did have individuals who had no antibody demonstrable by our technique, who experienced abortive infections of one, two, three, four, or five days, and who had no antibody when it was tested three months later.

DR. SABIN: By the tube test?

DR. GELFAND: Yes.

DR. SABIN: I interposed this, because as our published data show, with this Type 3 strain we have had individuals who have had even longer periods of multiplication, three to four weeks, and yet by the tube neutralization test had no demonstrable antibody and by the pH test showed titers of 32 and 64.

So this is another thing we must keep in mind in interpreting Dr. Gelfand's data.

DR. GELFAND: I was reminded also that I had not answered Dr. Paul's earlier question about the proper time to do antibody studies following vaccination. I must say that the purpose of our study was not to acquire information about serologic response and we made no effort, as we probably should have, to collect a series of blood

specimens. We were using serology only for the possibility of detecting infections by serologic conversions that we had missed by excretion studies.

Our interest was primarily on the spread phenomenon, and therefore we were interested mainly in the fecal excretion of virus. Therefore, I readily admit we were using very crude measures of antibody response.

Our own previous serologic studies are in agreement with the comments that have been made. We have seen low level responses that were very transient in nature. Even if the blood had been collected only a month later, these would have been missed. Therefore, our data really did not contribute very much on serologic response and were not presented for that purpose.

DR. MELNICK: The need for a more sensitive antibody test has been mentioned. We do have such a test, and it was described yesterday, when I mentioned the antigenic differentiation test which we use. Serum at varying dilutions is incorporated in the agar over monolayer cell sheets, and the virus challenge added as a disc on top of the agar.

By this test the National Foundation sera, which may have a titer of 1 to 1,000 or so in the standard CPE neutralization test, yield titers of over 1 to 300,000.

I do not recommend this test for ordinary use, because it requires the bottle or plate technique, but for very special purposes where one is looking for a sensitive measure of antibodies, I think it might be useful.

CHAIRMAN ZHDANOV: The next paper will be presented by Dr. Kimball, on "Minnesota Studies with Oral Poliomyelitis Vaccine. Community Spread of Orally Administered Attenuated Poliovirus Vaccine Strains." It will be followed by Dr. Paul's presentation on "The Capacity of Live Attenuated Poliovirus to Spread within Families."

13. MINNESOTA STUDIES WITH ORAL POLIOMYELITIS VACCINE. COMMUNITY SPREAD OF ORALLY ADMINISTERED ATTENUATED POLIOVIRUS VACCINE STRAINS*

ANNE C. KIMBALL, PH.D., ROBERT N. BARR, M.D., HENRY BAUER, PH. D., HERMAN KLEINMAN, M.D., EUGENE A. JOHNSON, PH.D., AND MARION COONEY, M.S.†

DR. KIMBALL *(presenting the paper):* In the 1958 Minnesota study of orally administered attenuated poliovirus vaccine[1] the feeding of virus to only half of the participating families during the control period provided an opportunity to study the spread of these strains of poliovirus within the community to the participants who received placebos. Prior to this study, intrafamily[2,3,4] and intra-institutional[5] spread of attenuated polio vaccine viruses had been reported. This study presents quantitative data in regard to the community spread of vaccine strains of poliovirus.

The poliovirus vaccine strains used in this study were developed by Cox. No harmful effects were attributed to the use of these strains in the 1958 Minnesota study nor in later studies conducted in Minnesota, Latin America and elsewhere. Natural human passage might be expected to produce changes in any property of these vaccine strains and intensive study of these passage strains is indicated. One crucial test for change is available from the medical history of the individuals receiving these strains by natural spread; a review of the detailed medical records of these individuals is presented. If after several natural passages in humans the strains do not show a persistent and progressive increase in virulence, the spread of these vaccine strains could have definite value; not only would some immunity be acquired by individuals not fed the vaccine strains but also the intermittent presence of these strains in a community could provide booster stimulation to previously vaccinated individuals. The magnitude of interfamily or community spread of these strains can indicate the extent of added benefit that can be anticipated.

MATERIALS AND METHODS

Participants were married University of Minnesota students and their children who lived in a crowded university housing development in Minneapolis, known as Como Village.

Design of the study, the vaccine strains used and the characteristics of the study population have been described.[1] All village residents (371 families) were invited to participate, and 149 families (40 per cent) volunteered. Thus members of 20 per cent of the households were fed vaccine (group B) and specimens were available from an additional 20 per cent of the households for the measure of community spread of the vaccine strains. The division of the participants into group A (74 families) and group B (75 families) was on a random basis. During the study period the identity of these groups was known only to one member of the team (E.A.J.) who made the random division and distributed the vaccine and placebo capsules into envelopes labeled for each family. The identity of the blood serum and stool specimens were not known to the laboratory personnel at the time of testing.

Three stool specimens were available from the control group (A) for isolation of each of the three respective poliovirus types. As can be observed in Table 1, showing the chronology and sequence of the feeding and collection of specimens, each participant submitted a total of six stool specimens. The first stool specimens were collected prior to the feeding of vaccine to either group. The second, third, and fourth

* This is one of a series of papers. Aided by grants from the Sister Elizabeth Kenny Foundation, Minneapolis, Minnesota and Lederle Laboratories, Division of the American Cyanamid Company, Pearl River, New York.

† Dr. Kimball, Dr. Barr, Dr. Bauer, Dr. Kleinman and Miss Cooney (Minnesota Department of Health); Dr. Johnson (School of Public Health, University of Minnesota).

stool specimens were collected after Type 2 had been fed to group B and before it was fed to group A. The third, fourth, and fifth stool specimens were collected after Type 1 had been fed to group B, and before it was fed to group A. The fourth, fifth, and sixth specimens were collected after Type 3 had been fed to group B, and before it was fed to group A. The community spread of each type of poliovirus was thus measured for a period of about eight weeks.

The collection of stool specimens was scheduled for about 14 days after the feeding of virus. The time elapsed between feeding and the collection of the stool specimens was calculated from the Wednesday of the week of feeding. The median day of receipt of the second stool specimens was 14 days, the third stool specimen 13 days, and the fourth stool specimen, 14 days after each immediately preceding virus feeding. More than 93 per cent of these three stool specimens were received within three days prior to, and six days following, the median day of receipt for each specimen. It will be noted in Table 1 that there was some overlap between the days for stool collection and the subsequent feeding days; however, no family was fed the vaccine (or placebo) until the expected stool specimens were submitted. The stool specimens were, in effect, the "ticket" for the receipt of the vaccine or placebo capsules. A total of only 15 stool specimens were missing; 10 of 1728 were missing from the 288 adults, four of 1224 were missing from the 204 children, and one of 318 were missing from the 53 infants. Only one specimen (from an infant) was unsatisfactory for virus isolation due to bacterial contamination which was not controlled by antibiotics.

Specimens were stored frozen (—20° C.) prior to processing. For isolation, 20 per cent emulsions of stool specimens were prepared in distilled water by shaking for five minutes, centrifugation at 1500 rpm. (I.E.C. No. 2) and 30 minutes at 13,000 rpm. (Servall). A portion of the supernatant was diluted 1-2 with double strength Hanks' balanced salt solution containing 600 units of penicillin and 600 micrograms of streptomycin per ml. Supernates of processed stool specimens were stored frozen and were thawed prior to inoculation when 0.1 ml. of each processed stool specimen was inoculated into each of two HeLa cell TC tubes. The procedure for preparation of the HeLa cell TC tubes has previously been published.[1] Inoculated TC tubes were examined after one, three, five, and seven days of incubation at 37° C. When characteristic cytopathogenicity was observed after at least

TABLE 1. CHRONOLOGY OF SPECIMEN COLLECTION AND VACCINE FEEDING MINNESOTA STUDY WITH ORAL POLIOMYELITIS VACCINE—1958

	GROUP A			GROUP B		
DATES	BLEEDINGS	STOOL SPECIMENS	FEEDINGS	BLEEDINGS	STOOL SPECIMENS	FEEDINGS
1–27, 2–1	1st			1st		
1–28, 2–6		1st			1st	
2–3, 2–8			Placebo			Type 2
2–16, 2–24		2nd			2nd	
2–24, 3–1			Placebo			Type 1
3–9, 3–19		3rd			3rd	
3–17, 3–22			Placebo			Type 3
3–30, 4–8		4th			4th	
3–31, 4–5	2nd			2nd		
4–7, 4–12			Type 2			Placebo *
4–20, 4–28		5th			5th	
4–25, 5–1			Type 1			Placebo
5–11, 5–20		6th			6th	
5–15, 5–21			Type 3			Placebo
6–2, 6–7	3rd			3rd		

* The time prior to the feeding of Type 2 vaccine to group A is the control period.

one transfer the isolated virus was typed in the conventional manner. Cytopathogenic agents other than poliovirus were isolated from 16 specimens; these proved to be adenovirus, Type 1 or 2. Selected stool specimens (143) from which poliovirus had not been isolated on HeLa TC were inoculated into monkey kidney TC tubes; no strains of ECHO virus were isolated.

Stool specimens from control group (A) individuals, from which Types 1, 2 or 3 poliovirus had been isolated prior to the feeding of each respective type of those individuals, have been sent to Dr. Herald R. Cox and co-workers for study of neurovirulence in monkeys. The results will be published later.

Evidence of community spread of these vaccine strains to the control group (A) was also shown by a significant rise (2 tube, 16-fold) in antibody titer observed in the blood specimen collected at the end of the control period as compared to the pre-feeding blood specimen. As is shown in Table 1, Type 3 virus was fed to group B on March 17 to 22, and the post-control-period blood specimen was collected on March 31 to April 5. The time elapsed was comparatively much shorter than was available between feeding of Types 2 and 1 virus and the collection of the post-control-period blood specimen. For this reason virus spread (as measured by antibody response in group A) was not as fully measured for Type 3 as for Types 2 and 1.

It is also possible that the full capacity for Type 3 to spread to group A participants was not fully measured or was reduced by the feeding of Type 2 and Type 1 vaccines to this group during the time period when the spread of Type 3 was potential (see Table 1). The spread of Type 3 vaccine strains may have been reduced by the feeding of Type 2 and Type 1 vaccines which interfered with the spread of Type 3 or the spread of Type 3 may have occurred but was not measured if the fed vaccine strain replaced the naturally acquired Type 3 virus. The full capacity for Type 1 to spread may also have been reduced by the feeding of Type 2 to group A.

Criteria used in evaluating the illnesses observed in group A (placebo fed) individuals who acquired the vaccine strains by natural passage are presented with the results. The medical follow-up was continuous and included numerous house visits during the study period.[4]

Climate in Minneapolis is the humid continental type and in general there exists a tendency to extremes in all climatic features. During the period of the study, the monthly temperatures (maximum, minimum, and mean) were slightly warmer than average except for February which was slightly colder than normal. Extremes of temperature during the study varied from —15° F. on February 16 to 88° F. on May 29. The climate during the study period was unusually dry with normal precipitation occurring only in April.[6]

RESULTS

The polioviruses isolated from stool specimens of Group B provide a rough measure of the source of supply of virus available for spread to the control group (A). The percentage of isolations effected from each of the six stool specimen sets received from the group B individuals (vaccine fed during the control period) is shown separately for 147 adults, 109 children and 23 infants in Fig. 1. Poliovirus was not isolated from stool specimens collected prior to vaccine feeding. The second stool specimen, submitted approximately two weeks after Type 2 virus had been fed, yielded Type 2 virus in 8.8 per cent of the adults, 38.5 per cent of the children, and 60.9 per cent of the infants. The third stool specimen, submitted after the feeding of Type 1 virus, yielded Type 1 virus in 12.9 per cent of the adults, 61.5 per cent of the children, and 87.0 per cent of infants. Thus Type 1 virus appears to have replaced Type 2 in the intestinal tract of many of the recipients. However, five children were still excreting Type 2 virus in the third stool specimen. The fourth stool specimen, submitted after the feeding of Type 3, yielded Type 3 virus in 12.2 per cent of the adults, 58.7 per cent of the children, and 65.2 per cent of the infants. Type 3 virus appears to have replaced Type 1 in the intestinal tract of many of the recipients, but two adults, one child, and one infant were still excreting Type 1 virus in the fourth stool specimen. Type 3 virus was isolated from a total of 60 of the fifth stool specimens and 31 of the sixth stool specimens from group B individuals. The excretion of Type 3 virus continued longest and was thus the largest source of virus "supply"

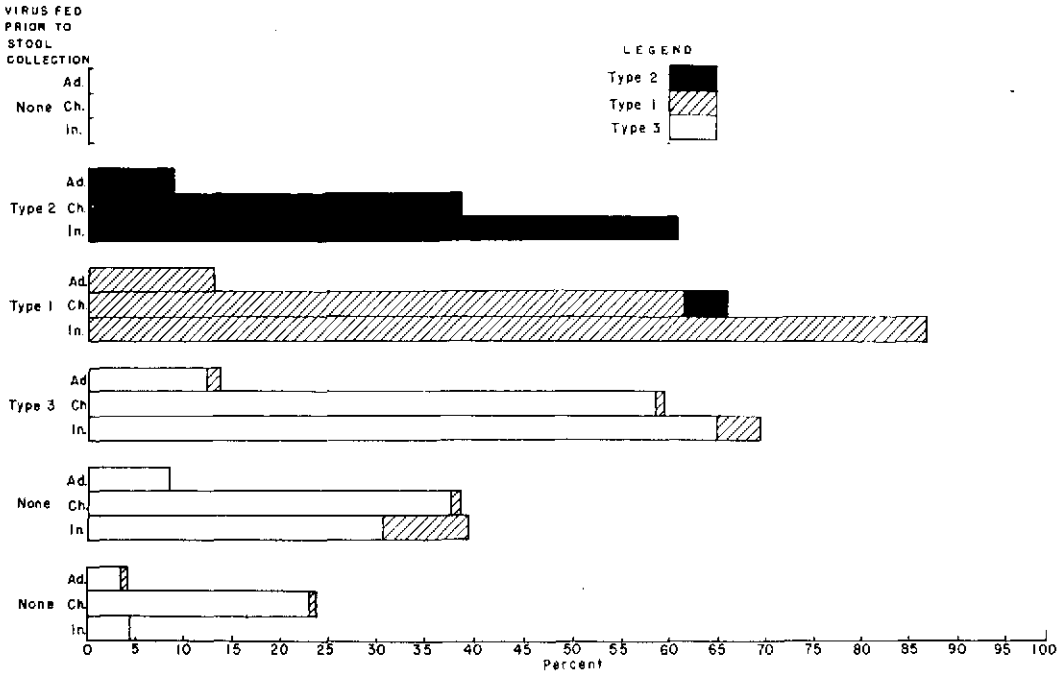


FIG. 1. Percentages of poliovirus isolated from six stool specimens collected from group B individuals (vaccine group).

Ad.—Specimens from 147 adults.
Ch.—Specimens from 109 children.
In.—Specimens from 23 infants.
Only 3 stool specimens were missing, no more than 1 from any group.

for spread to the control group as compared to the "supply" available for Types 2 and 1.

The percentage of isolations accomplished from each of the six stool specimens received from the group A individuals (placebo-fed during the control period) is shown separately for 141 adults, 95 children and 30 infants in Fig. 2. Isolations due to community spread are shown in bar-graph form. Isolations of Type 2 and 1 from specimens submitted *after* these types of poliovirus had been fed to group A are shown at the right of the bar-graph as percentages only. The total of 45 isolations from group A participants, shown in the bar-graph, Fig. 2, were from specimens submitted by 29 individuals.

There is a progressive increase in the number of isolations from the first to the fifth stool specimen, and then a decrease in the number of isolations in the sixth.

Poliovirus Type 2 was isolated from four participants (two children, two adults) in three group A families; the identity of these individuals, the chronological data of the stool specimens, and the antibody titers vs. Type 2 poliovirus are shown in Table 2. The stool specimens were collected 12 to 15 days after the group B participants were fed Type 2 virus. Two of the four persons were children in the same family (4-3 and 4-4) and neither child had antibodies to Type 2 virus detectable in the pre-feeding blood specimen but both showed a significant antibody response in the second (post-control) blood specimen. Both adults had antibody titers to Type 2 in the pre-study blood specimen and neither showed an increase in titer in the post-control blood specimen. Both adults had received Salk vaccine.

In addition to the four people who picked up Type 2 virus there were five other members of these three families. Four of the five additional persons had Type 2 antibodies. The fifth person, a child of 105-2, had no Type 2 antibodies detectable in serum dilution 1:4. There was no evi-

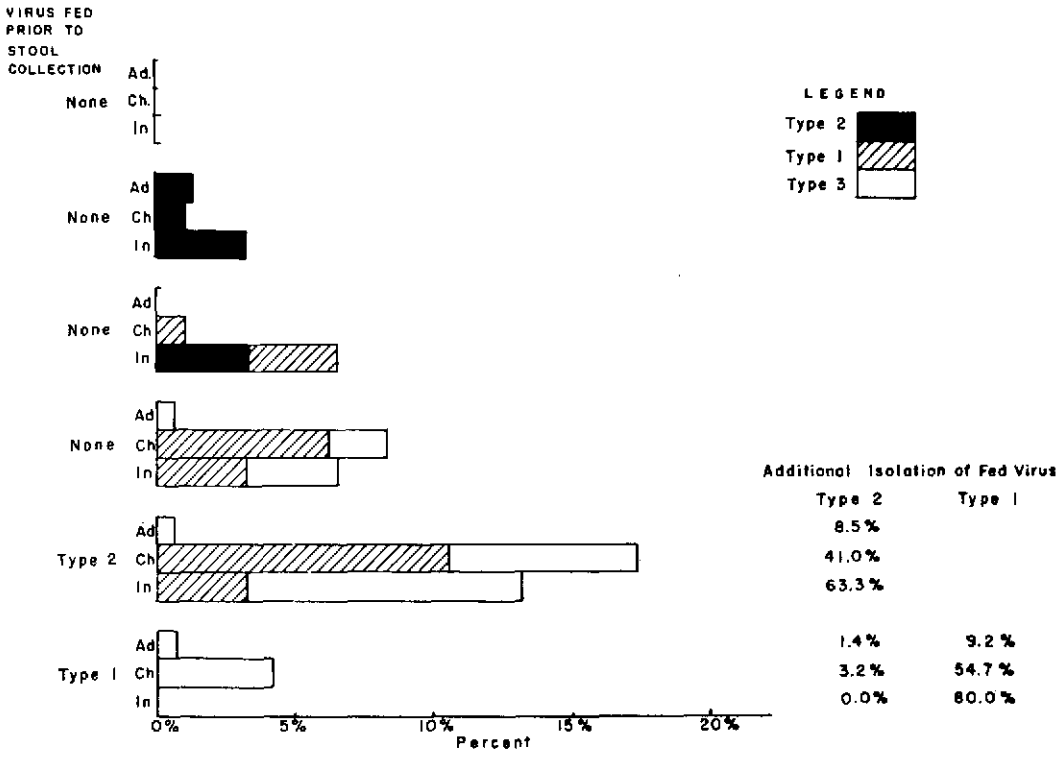


FIG. 2. Percentages of poliovirus isolated from six stool specimens collected from group A individuals (placebo group).

Ad.—Specimens from 141 adults.
Ch.—Specimens from 95 children.
In.—Specimens from 30 infants.
Only 13 stool specimens were missing; maximum of 4 from adults, 2 from children, 1 from infants.

TABLE 2. IDENTITY OF THE 4 PLACEBO-FED INDIVIDUALS FROM WHOM TYPE 2 POLIOVIRUS WAS ISOLATED AND THEIR TYPE 2 ANTIBODY TEST RESULTS

INDIVIDUAL IDENTITY				STOOL SPECIMEN DATA		TITER TYPE 2 POLIOVIRUS ANTIBODY		
FAMILY NO. AND PERSON NO.	SEX	AGE	NO. OF SALK SHOTS	ORDER NO.	NO. OF DAYS* SINCE VIRUS FEEDING	PRE-	POST CONTROL	POST FEEDING
4–3	Fe	34 mo.	2	2nd	14	<4	256	256
4–4	Fe	4 mo.	0	2nd 3rd	13 36	<4	16	64
12–1	Ma	32	1	2nd	15	16	16	64
105–2	Fe	20	3	2nd	12	64	64	1024

* Type 2 poliovirus had been fed to Group B families on February 3 to 8. Days elapsed to stool specimen collection date are calculated from February 5.

dence that any of these five individuals had picked up Type 2 poliovirus.

Poliovirus Type 1 was isolated from 12 children in seven group A families; the identity of these individuals, the chronological data of the stool specimens and the antibody titers vs. Type 1 poliovirus are shown in Table 3. Type 1 poliovirus was isolated from three children in family No. 29. The virus was isolated earliest (from the third stool specimen submitted) from the three-year-old (29-3) and later from her and her two younger brothers. Type 1 poliovirus was isolated from four individuals in family No. 165. The virus was isolated earliest from the eight-month-old infant (165-6) and later from her and her three older brothers. The remaining

TABLE 3. IDENTITY OF THE 12 PLACEBO-FED INDIVIDUALS FROM WHOM TYPE 1 POLIOVIRUS WAS ISOLATED AND THEIR TYPE 1 ANTIBODY TEST RESULTS

INDIVIDUAL IDENTITY				STOOL SPECIMEN DATA		TITER TYPE 1 POLIOVIRUS ANTIBODY		
FAMILY NO. AND PERSON NO.	SEX	AGE	NO. OF SALK SHOTS	ORDER NO.	NO. OF DAYS* SINCE VIRUS FEEDING	PRE-	POST CONTROL	POST FEEDING
29–3	Fe	3	3	3rd 4th 5th	6 26 49	<4	16	64
29–4	Ma	2	3	4th 5th	26 49	<4	64	256
29–5	Ma	1	3	4th 5th	26 49	<4	4	256
165–3	Ma	5½	3	4th 5th**	25 44	4	4	64
165–4	Ma	4	3	4th	26	4	16	256
165–5	Ma	2	3	4th 5th	23 44	<4	256	1024
165–6	Fe	8 mo.	3	3rd 4th 5th**	9 23 44	<4	4	256
27–3	Ma	4	2	5th†	49	<4	<4	256
47–4	Ma	2	2	5th†	53	<4	<4	1024
63–3	Fe	5	3	5th†	50	<4	<4	<4
67–3	Fe	2	3	5th†	50	<4	<4	16
103–3	Ma	2	3	5th†	50	<4	<4	1024

* Type 1 poliovirus was fed to Group B families on February 24 to March 1. Days elapsed to stool specimen collection date are calculated from February 26.

** Type 1 poliovirus also isolated from the 6th stool specimen but Type 1 had been fed to Group A prior to the collection of the 6th specimen.

† Type 1 and Type 2 poliovirus isolated; Type 2 virus had been fed prior to collection of the 5th stool specimen.

five Type 1 isolates share several characteristics: they were all from children age two to five years; they were late isolations, being found only in the fifth stool specimens; they were collected within a five-day period; Type 1 and Type 2 poliovirus were isolated from all five specimens (Type 2 virus had been fed to these children). None of these five children showed an antibody response to Type 1 virus in the post-control blood specimen which had been collected prior to the receipt of the fifth stool specimen.

In addition to the 12 people who picked up Type 1 virus there were 18 other members of these seven families. Eleven of the 18 additional family members had Type 1 antibodies and only one of these, 47-1, the father of 47-4, showed an antibody response to Type 1 during the control period. The seven other family members had no antibodies in dilution 1:4 vs. Type 1 and showed no antibody response during the control period.

TABLE 4. IDENTITY OF THE 13 PLACEBO-FED INDIVIDUALS FROM WHOM TYPE 3 POLIOVIRUS WAS ISOLATED AND THEIR TYPE 3 ANTIBODY TEST RESULTS

INDIVIDUAL IDENTITY				STOOL SPECIMEN DATA		TITER TYPE 3 POLIOVIRUS ANTIBODY		
FAMILY NO. AND PERSON NO.	SEX	AGE	NO. OF SALK SHOTS	ORDER NO.	NO. OF DAYS* SINCE VIRUS FEEDING	PRE-	POST CONTROL	POST FEEDING
41–3	Fe	3	3	6th	48	<4	<4	16
41–4	Fe	1	2	5th	25	4	<4	1024
45–5	Ma	6 mo.	0	5th	27	<4	<4	<4
69–3	Fe	2½	3	5th	29	<4	<4	16
69–4	Ma	6 mo.	0	5th	28	<4	<4	64
72–3	Fe	3	2	5th 6th	27 48	<4	<4	256
72–4	Fe	1½	0	6th	48	<4	<4	64
87–3	Ma	3½	2	4th 5th	11 33	<4	<4	4
92–3	Fe	5	1	5th	26	<4	<4	64
92–4	Ma	3	1	5th 6th	27 53	<4	<4	16
144–3	Ma	2½	3	4th 5th	7 26	<4	64	1024
144–4	Ma	11 mo.	2	4th 5th	8 25	<4	<4	1024
157–1	Ma	27	3	4th 5th 6th	9 26 47	<4	<4	16

* Type 3 poliovirus had been fed to Group B families on March 17 to 22. Days elapsed to stool specimen collection date are calculated from March 19.

Poliovirus Type 3 was isolated from 13 individuals (12 children and one adult) in eight group A families; the identity of these individuals, the chronological data of the stool specimens and the antibody titers vs. Type 3 poliovirus are shown in Table 4. Type 3 poliovirus was isolated from the fourth stool specimen of the adult (157-1) and three children (87-3, 144-3, 144-4); only one of these four individuals (144-3) showed an antibody response in the post-control blood specimen. Type 3 poliovirus was first isolated from the fifth or sixth stool specimens from the remaining nine children; no antibody response was observed in the post-control blood specimen which had been collected from these children prior to the time when they were shown to be excreting Type 3 virus.

In addition to the 13 people who picked up Type 3 poliovirus there were 21 additional persons in these eight families. Eight of the 21 individuals had antibodies vs. Type 3 poliovirus and one (144-1) of the eight showed an antibody response to Type 3 (1:4 to 1:64) during the control period. The remaining 13 individuals had no antibodies vs. Type 3 poliovirus detectable in serum dilution 1:4 and no antibody response was observed in the blood specimen collected at the end of the control period.

Serological evidence of community spread of the poliovirus vaccines that was unconfirmed by virus isolation was observed for seven people. As shown in Table 5, there were nine instances of poliovirus spread indicated by rise in antibody titer, four for Type 2, two for Type 1, and three for Type 3. Type 1 and Type 3 poliovirus both appear to have been picked up by two male adults (47-1 and 144-1). A child of 47-1 had also picked up Type 1 but not Type 3 (see Table 3) and two children of 144-1 had picked up Type 3 virus but not Type 1 (see Table 4).

Total community spread of these poliovirus vaccine strains, whether supported by virus isolation, antibody response, or both, is shown in Table 6. Among the 266 group A individuals Type 2 poliovirus had spread to eight, Type 1 to 14, and Type 3 to 16. These 38 instances of spread involved 36 individuals or 13.5 per cent of the control group. These 36 individuals were distributed in 23 families or 31.1 per cent of the 74 control group families.

Poliovirus was not isolated from any adults or children with antibody detectable in serum dilu-

TABLE 5. SEROLOGICAL EVIDENCE OF SPREAD OF POLIOVIRUSES TO CONTROL GROUP (A) INDIVIDUALS UNCONFIRMED BY VIRUS ISOLATION FROM STOOL SPECIMENS

POLIO-VIRUS TYPE	INDIVIDUAL IDENTITY				ANTIBODY TITER		
	FAMILY NO. AND PERSON NO.	SEX	AGE	NO. OF SALK SHOTS	PRE-	POST CONTROL	POST VACCINE
2	5–3	Ma	3½		16	256	1024
	43–1	Ma	25	0	4	256	64
	56–1	Ma	31	1	64	1024	256
	107–1	Ma	25		16	256	256
1	47–1*	Ma	26	2	16	256	256
	144–1**	Ma	25	3	16	256	64
3	33–2	Fe	26	3	64	1024	256
	47–1*	Ma	26	2	64	1024	256
	144–1**	Ma	25	3	4	64	64

* Type 1 poliovirus isolated from 1 child of 47-1.

** Type 3 poliovirus isolated from 2 children of 144-1.

TABLE 6. TOTAL SPREAD OF POLIOVIRUS VACCINE STRAINS TO GROUP A INDIVIDUALS AS EVIDENCED BY ISOLATION, SEROLOGICAL RESPONSE, OR BOTH

GROUP A (CONTROL)	TOTAL No.	TYPE 2 No.	TYPE 1 No.	TYPE 3 No.	TOTAL No.	TOTAL %
Adults	141	5	2	4	9*	6.4
Children	95	2	11	9	22	23.2
Infants	30	1	1	3	5	16.7
Total Persons	266	8	14	16	36*	13.5
Families No.	74	7	8	10	23*	31.1
%		9.5	10.5	13.4		

*2 less than apparent total; evidence of spread of both Types 1 and 3 poliovirus to 2 adults, reduces the person and family totals.

tion 1:4 unless the individual was known to have received Salk vaccine. Virus isolation rates from stool specimens as a function of antibody status on the initial blood specimen and Salk vaccine experience is shown separately for adults, children and infants in Table 7. Among the adults the majority (63 per cent or more) had polio antibodies detectable in serum dilution 1:4 or greater in the initial specimen, but virus isolation was accomplished from few of these individuals as compared to the isolation rates from those without antibodies. Furthermore, virus isolation from adults in the presence of serum antibody was limited to those who had had two or more doses of Salk vaccine with one exception and this individual had had one dose of Salk vaccine. Among the children, the majority had no detectable antibodies to Types 1 and 3 and over one-third had no detectable antibodies to Type 2. Virus isolation rates were high from those children without polio antibodies regardless of their Salk status. Virus isolation rates from children were significantly lower if poliovirus antibodies were demonstrable than if they were absent. It is not possible to determine whether the antibodies detected in these children were natural in origin, Salk-induced, or both. Since it is established that naturally acquired antibodies are associated with a short period of virus multiplication in the intestinal tract, it is logical to assume that the lower percentage of isolations affected in children with detectable antibodies as compared to those without detectable antibodies, was largely due to that portion of the children in whom the antibodies were natural in origin. Virus isolation rates were high from infants regardless of the presence of poliovirus antibodies in the initial blood specimen and regardless of the Salk status. The presence of poliovirus antibodies in the serum of the infants in almost every instance appeared to be the result of placental transfer as judged by the presence of the same type of antibody in the mother's serum and the extrapolated fall in titer as related to the age of the infant.

Evaluation of Symptoms During the Control Period. Study of the occurrence of symptoms in individuals to whom the virus had spread during the control period revealed no illnesses which were attributable to the vaccine strains. For individuals to whom spread was proven by isolation, the time period between two weeks prior to the collection of the stool specimen from which virus was first isolated and two weeks later (or until the end of the control period) was classified as pertinent. During this pertinent period gastrointestinal disturbances were reported for one child and one adult; the remainder of the individuals from whom virus was isolated reported no illnesses or symptoms. For individuals for whom the evidence of spread was serological only, the time period for evaluation of occurrence of symptoms was from the date of feeding of the respective type to group B to the end of the control period. During this time period one child and one adult reported gastrointestinal disturb-

TABLE 7. VIRUS ISOLATION RATES FROM STOOL SPECIMENS AS A FUNCTION OF ANTIBODY STATUS AND SALK VACCINE EXPERIENCE

SALK VACCINE EXPERIENCE		INITIAL TITER <4		INITIAL TITER 4 OR >	
		0—1 DOSE	2 OR MORE DOSES	0—1 DOSE	2 OR MORE DOSES
Adults	Type 1	12*/29	13/41	0/46	8/166
	Type 2	12/32	3/25	1/43	13/182
	Type 3	8/39	15/65	0/36	3/141
Children	Type 1	14/16	86/119	0/1	28/65
	Type 2	8/16	35/53	0/1	47/131
	Type 3	7/17	72/146	0/0	3/38
Infants	Type 1	28/35	4/5	10/11	3/3
	Type 2	22/30	3/5	12/16	1/3
	Type 3	12/37	1/7	5/9	0/1

* Numerator: number of virus isolations from stool specimens; denominator: total number of persons from whom the isolations were accomplished and who had the pre-vaccine antibody titer and Salk experience as indicated in column headings.

ances, one adult reported a respiratory illness, and one adult reported both a respiratory illness and later a gastrointestinal disturbance. Additional members of the 23 families of individuals to whom the vaccine strains had spread reported essentially the same rates of respiratory illnesses and gastrointestinal disturbances as were observed in the study group as a whole.[1] In the individuals to whom spread was indicated by laboratory evidence, as well as in other members of their families, the gastrointestinal disturbances occurred in the same period (March 9 through March 22) as those observed for the entire study group.

The medical records are presented for the two families (see Table 3) in which considerable intrafamily spread of the virus appears to have followed the entrance of Type 1 vaccine strain into the household. The medical records for family 165 (two adults, four children) show no illnesses whatsoever during the entire study period in any of the individuals. The medical record for family 29 (two adults, three children) indicates that both adults had a gastrointestinal disturbance in the middle of March; one child had a nasal discharge in late February and loose stools on one day in late March; one child had loose stools for two days in late March; the third child was well throughout the entire study period.

DISCUSSION

Using the time interval of about three weeks between feedings of the three types of poliovirus, it appears, as judged by virus isolations, that Type 1 replaced Type 2 and Type 3 replaced Type 1 very well (see Figure 1). The presence of one virus is usually expected to interfere with the establishment of another virus. Since there was a time lapse of about one week between the collection of the stool specimens and the later feeding of another type, actual replacement is not proven but is strongly suggested by the evidence presented and is a most striking phenomenon. The continued excretion of Type 3 poliovirus in group B individuals, as shown by isolation from the fifth and sixth stool specimens, is in marked contrast to the small amount of continued excretion of Types 2 and 1 poliovirus when followed by the feeding of another type. This long excretion may be a characteristic of the Type 3 vaccine strain and it is not known whether it would have been well replaced if fed ahead of Types 1 or 2. The possibility must also be considered that the long continued excretion was observed because no other virus was fed and therefore there was less competition for lebensraum.

The absence of "wild" poliovirus in this community was demonstrated by the absence of poliovirus in the stool specimens collected prior to feeding the vaccine strains and by the sequential appearance of each type of poliovirus in stool specimens only after each specific type had been fed. Since only 16 cytopathogenic agents (adeno Types 1 and 2) other than poliovirus were isolated during this study the possible effect of enteric viruses other than poliovirus in interfering with the establishment of the vaccine strains of poliovirus has not been measured.

The number of isolations shown for group B individuals in Fig. 1 is not an accurate measure of all of the persons who excreted virus. The specimens were collected about two weeks after virus feeding and it can be assumed that additional group B individuals excreted virus for shorter periods of time. This assumption is supported by the previously published results showing antibody response[1] which occurred for each type in a larger proportion of the individuals than those yielding virus in the stool specimen collected two weeks after feeding. The value of the isolation rates shown in Fig. 1 is therefore limited to a comparison of the differences in amounts of virus of each type available for community spread rather than as a graphic representation of the total number of excretors in the community.

The difference in the amount of spread observed with the three types is probably related to the number of susceptibles in the community.[1] Considering susceptibles as those without antibody, the smallest number were susceptible to Type 2, and Type 2 spread the least; the largest number were susceptible to Type 3, and Type 3 spread the most. The difference in observed spread of Types 1 and 3 was small, but as has been pointed out, we have reason to believe that the spread of Type 3 was less completely measured in this study than were the spread of Types 1 and 2.

The presence of antibody in the pre-feeding blood serum specimen does not always indicate protection against the vaccine strains becoming established in the intestinal tract (see Table 7). It has previously been established by several investigators that Salk vaccine is not effective in preventing multiplication of poliovirus in the intestinal tract. It also appears to be true that Salk vaccine induced antibodies are not effective in preventing multiplication of the vaccine strains of poliovirus used in this study (see Table 7). In the absence of detectable poliovirus antibodies, prior Salk experience does not markedly reduce the virus isolation rates in either adults or children. Differences in natural and Salk induced antibodies cannot be determined, but it is logical to assume that poliovirus antibodies are more often natural in origin in adults than in children. On the basis of this assumption the differences in virus isolation rates observed in adults and children who have detectable antibodies and Salk experience is explainable (see Table 7, last column). If the poliovirus antibodies in the majority of the adults were natural in origin the low rates of virus isolation which were obtained (2.1 per cent to 7.1 per cent for the various types) are to be expected; in a larger proportion of the children showing antibodies these antibodies can be assumed to be Salk induced because the virus isolation rates are higher (7.9 per cent to 43.1 per cent). It follows that

community spread of these vaccine strains was not as effectively reduced by Salk induced antibodies as by natural antibodies.

Recently Gelfand and co-workers have reported intrafamily [4] and community [7] spread of the attenuated poliovirus vaccine strains developed by Sabin. Spread of the vaccine strains was not observed when an attempt was made to observe long continued interfamily spread.[4] When experimental design maximized known factors which could be expected to facilitate interfamily spread of virus only 30 per cent of the contact children excreted virus.[7] These results are basically similar to the very limited extent of community spread reported in this study.

The rate of spread of these vaccine strains of poliovirus can be expected to vary in different communities and more spread could be expected in this community if a study were conducted during the summer months. There is a need to measure the rate of interfamily spread in a variety of community settings.

The findings reported in this paper suggest that the extent of spread will vary inversely with a community's prior experience with "wild" poliovirus. The immunity producing potential of live attenuated poliovirus vaccine is such that one can expect that the spread of vaccine strains may also vary inversely with a community's prior experience with live attenuated poliovirus vaccine. It may be practical in the future to maintain immunity to poliomyelitis in a population by administering the oral vaccine to newborn infants and the family of the infant. Study may later indicate that the feeding of only the infants may be effective in maintaining immune status for a community by the potential the infant has shown as a source of intrafamily spread which would supply booster exposure to the vaccine.

The modest rate of community spread of poliovirus vaccine strains observed in this study indicates that the benefit of inadvertent polio-immunization will be limited beyond the households of vaccinees.

It is noteworthy that in this study a large proportion of this community participated; members of 20 per cent of the households were fed vaccine and an additional 20 per cent of the households were studied as contact families.

SUMMARY

The community spread of poliovirus vaccine strains was studied in a community of 371 households. Oral poliovirus vaccine was fed to 279 individuals in 75 families (group B) and placebos were fed to 266 individuals in 74 families (group A). The "supply" of virus available for spread was measured by virus isolations from group B. Spread to the control group (A) was measured by virus isolations and antibody response.

Poliovirus Type 2 spread to eight individuals in seven families, Type 1 to 14 individuals in eight families, and Type 3 to 16 individuals in 10 families. The variation in the rates of spread among the three types appears to be related to the proportion of susceptible individuals rather than any measurable variation in the potential for spread among the three types of virus.

The 38 instances of virus spread involved 36 individuals (13.5 per cent of group A) consisting of nine adults, 22 children and five infants.

The 36 individuals were distributed in 23 of the 74 group A families, thus one or more persons in one third of the group A families were infected.

These results indicate that the community (interfamily) spread of these poliovirus vaccine strains to be markedly less than has been observed for intrafamily spread.

No illnesses attributable to the infection with the vaccine strains were observed among the 36 individuals who acquired the poliovirus vaccine strains by natural interfamily spread.

REFERENCES

1. Barr, R. N., Bauer, H., Kleinman, H., Johnson, E. A., Martins da Silva, M., Kimball, A. C., and Cooney, M. K.: Use of orally administered live attenuated polioviruses as a vaccine in a community setting. *J. Am. M. Assn.* **170:** 893-905 (June 20) 1959.
2. Martins da Silva, M., McKelvey, J. L., Prem, K. A., Bauer, H., Cooney, M. K., and Johnson, E. A.: Studies of orally administered attenuated live virus poliomyelitis vaccine in newborns and infants under six months. *Univ. Minnesota M. Bull.* **29:** 133-150 (Dec. 15) 1957.

3. Gard, S., Böttiger, M., and Lagercrantz, R.: Vaccination with attenuated poliovirus Type 1, the CHAT strain. In: *Live Poliovirus Vaccines, First International Conference.* Scient. Pub. no. 44 of the Pan American Health Organization, pp. 350-354, 1959.
4. Gelfand, H. M., Potash, L., LeBlanc, D. R., and Fox, J. P.: Intrafamilial and interfamilial spread of living vaccine strains of polioviruses. *J. Am. M. Assn.* **170:** 2039-2048 (Aug. 22) 1959.
5. Horstmann, D. N., Niederman, J. C., and Paul J. R.: Attenuated Type 1 poliovirus vaccine. Its capacity to infect and to spread from "vaccinees" within an institutional population. *J. Am. M. Assn.* **170:** 1-8 (May 2) 1959.
6. U. S. Department of Commerce, Weather Bureau: Local climatological data with comparative data, 1958, Minneapolis—St. Paul, Minnesota.
7. Gelfand, H. M., LeBlanc, D. R., Potash, L., Clemmer, D. J., and Fox, J. P.: The spread of living attenuated strains of polioviruses in two communities in Southern Louisiana. Presented before the Epidemiology Section, American Public Health Association, Atlantic City, Oct. 19, 1959.

14. THE CAPACITY OF LIVE ATTENUATED POLIOVIRUSES TO CAUSE HUMAN INFECTION AND TO SPREAD WITHIN FAMILIES*

JOHN R. PAUL, DOROTHY M. HORSTMANN, JOHN T. RIORDAN, E. M. OPTON, AND R. H. GREEN

Section of Epidemiology and Preventive Medicine, Yale University School of Medicine, and the WHO Regional Poliomyelitis Laboratory for the Americas

DR. PAUL *(presenting the paper)*: The purpose of this report is to document the capacity of three attenuated polioviruses to take and to spread within families when given as a trivalent vaccine, according to the outline of the field trial described earlier today in the paper by Dr. Horstmann and her colleagues.[1]

This work is essentially a study by the Yale Poliomyelitis Study Unit, in which I am only a spokesman. Others who did most of the work are listed on the title page.

Mention has already been made of the degree to which alimentary infection by these strains was induced in this particular field trial, involving as it did approximately 50 families, about the size of Dr. Verlinde's study, although the environment was different. The setting was in a village in Costa Rica.

For the privilege of carrying out this study there, we are much indebted to the Minister of Health, Dr. Quirce, and to the Director General of Health, Dr. Vargas-Méndez.

The strains of the attenuated poliovirus used were kindly supplied by Dr. Cox, and are known as the Lederle attenuated poliovirus strains.

I want to mention again that the design of the trial called for the feeding of an index child under the age of two years in each of 48 separate families in which the siblings, who were all under the age of five, were left unfed, so they could be included in the study as susceptibles.

In other words, an object of the trial was to see how far these polioviruses got in this particular setting, why they got where they did, or why not. And again may I emphasize that the objective of this study was not to compare the capacities of individual attenuated poliovirus strains, but rather to consider the problem as one of experimental epidemiology.

A special framework has been established here, and it should be recognized from the start that the findings reflect the effectiveness of this vaccine when administered within this particular environment.

This is a point which Professor Stuart-Harris made yesterday, and Professor Zhdanov has emphasized it in his use of the term "ecology." In other words, our study is more academic than practical, and could be said to recall Topley and Webster's observations of a generation ago on experimental epidemiology in mice. Their results were wholly dependent upon the age and immune status of their mice, the size of the box in which their mice were placed, its environment, and the presence of extraneous infections.[2] Our results are dependent upon similar factors.

RESULTS

A theoretical schema for depicting the *infectability* of an attenuated poliovirus strain when given as a vaccine is presented first in a series of diagrams.

Figure 1 is a schematic diagram to illustrate hypothetically the results in a group of 48 potential vaccinees (designated by 48 horizontal lines) who have been given two doses of triple, live poliovirus vaccine one month apart, on 30 July and 30 August. The blank background would indicate that the vaccine did not give rise to antibodies in any of this group.

Figure 2 is a continuation of the diagram in Fig. 1 to illustrate hypothetically, complete suc-

* Representing studies planned and carried out by the Yale Poliomyelitis Study Unit under the auspices of the Pan American Sanitary Bureau and in close cooperation with the Ministry of Health of Costa Rica.

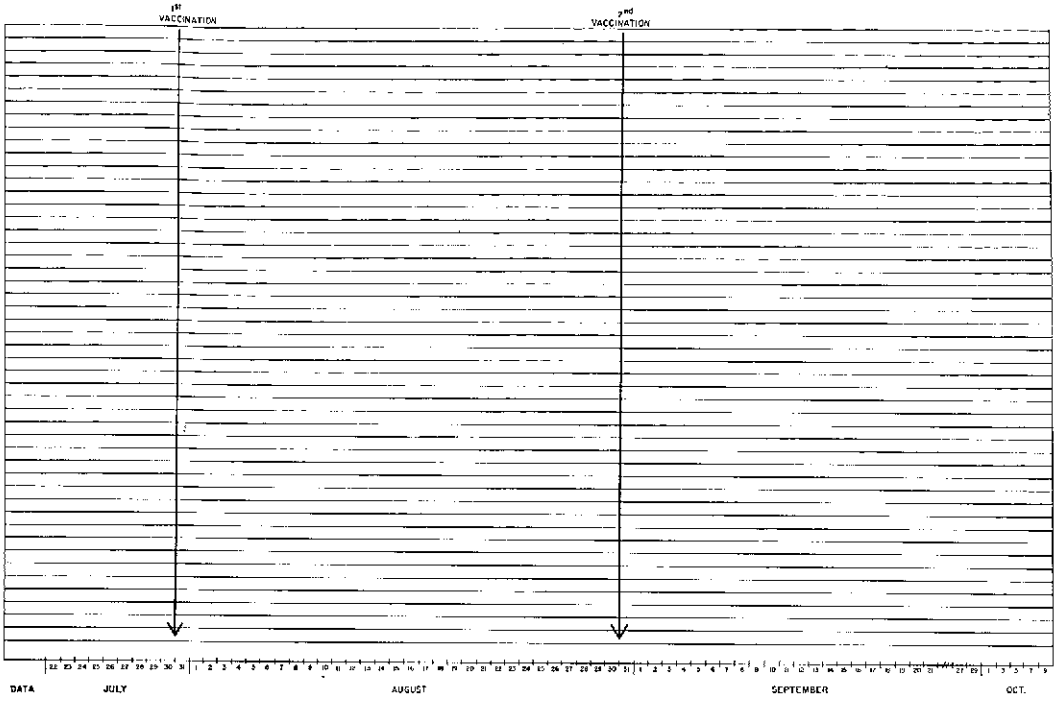

1st VACCINATION
2nd VACCINATION
DATA
JULY
AUGUST
SEPTEMBER
OCT.

FIG. 1

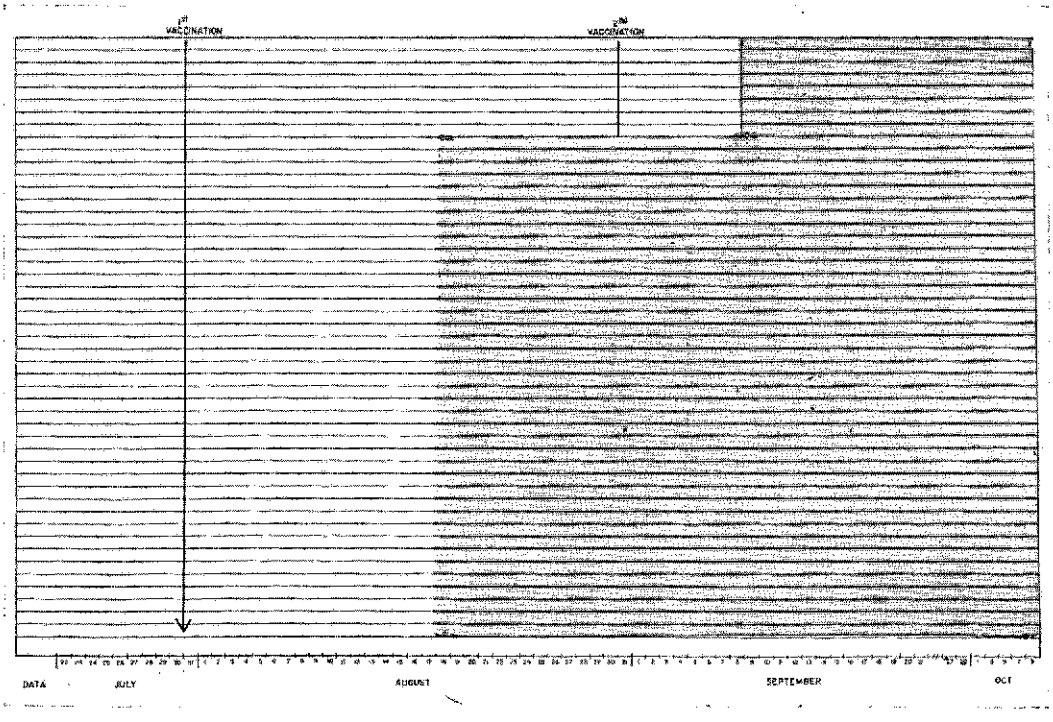

1st VACCINATION
2nd VACCINATION
DATA
JULY
AUGUST
SEPTEMBER
OCT

FIG. 2

cess of vaccination to one type of poliovirus. Shaded areas on the right-hand side of the chart represent a significant rise in antibodies for let us say, Type 1 poliovirus. Here, in mid-August it has been assumed that 40 of the 48 potential vaccinees developed Type 1 antibodies after receiving the first dose of triple vaccine and the remaining 8 candidates responded to the second dose in early September giving a 100 per cent favorable response by early October.

Next the infectability of the strains used in the trial depicted only in terms of antibody responses in each of the 48 index (vaccinated) children is given in Figures 3, 4, and 5 to illustrate the results with Types 1, 2, and 3, respectively.

In Figure 3 is shown the actual response in the development of Type 1 antibodies in the Costa Rican village population. The shaded area running across the right lower part of the chart indicates that 31 of the 48 vaccinees developed antibodies in two waves after the two doses of triple vaccine. None possessed antibodies prior to receiving the first dose of vaccine. Before the study was over six vaccinees had been lost and so their final antibody status (as of early October) remains undetermined.

Figure 4 illustrates results with Type 2 antibodies in the Costa Rican village population. The shaded area running across the entire lower third of the chart indicates that 13 of the potential vaccinees possessed antibodies to Type 2 prior to the first dose of vaccine. Ten others converted from negative to positive during the period of observation.

Figure 5 illustrates the antibody response to Type 3 in terms of the diagram in the previous charts.

Superimposed on this type of diagram there is shown, in Figure 6, not only the antibody responses but the days on which Type 1 poliovirus was isolated and the days on which non-poliovirus enteroviruses were isolated, and the complex relationship between the two.

Figure 6 illustrates the antibody response to Type 1 attenuated poliovirus as already shown in Fig. 3, and on it has been superimposed a system for demonstrating the actual days in which Type 1 poliovirus was isolated either for brief or

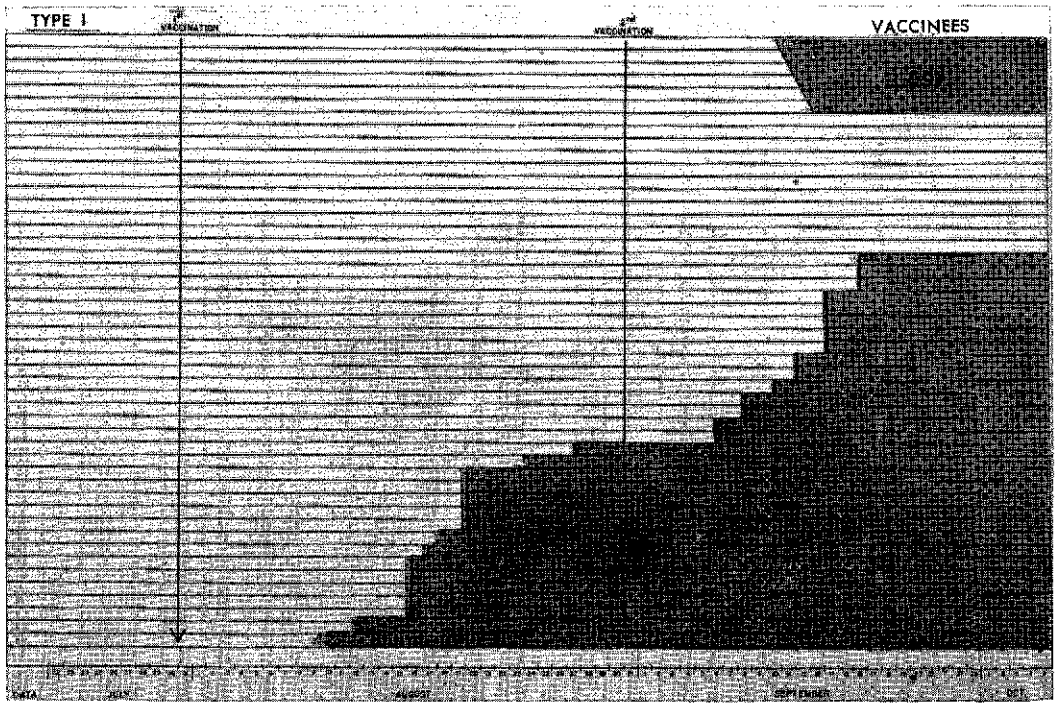


FIG. 3

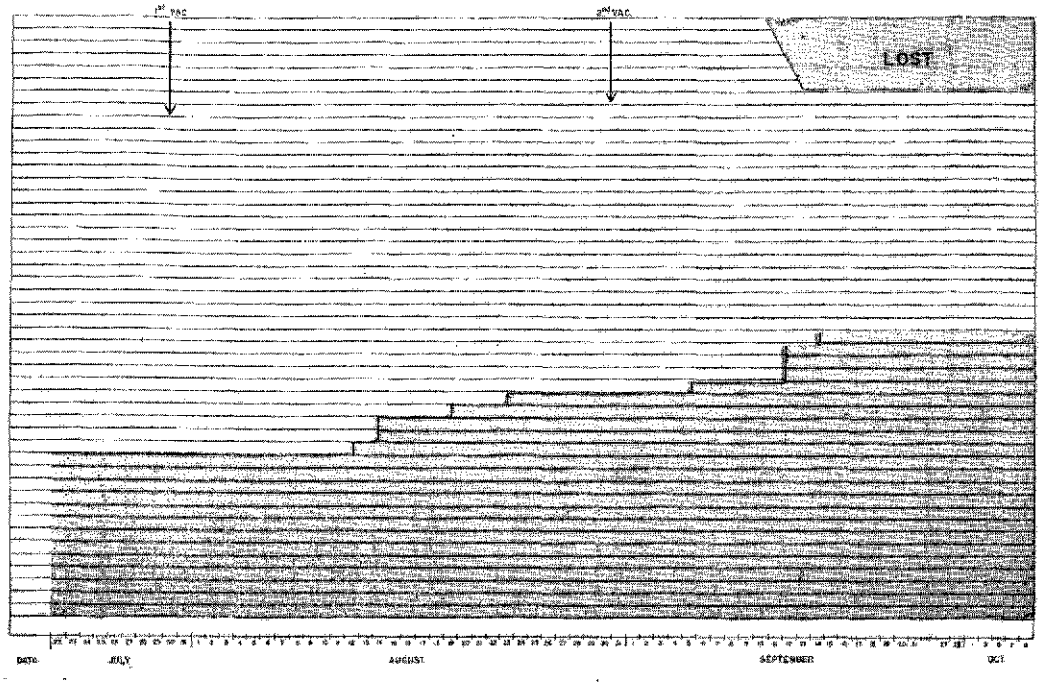

1ST VAC.
2ND VAC.
LOST
DATE
JULY
AUGUST
SEPTEMBER
OCT.

FIG. 4

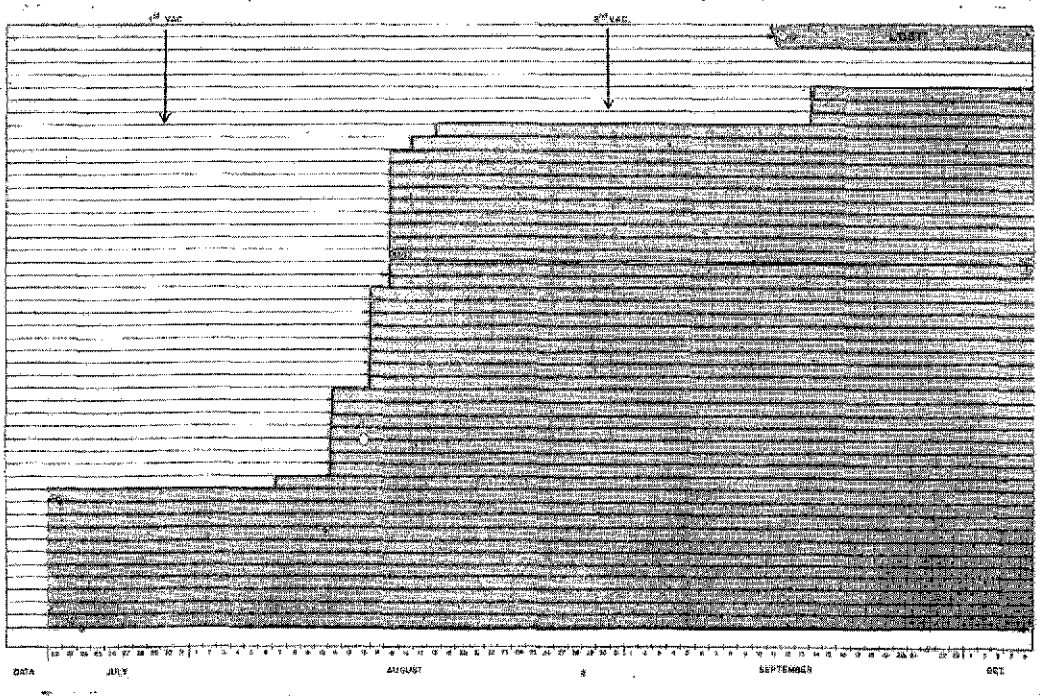

1ST VAC.
2ND VAC.
LOST
DATE
JULY
AUGUST
SEPTEMBER
OCT.

FIG. 5

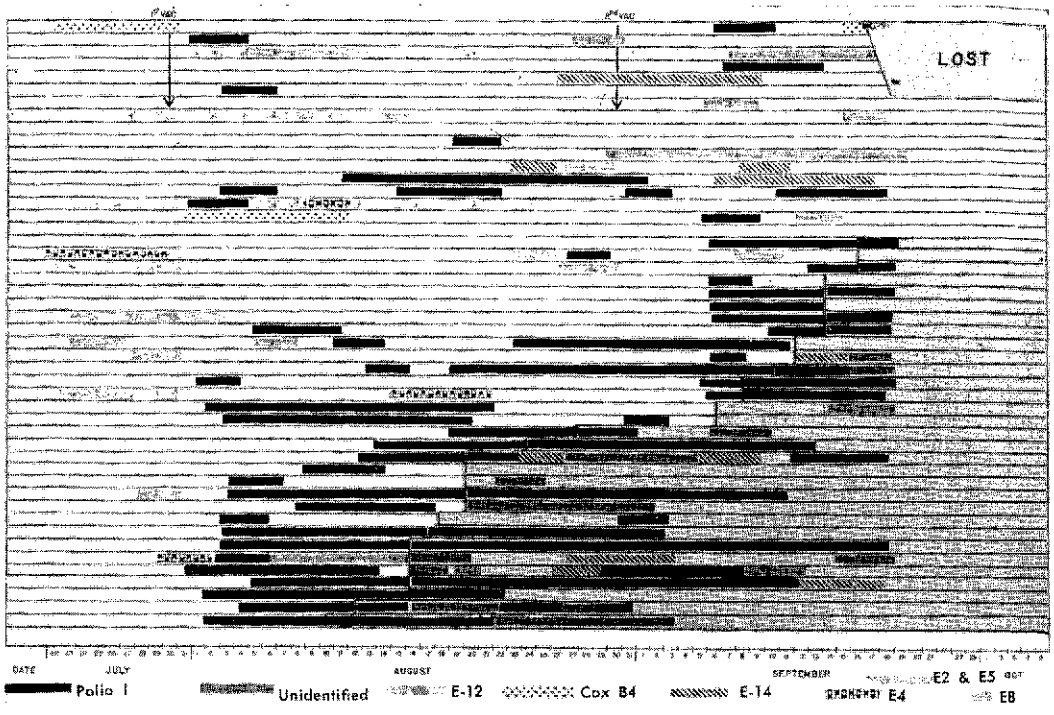

LOST
DATE
JULY
AUGUST
SEPTEMBER
Polio 1
Unidentified
E-12
Cox B4
E-14
E4
E2 & E5
E8

FIG. 6

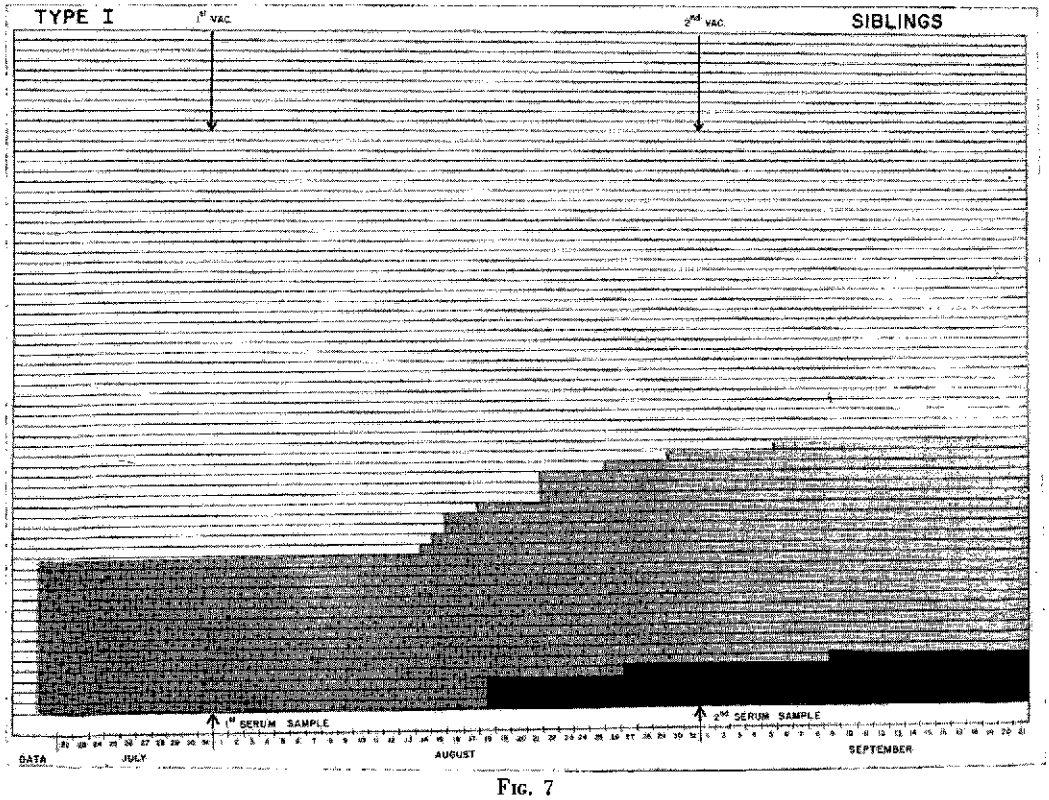

TYPE I
1ST VAC.
2nd VAC.
SIBLINGS
1ST SERUM SAMPLE
2ND SERUM SAMPLE
DATA
JULY
AUGUST
SEPTEMBER

FIG. 7

prolonged periods, some lasting seven weeks. Poliovirus infections are shown by the black stripes. Coincidentally, many enterovirus infections were present and these are shown by various kinds of shaded stripes.

And, as the final group in this series of diagrams the same general situation is shown for the 67 exposed siblings within these same 48 families (see Figures 7 and 8).

Figure 7 illustrates Type 1 infections in the population of 67 siblings (under the age of five) who were members of those families in which intrafamilial exposure to an infected vaccinee occurred. Fifteen siblings possessed Type 1 poliovirus antibodies prior to the administration of the first dose of triple vaccine. Eleven negative siblings converted during the 9-week-period of observation—a rate of 21 per cent. Of those 15 children already possessing antibodies to Type 1, 5 showed a fourfold rise associated with reinfection as indicated by the dark area in the lower right-hand corner.

Figure 8 similarly illustrates Type 3 infections among 67 siblings as a result of intrafamilial exposure. Forty of these siblings possessed antibodies to Type 3 poliovirus before vaccine was introduced into the family, and of these, 18 became re-infected. Of those 27 who lacked antibodies at the onset, 15 converted.

Non-poliovirus Enteroviruses. The degree to which the children in this study were harboring non-poliovirus enteroviruses during the prevaccinal period of one week appears in Fig. 9.

In Figure 9 the age-specific rates are shown at which participants in this trial excreted polioviruses and non-poliovirus enteroviruses prior to the first administration of vaccine. Our impression is that the impact of the non-poliovirus enteroviruses upon the poliovirus responses in the vaccinees, or the actual degree of interference which they exerted in this trial was appreciable but not great. When a comparative rate analysis was made to determine whether those children harboring such enteroviruses at the *beginning of the trial* had a higher failure rate than those without enteroviruses, there was evidence that, as far

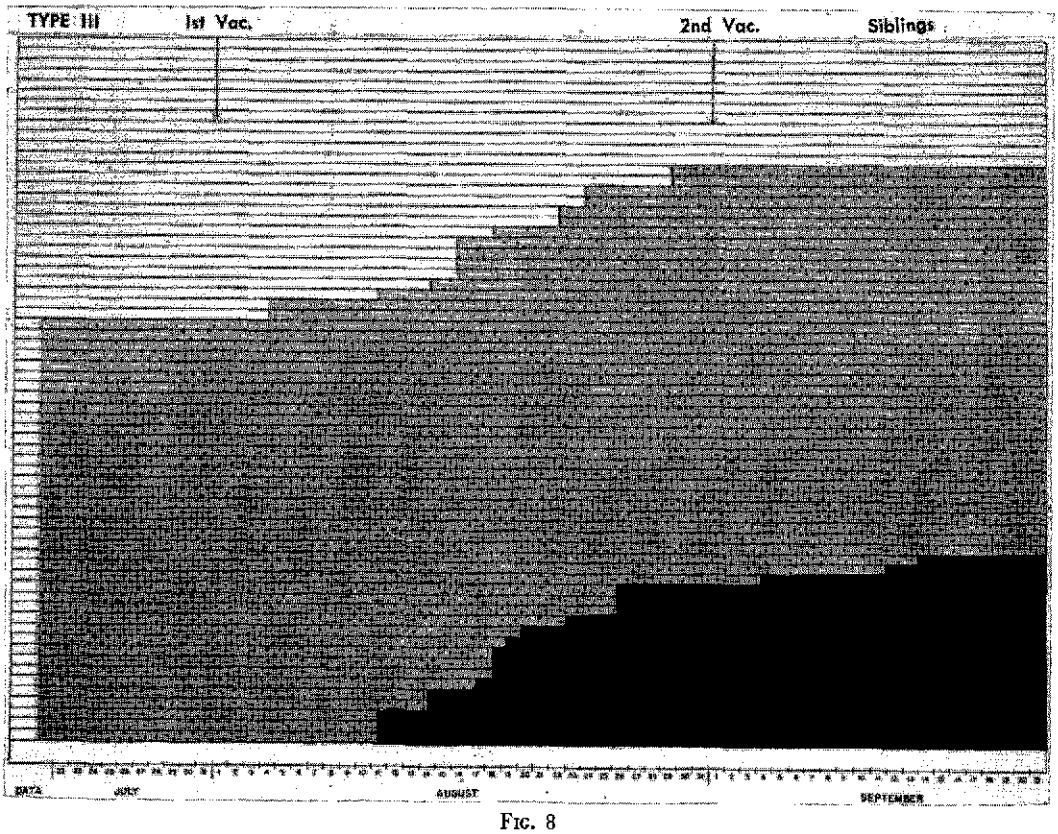


FIG. 8

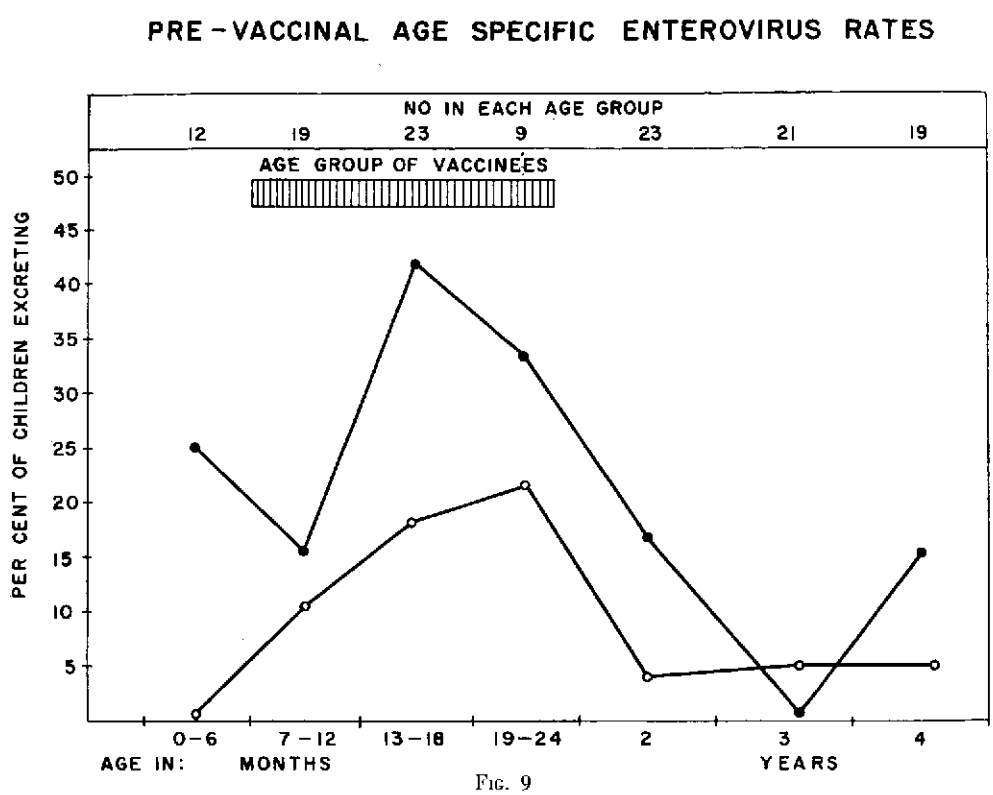

PRE-VACCINAL AGE SPECIFIC ENTEROVIRUS RATES
NO IN EACH AGE GROUP
12 19 23 9 23 21 19
AGE GROUP OF VACCINEES
PER CENT OF CHILDREN EXCRETING
50 45 40 35 30 25 20 15 10 5
0-6 7-12 13-18 19-24 2 3 4
AGE IN: MONTHS YEARS

FIG. 9

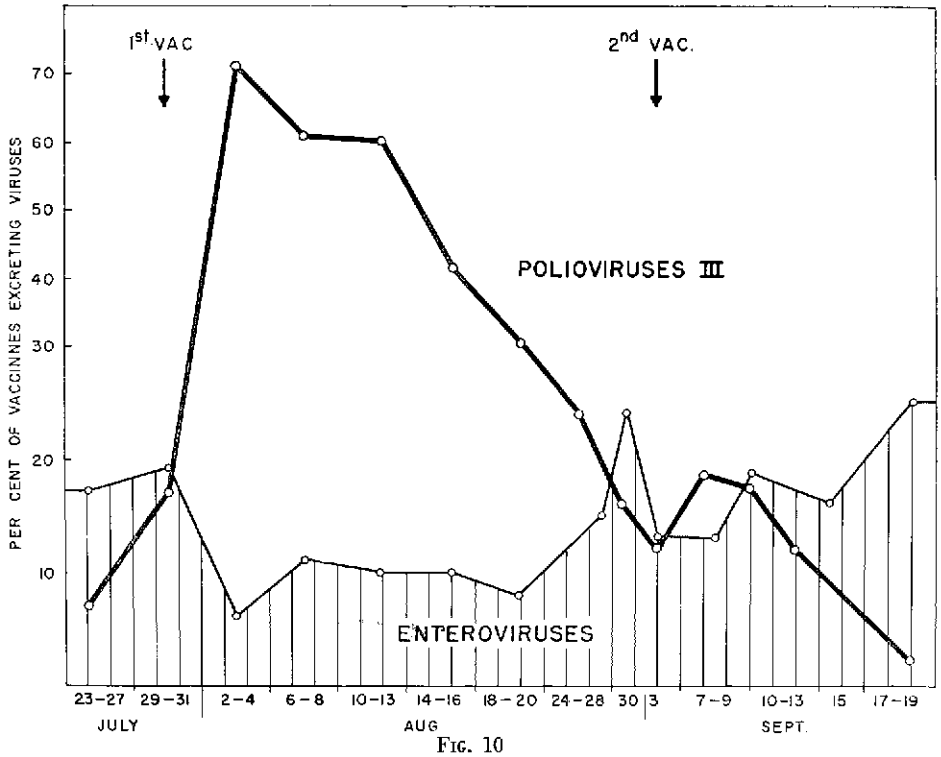

1st VAC
2nd VAC.
PER CENT OF VACCINNES EXCRETING VIRUSES
70 60 50 40 30 20 10
POLIOVIRUSES III
ENTEROVIRUSES
23-27 29-31 2-4 6-8 10-13 14-16 18-20 24-28 30 3 7-9 10-13 15 17-19
JULY AUG SEPT.

FIG. 10

as the response to Type 1 attenuated poliovirus was concerned, an interfering role on the part of non-poliovirus enteroviruses was appreciable. If any particular enterovirus could be incriminated, it may have been ECHO 12. With Type 3, however, there was no evidence of interference. Actually, the opposite may have occurred (see Fig. 10).

Figure 10 shows the rates at which potential vaccinees excreted Type 1 poliovirus as compared with rates at which members of the same group were excreting non-poliovirus enteroviruses. Prior to the administration of the triple vaccine and two months later, the incidence of non-polioviruses was about 20 per cent, but during the period of greatest infection with polioviruses this level was reduced to about 10 per cent.

Thus, the impression we have derived from this study is that, if any virus can be regarded as an "interfering agent," it was the Type 3 attenuated poliovirus used in the vaccine. This "took" so well and was so *efficient* in producing lasting infection that it dominated the scene and appeared to crowd out the other competing attenuated polioviruses, and perhaps, the "competing" non-poliovirus enteroviruses as well. The ultimate extent to which this dominant effect of Type 3 poliovirus actually excluded or merely postponed ultimate infection in the vaccinees by the two other attenuated polioviruses is not known, for our observations on antibody determinations cover only 11 weeks in these index children.

Spread within Households. The findings on intra-familial spread reflect fairly closely the infectivity rates which resulted from feeding these three attenuated strains to the index children in the form of a trivalent vaccine given on two occasions a month apart. The strain which "took" the best spread the best. Infectivity and spreading capacity seem to be closely related properties. I cannot say that they are identical. Thus, during the 11-week-period of observation, Type 3 poliovirus, given at a dose of $10^{5.5}$, which had the capacity to infect at a rate of about 91 per cent

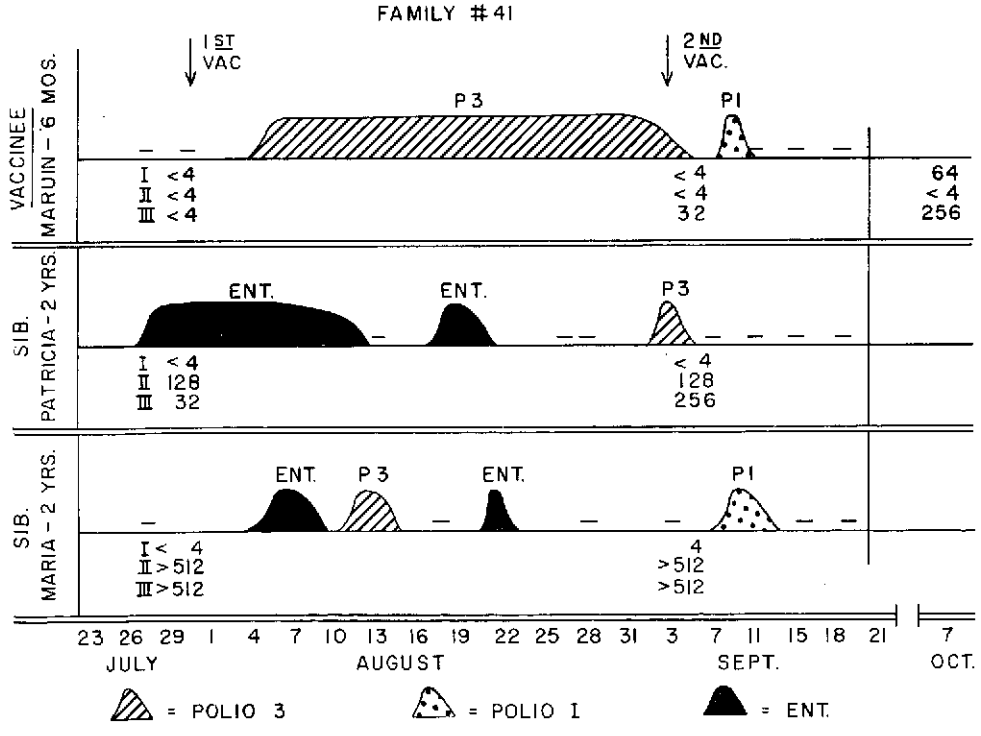


FIG. 11. A family diagram illustrating relationships between the infection periods during which three of its members, one vaccinee (Maruin), and two siblings, excreted polioviruses Type 3 and Type 1 and a non-poliovirus enterovirus designated as Ent.

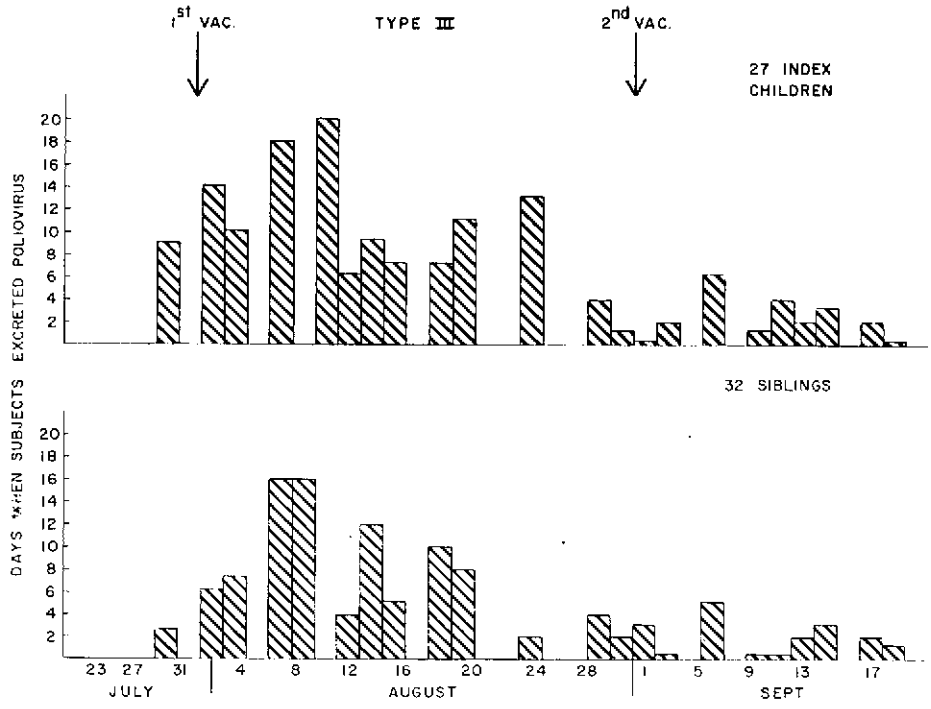


FIG. 12. A comparison between the days on which 27 index children excreted Type 3 poliovirus with the days on which 32 siblings excreted this same virus. The siblings' infections are fairly coincidental to those of the index children.

(see Fig. 5), spread the most, infecting 55 per cent of the homotypic negative siblings and re-infecting about 44 per cent of those who already had homotypic antibodies (Fig. 8). With Type 1 (see Fig. 7), the response was less with a spread of 21 and 33 per cent, respectively. With Type 2, with a low infectivity rate, practically no spread was noted.

It is to be emphasized again that none of these siblings were more than five years old, and for these results to be meaningful, the young age of the potential hosts in this population should be kept in mind, as well as the contemporary existing pattern of interfering non-poliovirus enteroviruses which found its highest prevalence among the 12-24-month-old children.

As to the speed with which these attenuated poliovirus infections spread from the index child to siblings, these data appear in Figs. 11 and 12.

In some of the families the index child only excreted the virus for a day or two, and the same was true with the siblings. In others, virus excretion continued for two to three weeks (see Fig. 11 for an example of what happened in a single family). Data in Fig. 12 record *all the days* in which index children excreted virus and all the days in which their siblings also excreted a poliovirus of similar type. This is a demonstration that there was no dearth of polioviruses going around in these families during the vaccination period. Figure 13 records the first recognized *day of onset* of the index child infection.

It would seem from this last figure that a fair percentage of the siblings picked up their attenuated poliovirus from the index child almost at once with Type 3—indeed 63 per cent of them picked up the virus within five days from the known onset of exposure. With Type 1 this almost simultaneous onset was less, 37 per cent. The phenomenon of rapid spread which occurs in a fair percentage of families, can give the false impression that these infections were derived from a common extra-familial source, and not as a result of one human passage within the family. The observation recalls to the senior author many previous discussions in pre-vaccinal days, in which the attempt was made to interpret the spread of *wild* polioviruses through families.

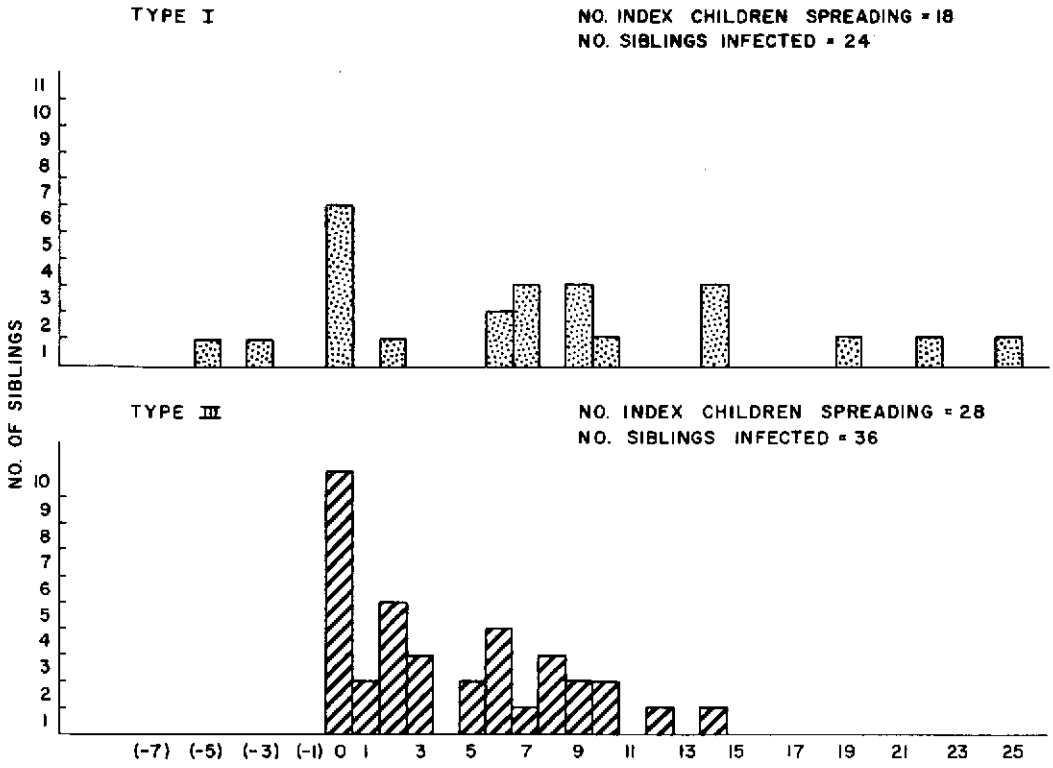


FIG. 13. Days of onset of the siblings' infection as related to the index child's infection with Type 1 and also with Type 3.

Almost 20 years ago, I made the mistake of saying that the situation with regard to family epidemics would "seem to indicate that most families become infected through a common source . . . ".[3] Our present data would belie this explanation and point rather to the fact that the attenuated viruses used in this trial spread so quickly from the index child in the family that it may well be that within 12 to 18 hours after the ingestion of attenuated polioviruses an index child might be considered as capable of infecting his intimate playmates. This eliminates the necessity of assuming that an almost simultaneous infection in several members of a family necessarily means an extra-familial common source of virus.

COMMENT

This report on the experimental epidemiology of poliomyelitis, based on a short but rather intimate study of a trivalent, oral poliovirus vaccine trial administered twice and under rather special host and environmental circumstances, has brought out some of the complexities of achieving an optimum rate of 100 per cent "takes" by the current procedures used. However, there is no intimation presented here to suggest that this goal cannot be achieved. The report has pointed out a considerable difference in the capacities of these three particular attenuated poliovirus strains to "take," even though all were given in presumably the same dosages, namely about $10^{6.5}$. The rate of "takes," as measured by antibody *conversion rates*, was more than 90 per cent with Type 3, about 75 per cent with Type 1, and much lower for Type 2. This differential capacity to infect the index children was mirrored by a parallel capacity for these viruses to spread to siblings. Indeed, the ease and the speed of intrafamilial spread on the part of these attenuated polioviruses was striking.

Finally it should be emphasized that the results of this trial, utilizing as it did a rather special and unofficial method of giving the vac-

cine, was designed for an *epidemiological study*. Such results do not have direct bearing on other programs of different design calculated to immunize a whole population against poliomyelitis, particularly by mass administration of the vaccine such as the program in progress in the same general area as our study. We do not as yet have data from this larger program, but are particularly grateful to the local health authorities of Costa Rica for the privilege of carrying out this trial in an area where considerable experience had been gained with regard to live poliovirus vaccination.

CONCLUSIONS

Emphasis has been placed in this small study of 48 families living in a subtropical environment, on what can be learned from the standpoint of human experimental epidemiology of poliomyelitis. Major aims have been to determine the capacity to infect on the part of the Lederle strains of attenuated poliovirus when given in the form of a trivalent vaccine in two doses one month apart. Particularly have we tried to determine the reasons which can be ascribed for the failure of some strains to "take." We have also studied the pattern of intrafamilial spread of these infections. It is to be emphasized that the conclusions reached apply only to one small study carried on within a special framework.

ACKNOWLEDGMENTS

Grateful acknowledgment is expressed by the authors to the following: Dr. José Manuel Quirce, Minister of Health, and Dr. Oscar Vargas-Méndez, Director General of Health, Costa Rica; Dr. Juan A. Montoya, Pan American Sanitary Bureau Epidemiology Consultant, and Dr. Joaquín Núñez, Technical Director, National Campaign against Poliomyelitis, Costa Rica; also to Mrs. María José Ramírez Vargas, and Miss Jael Chacón Zamora, Santo Domingo de Heredia Health Center, Costa Rica. Without the cooperation of these individuals this study would not have been possible.

REFERENCES

1. Horstmann, D. M., Paul, J. R., Isacson, E. P., and Niederman, J. C.: Effectiveness of trivalent vaccine in immunizing young children. Studies on the stability of the vaccine strains after human passage. (See this volume, pp. 113-120).
2. Webster, L. T.: Experimental epidemiology. *Medicine, Balt.* **25:** 77-109, 1946.
3. Paul, J. R.: The epidemiology of poliomyelitis. Delivered at a Symposium on Infantile Paralysis, Vanderbilt University, April 1941. Balto., The Waverly Press, 1941.

FOURTH SESSION

TUESDAY, 7 JUNE 1960, 2:00 P.M.

Chairman

DR. JAMES H. S. GEAR

Director, South African Institute for Medical Research
Johannesburg, South Africa

TOPIC II. SAFETY. (B) FIELD EVIDENCE OF SAFETY

(continuation)

(2) SPREAD OF VIRUS IN THE COMMUNITY

Presentation of Papers by:

Dr. Sven Gard

Dr. Masami Kitaoka

(DISCUSSION)

(3) SAFETY IN PREGNANCY AND FOR THE NEWBORN

Dr. Konald A. Prem

TOPIC III. EFFICACY. (A) LABORATORY EVIDENCE

(1) ANTIBODY RESPONSE

(a) Methodology

Presentation of Papers by:

Dr. V. Vonka

Dr. Marina K. Voroshilova

(DISCUSSION)

(b) Influence of Age

Dr. Natan Goldblum

Dr. Joseph S. Pagano

TOPIC II. SAFETY. (B) FIELD EVIDENCE OF SAFETY
(continuation)

15. FIELD AND LABORATORY EXPERIENCES WITH THE CHAT STRAIN TYPE 1 POLIOVIRUS*

SVEN GARD

The Department of Virus Research, Karolinska Institutet Medical School, and the State Bacteriological Laboratory, Stockholm, Sweden

DR. GARD: At the First International Conference on Live Poliovirus Vaccines a brief report [1] on a Swedish small-scale field trial with the CHAT strain Type 1 virus was presented. This trial was initiated in November 1957 with participation of 20 volunteer families. A second test vaccination of 107 families was performed in the winter of 1958-59. In 1959-60, finally, three separate controlled studies have been conducted and, in addition, less rigidly controlled group vaccinations have been carried out. A total of about 1000 persons have now been vaccinated.

A detailed report of our various findings will be presented elsewhere. I should mention, however, or point out, that we have applied live virus only under cover of a previous basic serologic immunity produced by administration of inactivated vaccine. A dose of 10^6 $TCID_{50}$ was used throughout. In 275 persons without pre-existing natural immunity this dose never failed to produce infection. It should be pointed out, however, that in more than 2500 stool specimens from about 750 persons participating in the various projects non-polio viruses were encountered on two occasions only, one enterovirus and one adenovirus. Apparently, therefore, the experiments were conducted in the absence of interference. All feedings were done in the period January through April.

* This work was supported by grants from the Swedish Society against Poliomyelitis.

In the present connection, I will touch on two aspects of the use of live virus vaccine. The first concerns spread of virus to contacts.

Spread of virus to contacts. It was previously reported [1] that, in the first trial, a spread of virus to susceptible family contacts occurred mainly from vaccinees less than two years old. Of 12 children below the age of two years, seven were spreaders as against none of seven children above that age.

In the subsequent trial all index children were four years old; of 63 children with susceptible family contacts only three became spreaders.

In 1960 two separate studies of this question were conducted. In one trial [2] two comparable groups were included. Among 32 families with index children less than two years old spread of virus occurred on 13 occasions, whereas only one instance of contact infection was observed in 30 families with index children above the age of two years. The results summarized in Table 1 indicate that the young children cause contact infections more than 10 times as often as the older ones.

A similar study [3] was set up in an institutional school for blind children with a total of 152 inmates, aged seven to 19 years. Dormitories for girls and boys were located in separate wings or buildings; play rooms and dining rooms were shared; teaching was coeducational in nine grades and 14 separate classes according to age.

TABLE 1. INTRAFAMILIAL SPREAD OF CHAT VIRUS INFECTION FROM INDEX CHILDREN BELOW AND ABOVE THE AGE OF TWO YEARS

	<2 YEARS	>2 YEARS
1957/58	7/12	0/7
1958/59	—	3/63
1959/60	13/32	1/30
per cent	45.5	4.0

Denominator: number of families studied.
Numerator: number of families where spread occurred.

After a general vaccination with inactivated virus live virus was fed to one or two children in each class. Altogether 20 children served as index children, 11 girls and nine boys. Excretion was followed in weekly specimens. Of the 112 contacts studied only three children became infected, two girls in the first and one in the second grade. The probable source of infection was a girl in the first grade. Later all participating children were challenged with CHAT virus. In the four lower grades 90 per cent, and among the older children 60 per cent proved to be susceptible.

Apparently, then, the CHAT virus does not spread easily by contact. It would seem that the last-mentioned study is particularly illustrative in this respect, inasmuch as the mode of intercommunication among blind children, to a large extent by touch, and the particular hygienic difficulties associated with these children's physical handicap should facilitate the dissemination of enteric viruses.

In attempts to find the reasons for the marked age dependence of the capacity for transmission of infection we have measured the amount of virus excreted by subjects of different ages, without finding any explanation. Thus, in 12 children, less than two years old, the maximum virus content in the stools, regularly observed at the beginning of the excretion period, varied between $10^{4.4}$ and $10^{6.6}$ $TCID_{50}$ per gram, average $10^{5.55}$; in 15 children above two years the corresponding figures were $10^{3.8}$—$10^{6.8}$ and $10^{5.58}$, respectively. We have not systematically studied throat excretion; according to other authors, however, no definite correlation seems to exist between appearance of virus in the throat and spread by contact. The coincidence of the shift in spreading capacity with the time when children generally are house-broken seems to us to be the most significant fact in the present connection.

The H Marker. In the course of comparative studies of the plaque-forming capacity of various strains on human embryonic and monkey kidney cultures Dr. Margareta Böttiger observed certain characteristic strain differences. A detailed study of the phenomena involved is in progress and will, in due time, be reported in detail. Since, however, the present observations seem to be of general interest, a brief description of some preliminary results appears justifiable.

Early observations indicated that the plaque-forming capacity of most strains was the same in fresh explants of monkey kidney as in human embryonic kidney. With certain strains, however, consistently lower plaque counts were obtained on human tissue. With the CHAT strain the difference amounted to about 2 logs. In most cases this characteristic was maintained after one passage through the human intestinal tract, which was considered to indicate that the property might serve as a strain marker. It was tentatively called the *H* (for human) marker.

The same pattern as first observed repeated itself in tests with 29 consecutive batches of human embryonic and monkey kidney cultures. In the 30th experiment, however, much higher counts were obtained with human cultures. The reason for this abrupt change was finally traced to a new batch of the balanced salt solution used which inadvertently had been given a too high bicarbonate content. Consequently, systematic studies on the effects of bicarbonate and temperature on the plaque formation in various tissues were initiated. Exact reproducibility has not yet been achieved and it is therefore too early to draw any definite conclusions. Certain trends are apparent, however, as illustrated by the following tables.

Table 2 shows the effect of variation in the bicarbonate content at constant temperature (37° C.). In this experiment the *d* character of the CHAT strain is clearly manifest in human tissue at 0.2 per cent bicarbonate, whereas the reduction in plaque counts on simian tissue is question-

TABLE 2. PLAQUE-FORMING CAPACITY OF TWO STRAINS IN HUMAN AND SIMIAN TISSUE AT 37° C. AND VARYING BICARBONATE CONCENTRATION

BICARB. CONC.	HUMAN			SIMIAN		
	CHAT		E 206	CHAT		E 206
	10^{-4}	10^{-5}		10^{-4}	10^{-5}	
0.4%	C	106	46	C	63	28
0.2%	10	1	50	138	18	18
0.1%	2	0	21	92	33	30

C = confluent lysis.
Figures represent averages of counts on three cultures each.

TABLE 3. PLAQUE-FORMING CAPACITY OF TWO STRAINS IN HUMAN AND SIMIAN TISSUE AT VARIOUS TEMPERATURES. BICARBONATE CONTENT 0.2 PER CENT

TEMP.	HUMAN			SIMIAN		
	CHAT		E 206	CHAT		E 206
	10^{-4}	10^{-5}		10^{-4}	10^{-5}	
35°	>140	25	25	129	33	8
37°	10	1	50	138	18	18
39°	0	0	5	0	0.3	16

Plaque = averages from three cultures each.

able even at 0.1 per cent. The wild strain E 206 is largely unaffected in both tissues.

In the experiment shown in Table 3 the bicarbonate content was 0.2 per cent throughout. Under these conditions a temperature effect on the CHAT strain was demonstrable at 37° C. in human tissue but only at 39° C. in monkey cells. The wild strain was possibly affected by the higher temperature in human but apparently not in simian tissue.

Table 4, finally, shows a comparison in human tissue of three strains: the attenuated CHAT, the LSc 2ab, and the wild E 206. In this case the *d* character of CHAT is again clearly manifest at 0.2 per cent bicarbonate, whereas the others may be affected at 0.1 per cent but definitely only when the concentration is lowered to 0.05 per cent.

These observations indicate that the *d* and *T* characteristics are interdependent and that a certain strain might be best described by recordings of its reactions in a three-dimensional diagram. Furthermore the pattern seems to vary with the type of tissue used in the test. In the present connection it is of interest to note that some strains seem to be adapted to simian, where-

TABLE 4. CORRELATION BETWEEN BICARBONATE CONCENTRATION AND PLAQUE COUNTS IN *Human* TISSUE AT 37° C.

BICARB. CONC.	CHAT	E 206	LSc 2AB
0.8%	92	61	52
0.4%	92	65	69
0.2%	0	56	56
0.1%	0	24	29
0.05%	0	0	0

as others might grow better in human tissue. The differences usually become manifest *in vitro* only when the virus is grown under suboptimal conditions.

The phenomena described may have a bearing on the problem of selecting suitable live vaccine strains. In principle the choice is now primarily based on the results of neurovirulence tests in monkeys on the assumption of a correlation between monkey neurovirulence and pathogenicity to man. To what extent this assumption holds is not known, however. As a matter of fact some strains isolated from paralytic human cases produce only inapparent infections in monkeys, while on the other hand strains obtained from healthy children in interepidemic times not infrequently turn out to be highly pathogenic to monkeys. A systematic study of the differential *in vitro* growth potential of strains of various origin might shed some light on this important problem.

I should like to add here that the designation of a strain as $t+$ or $t-$ really does not tell very much about the characteristics of the strain. This statement should be to a certain extent qualified. Certainly, small variations in temperature may cause rather great differences in the result, and particularly the conditions in the culture, such as bicarbonate content, should also be taken into account.

I believe also that the great variability in these two respects observed in different strains of viruses makes one hesitate a little to talk about genetic markers. It seems to me that the RNA viruses present problems that cannot be solved with the classical methods we have learned to use in the study of DNA genetics. For the time being, I would prefer to talk about strain patterns, behavioral patterns, rather than genetic characteristics.

REFERENCES

1. Gard, S., Böttiger, M., and Lagercrantz, R.: Vaccination with attenuated poliovirus Type 1, the CHAT strain. In: *Live Poliovirus Vaccines, First International Conference*, Scient. Pub. no. 44 of the Pan American Health Organization, pp. 350-354, 1959.
2. Böttiger, Margareta, Gard, S., Kaijser, K., Lundström, R. To be published.
3. Böttiger, Margareta. To be published.

16. STUDIES ON LIVE POLIOVIRUS VACCINE IN JAPAN

MASAMI KITAOKA, M.D.

WHO Regional Polio Center, Tokyo National Institute of Health, Tokyo, Japan

DR. KITAOKA: According to the recommendation of the WHO Expert Committee on Poliomyelitis, Geneva, 1957, formaldehyde inactivated vaccine (Salk type) has been prepared in my laboratory on a small scale. Four private manufacturers of biological products have now started to make an adequate amount of the vaccine to immunize the community against poliomyelitis in endemic areas, especially infants, although some amount of the vaccine is still imported for this purpose. At the same time, the WHO Expert Committee recommended that further investigation be carried out on the live attenuated vaccine in the laboratory, as well as in field trials.

In reviewing the epidemiology ' of poliomyelitis in Japan, our attention should be focused more on the live attenuated vaccine than on the Salk vaccine, taking the following into account: (1) the age distribution of the incidence of paralytic polio is almost limited to the < 1 to 3 year-old group (508/608=83.8 per cent), especially affecting the children < 1 to 1 year-old (376/608=61.8 per cent (Table 1); (2) any type of poliovirus could be isolated from the stools of healthy-looking children; in the summer of 1956, for example, six Type 2 polioviruses were isolated from six infants aged two months to two years at the Infant Home in Tokyo, where no case of paralytic polio has been recognized in the past several years (Table 2); (3) not all of these six strains proved to have the same high paralytogenic properties, but some, for example the SK-50 strain, proved to be of very low virulence for mice and monkeys after their inoculation by intracerebral (IC) and intraspinal (IS) routes, as shown in the table; (4) the blood level of neutralizing antibodies in children was found generally to reach the maximum in titer against all types of poliovirus without manifest infection in the five-year age group; and (5) under poor sanitary conditions, when a polio epidemic caused by Type 1 poliovirus broke out in 1956 in Tataki village, almost all infections were in children under seven (Table 3).

From the foregoing, it can be presumed that many children reared in the endemic area are acquiring immunity against polio, following subclinical infection with such a strain of low paralytogenic properties of varying degree, which could multiply in the intestines the same as a saprophytic microorganism (*E. coli*., for ex-

TABLE 1. AGE AND SEX DISTRIBUTION OF POLIO CASE INCIDENCE IN 16 CITIES AND TOWNS IN JAPAN, 1956-1958

Age	<1	1	2	3	4	5	6	7	8	9	10-19	20<	Total
m.	39	137	78	28	12	19	5	7	2	3	4	3	337
f.	36	104	57	29	19	7	5	4	2	1	4	3	271
Total	75	241	135	57	31	26	10	11	4	4	8	6	608

508 cases (83.8%) under 3 years old.
376 cases (61.8%) under 1 year old.

TABLE 2. MOUSE PATHOGENICITY OF POLIOVIRUS TYPE 2 FROM HEALTHY LOOKING INFANTS UNDER TWO YEARS OLD IN TOKYO

Name of Strain	Intracerebral Inoculation	Intraspinal Inoculation	$TCID_{50}$
SK–1	9/26*	16/20	$10^{6.0}$
SK–2	1/29	9/15	$10^{5.7}$
SK–8	8/28	16/18	$10^{6.0}$
SK–40	4/30	16/18	$10^{6.5}$
SK–50†	0/30	9/27	$10^{7.0}$
SK–53	6/30	16/20	$10^{6.3}$
Control Type 2 poliovirus strain isolated from a paralytic polio patient		23/27	$10^{6.0-7.0}$

* Numerator: number of deaths.
Denominator: number of inoculated mice.
† Inoculated intraspinally into 2 cynomolgus monkeys, one suffering from paralytic polio and the other remaining well.

TABLE 3. VIRUS ISOLATIONS FROM STOOLS OF ALL INHABITANTS INCLUDING POLIO CASES IN TATAKI VILLAGE, EHIME PREFECTURE, 1956

Age (years)	Polio Cases	All Healthy Inhabitants
0 — 1	5/19* (5)	7/22
2 — 3	5/11 (2)	10/28
4 — 5	5/11	8/26
6 — 7	2/8 (1)	3/40 (3)
8 — 9	0/3	0/41
10 — 11	1/1	1/31
12 — 13	1/1	0/23
14 — 15	1/1	0/10
16 — 20		0/42
21 — 30		1/60
31 — 40		0/54
41 — 50		0/50
50 — over		0/83
Unknown		0/20
Total	20/55 (8)	30/513 (7)

* : Numerator: No. of positive isolations.
Denominator: No. of test samples.
( ): No. of other viruses such as Coxsackie, Echo and Adenoviruses.

ample) and which are mixed in nature with the highly virulent strains.

Needless to say, in order to match the epidemiological features in the endemic area, a strain of extremely low virulence may be made available as a vaccine, provided it is proven to be capable of immunizing the subject, of being genetically stable, and of not becoming virulent through serial passage from man to man. Furthermore, it is highly possible to replace the virulent wild strain by such a low virulent strain in the endemic area. In 1954, at the Third International Poliomyelitis Conference in Rome, it was reported that two strains, namely B-34 (isolated from the father of a polio patient in Tokyo) and Lansing, had become avirulent for mice by the IC route through passage in tissue culture; later, however, on the advice of Dr. Sabin, virulence tests by the IS route showed that the strains were not yet completely avirulent. During the experiments on poliovirus mutation problems, the *mit*+ strain, derived from the wild strain MEF-1, was decreased in virulence to the point of causing only one death out of 50 mice inoculated by

the 1S route. Prior to the use of such a low virulent strain as a vaccine, there were many tests to prove the strain to be safe in neurovirulence and in genetic stability, and not to be contaminated with other viruses.

At present, attenuated vaccines have been developed through the efforts of Dr. Sabin,[2] Dr. Koprowski,[3] and Dr. Cox,[4] respectively. The use of these vaccines is supported by basic studies, and by small- or large-scale field trials in various parts of the world, especially in Singapore, where mass vaccination was carried out with significant results without accident. These results encouraged me to make up my mind to use, without fear of risking an accident, the live poliovirus vaccine in the field in the known endemic area. Since 1957, I have requested permission from the Welfare Ministry several times to use the oral vaccine in the field. The answer was that it was not yet the proper time for its use, since it was unreliable in genetic stability and because of the probability that it might convert into the wild strain. However, both kinds of vaccine, one being a frozen virus suspension and the other in the form of granules contained in a capsule, were already at my disposal through the courtesy of Dr. Sabin and Dr. Cox, respectively, as requested by us.

The Sabin vaccine was preserved in a deep freezer (—20° C.) and the Cox vaccine was kept in the refrigerator until used. A part of the Cox vaccine was given to Dr. Nishizawa of Osaka University for its trial on infants and for experiments on monkeys. Since 1959, Dr. Enjoji of Fukuoka University has been joining our study groups on live poliovirus vaccine in Japan and has been trying to administer both types of the Cox vaccines (monovalent and trivalent), to infants. Thus, Dr. Nishizawa, Dr. Enjoji, Dr. Asano (First National Hospital), and I had carried out the vaccine trial in volunteers in each hospital to follow up any side reaction, to ascertain the extent of immunity and its duration, and to check antigenic stability of the virus excreted by the vaccinees, as well as the duration of virus excretion and spread of virus excreted among contacts, before the Welfare Ministry decided to grant permission for field trials. In the meantime, the Welfare Ministry showed a tendency toward granting tacit permission to use the vaccine in the field, supervised by myself. Nagaoka City, Niigata Prefecture, and Kobe City, Hyogo Prefecture, were selected for the vaccine trial started last March and April. This paper represents the outline of the results so far obtained on live poliovirus vaccine in Japan.

The Cox monovalent vaccine. Eight children, six males and two females, aged from one year and five months to eight years and three months, all staying in the same room, were fed successively with one capsule each of Type 1 and Type 3, and then two capsules of Type 2 Cox monovalent vaccine, at four-week intervals. The virus titers of Types 1, 2, and 3 vaccines were estimated at 4.0×10^5, 3.2×10^5, and 1.1×10^5 plaque-forming units per capsule, respectively. These titers might be underestimated owing to the technical difficulties encountered in separating viruses from granules in capsule.

Blood samples for the determination of neutralizing antibody were taken once just before initial feeding, and three times every four weeks, after successive feeding of each monovalent vaccine. Virus isolation attempts were undertaken from stools collected weekly for 12 weeks after the initial feeding. As a whole, an antibody rise was recognized in all vaccinees except one fed with the Type 3 vaccine, and some of them showed a titer of more than 7940. It seems likely that the administration of Type 1 vaccine stimulated formation of antibody against Type 2 and viceversa, but poorly for Type 3 poliovirus (Table 4).

Fourteen strains of Type 1 poliovirus, three strains of Type 2, and 13 strains of Type 3 were isolated. Most of them were isolated for three weeks after feeding and persisted for six weeks (Table 5).

The virus isolation from stools was found to be strongly influenced by the status of each child, that is, three types of virus were usually isolated from a triple-negative child. If the vaccinee has not the naturally acquired antibody against any type of poliovirus, the virus corresponding to the deficient antibody can be isolated without difficulty, following oral administration of live poliovirus vaccine. However, it can be observed that, with an antibody titer for Type 1 or Type 2 poliovirus as high as 1:51-1:1300, sometimes the remaining type of poliovirus could not be isolated,

TABLE 4. IMMUNE RESPONSE (NEUTRALIZING ANTIBODY) AFTER ORAL ADMINISTRATION OF THE COX MONOVALENT VACCINE IN INFANTS (VACCINE GROUP)

No.	Name	Sex	Age	Virus Isolation*	Status at Prefeeding			Type 1 Antibody Postfeeding of			Type 2 Antibody Postfeeding of			Type 3 Antibody Postfeeding of		
					Type 1	Type 2	Type 3	Type 1	Type 3	Type 2	Type 1	Type 3	Type 2	Type 1	Type 3	Type 2
1	T.I.	m	1.5	1, 2, 3	<4	<4	<4	130	32	1600	<4	16	160	<4	130	<7940
2	Y.T.	m	3.2	1	<4	1300	<4	2500	130	>6300	>7940	>6300	>6300	<4	<4	<4
3	T.T.	m	3.10	1,3	<4	130	<4	400	160	500	2000	1024	4096	<4	32	32
4	S.S.	m	3.1	1,3	<4	130	<4	130	130	130	>6300	>7940	2500	6	25	<4
5	M.A.	f	3.2	1,3	<4	320	<4	2000	500	160	>7940	1600	>7940	13	32	40
6	M.M.	m	8.3	1	<4	32	<4	3200		64	>7940		500	130		25
7	S.O.	f	5.6	1,3	<4	32	<4	400	32	16	>6300	790	500	32	250	500
8	H.K.	m	5.10		51	<8	<8	320	32	100	25	25	32	32	32	16

* Virus isolation from stools.

Test serum samples were collected every four weeks after feeding.

Pfu of Type 1, Type 2, and Type 3 virus in each capsule, 4.0×10^2, 3.2×10^3, and 1.1×10^2 and each capsule of Type 1, Type 3, and Type 2 given with four-week intervals, successively.

TABLE 5. ISOLATION OF POLIOVIRUSES FROM THE STOOLS IN VACCINE AND CONTACT GROUPS

	Vaccine Group								Contact Group																			
	1	2	3	4	5	6	7	8	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20
Prefeeding	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
Postfeeding	Type 1			4.0 x 10^2 Pfu																								
7 days	1	1	1	1	1	1	1	—	—	1	—	—	—	—	—	—	—	—	1	—	—	—	—	—	—	1	—
14 "	1	—	—	—	1	—	1	—	—	1	—	—	—	—	—	—	—	—	—	—	—	—	1	—	—	1	—	—
21 "	1	1	—	—	—	—	1	—	1	—	—	—	—	—	—	—	—	—	—	—	—	—	1	1	—	—	—	
28 "	1	—	—	—	—	—	—	—	—	—	—	—	—	—	1	—	—	—	—	—	—	—	1	1	1		—	
Postfeeding	Type 3			3.2 x 10^3 Pfu																								
7 days	3	—	3	3	—	—	3	—	—	3	—	—	—	—	—	—	—	—	—	—	—	—	—	1	—	—	—	—
14 "	3	—	—	3	—		—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	1	—	3	—	3	—
21 "	3	—	3	3	—		3	—	3	—	—	—	—	—	3	3	3	—	—	—	—	—	3	—	3	—	3	—
28 "	3	—	—	—	—		—	—	—	—	—	—	—	3	3	—	3	—	—	—	—	—	3	—	—	—		—
Postfeeding	Type 2			1.1 x 10^2 Pfu																								
7 days	2	—	—	—	3		—	—	—	—	—	—	3	3	3	—	3	—	—	—	—	—	3	3	—	—		—
14 "	2	—	—	—	3		—	—	3	—	—	—	3	—	—	3	—	—	—	—	—	—	3	3	—	—		—
21 "	2	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	2	—	—	—		—	2	—	—		—
28 "	—	—	—	—	—	—	—	—	—	—	—	—	3	—	—	—	—	—	—	—	—		—	—	—	—		—

1 = poliovirus Type 1 isolated.
2 = poliovirus Type 2 isolated.
3 = poliovirus Type 3 isolated.
– = no poliovirus isolated.

TABLE 6. RELATION BETWEEN POSITIVE POLIOVIRUS ISOLATION FROM STOOLS AFTER ORAL ADMINISTRATION OF LIVE POLIOVIRUS VACCINE AND STATUS OF INFANTS BEFORE FEEDING (SEMI-SCHEMATIC)

VIRUS TYPE	TYPE 1	TYPE 2	TYPE 3	TYPE 1	TYPE 2	TYPE 3	TYPE 1	TYPE 2	TYPE 3
Positive virus isolation after feeding	+	+	+	−	(+) (−)	(+) (−)	(+) (−)	−	(+) (−)
Titers of neutralizing antibody before feeding	<4	<4	<4	51	<4	<4	<4	32–1300	<4
Positive virus isolation after feeding	(−) (+)	−	(+) (−)	+	−	(+) (−)	−	+	−
Titers of neutralizing antibody before feeding	100–2000	32–1024	<4	<4	160	250	130	16	25

even if the antibody for this type is deficient, as shown in Table 6.

Twenty children, 10 males and 10 females, aged from eighteen months to 10 years and three months and living in separate quarters from the eight vaccinees, had opportunity to come in contact with them in and outside the house. They were followed up weekly for immune response (Table 7) and virus isolation from the stools was attempted in the same manner as the vaccinees had been after the feeding of the vaccine. Out of 20 children, 11 were bled before feeding,

TABLE 7. IMMUNE RESPONSE (NEUTRALIZING ANTIBODY) IN CONTACTS WITH INFANTS FED WITH THE COX MONOVALENT VACCINE (CONTACT GROUP)

Number	Name	Sex	Age	Virus isolation	Status at Prefeeding			Type 1 antibody Postfeeding			Type 2 antibody Postfeeding			Type 3 antibody Postfeeding		
					type 1	type 2	type 3	type 1	type 3	type 2	type 1	type 3	type 2	type 1	type 3	type 2
1	T.K.	f	3.8	1,3	< 4	32	256	500	25	8	>7940	2500	>6300	>7940	64	4096
2	T.M.	f	2.7	1,3	< 4	160	130	400	32	32	>7940	2000	2500	500	130	130
3	S.K.	f	7.9		500	130	<8	500	160	160	130	32	100	< 4	< 4	< 4
4	Y.H.	f	6.6		130	1024	< 4	400	100	32	2000	1600	500	< 4	< 4	< 4
5	Y.K.	f	5.3	3	100	32	< 4	130	160	500	32	40	130	< 4	< 4	20
6	K.O.	m	4.2	3	2000	130	< 4	2000	256	2500	130	32	400	< 4	< 4	130
7	A.A.	f	7.11	1,3	160	32	< 8	160	>7940	630	32	>7940	1600	< 8	< 8	130
8	K.I.	f	9.3	2,3	160	1024	< 4	400	500	500	630	500	>6300	< 4	10	8
9	S.K.	f	6.3	3	400	130	< 4	130	500	1600	160	500	>7940	< 4	10	64
10	M.K.	f	8.11		130	16	25	40	32	32	< 4	< 4	< 4	6	< 4	< 4
11	M.N.	f	4.10	1				< 4	< 4	< 4	>7940	2000	2500	< 4	< 4	< 4
12	H.M.	m	3.3					< 4	25	32	500	2000	2000	< 4	< 4	< 4
13	E.M.	m	10.3					25	32	32	160	130	130	< 4	< 4	< 4
14	K.A.	m	7.3					100	130		< 4	< 4		16	8	
15	N.U.	m	1.7	1,3				64	32	400	< 4	< 4	130	< 4	25	500
16	T.T.	m	2.0	1,2,3				32	32	400	< 4	< 4	130	130	64	2000
17	S.O.	m	6.8	1,3				500	>7940	>7940	8	2000	500	< 4	40	250
18	T.K.	m	1.6	1				25			>7940			< 4		
19	H.T.	m	2.4	1,3				160			>7940			< 4		
20	H.T.	m	4.3		64	25	320	500	40	16	400	32	25	2000	100	130

Date of bleeding: the same as day infants fed vaccine.
Blank indicates that the test samples were not collected.

and of those, nine were single negative and two triple positive. Three of them became infected one week after the vaccine trial started. In another experiment, 18 infants were divided into two groups of nine each, one being one to four months old and the other six to 18 months old. Three of each group were fed with the Cox monovalent vaccine and the remaining six served as contacts. Virus isolation was undertaken from the stools in order to follow up the spread of virus to contacts. The contact babies who were older than six months were easily infected with poliovirus, while those younger than four months, who could not move by themselves, did not become infected as easily within four weeks as the older babies (Table 8). The maternal antibody was found not to be capable of inhibiting multiplication of such an attenuated virus in the intestines. The stimulation of antibody formation for Type 2 poliovirus was seen in the contact group after Type 1 vaccine was fed to the vaccine group and viceversa. All of them except two showed positive immune response after feeding. However, the immune response for Type 3 poliovirus was poor in the vaccine group after feeding of any type of vaccine.

Fifteen strains of Type 1, two strains of Type 2, and 26 strains of Type 3 poliovirus were isolated from the stools. They were isolated sooner or later within three weeks and sometimes persisted for eight weeks in the contact group after the vaccine was fed. The type of poliovirus recovered from the contact group was related to the immune status in a manner similar to that of the vaccine group.

As for the genetic stability of poliovirus excreted by the vaccinees and the contacts, 20 new isolates of Type 1 poliovirus did not show any change in *MS* marker. On the other hand, out of 24 strains of Type 3 poliovirus, three were found to change from *MS* to *MS*+ marker; two of them came directly from two vaccinees one and three weeks after feeding, respectively, and another one from a contact presumably infected from the vaccinees four weeks later. Such an experiment is still in progress together with those of *d*+ and *T*+ markers on new isolates. At any rate, the genetic properties of Type 3 proved not always to be stable.

Dr. Nishizawa had an opportunity to observe the degree of neurovirulence of the Cox monovalent vaccine in a girl nine months old who had been fed successively four weeks apart with the Cox Types 1, 2, and 3 monovalent vaccines, respectively. During the course of vaccination, she got severe diphtheria and died 13 weeks after the initial feeding; at that moment she had positive antibody against Type 1 poliovirus. The autopsy findings confirmed diphtheria and bronchopneumonia. Pathological-morphological changes caused by poliovirus were not recognized in the section of the spinal cord. The virus isolation results from the brain, spinal cord, tonsils, heart, lung, liver, kidney, large and small intestines, and stool in the intestines, were all negative. From the foregoing, any type of the Cox mono-

TABLE 8. SPREAD OF VIRUS FROM THE VACCINEES TO THEIR CONTACTS IN BOTH GROUPS, 1-4 MONTHS OLD AND 6-18 MONTHS OLD

Age group (month old)	No. of infants	Weeks after feeding 1	2	3	4	5	7	7	8	10
		Type 1 fed				Type 3 fed		Type 2 fed		
6 - 18	3 vaccinees	2/3*	2/3	2/3		3/3	1/3		1/3	1/3
	6 contacts	2/6	3/6	3/6		3/5	0/5		0/5	1/5
1 - 4	3 vaccinees	2/3	1/3	1/3		2/3	0/3		2/3	1/3
	6 contacts	0/6	0/6	0/6						

* Numerator: No. of virus isolations.
Denominator: No. of test samples.

valent vaccine seems to be safe so far as it is concerned.

Study of the immune response and virus isolation from stools after oral administration with the Cox monovalent vaccine was undertaken in eight infants aged 1 to 14 months old in Osaka (Dr. Nishizawa) and in seven children aged six months to four years in Fukuoka (Dr. Enjoji). The results obtained thus far were almost the same as those obtained in Tokyo.

Naturally infected poliovirus replaced by the oral administered attenuated virus. Dr. Nishizawa gave two paralytic polio patients, whose causative agents were identified as Types 2 and 3, respectively, the different types of attenuated poliovirus, such as Type 1 and Type 2 monovalent vaccine. The fact that the Type 2 originally in the patient was replaced by the Cox Type 1 poliovirus, was proven by isolation of virus and its identification, its pathogenicity for mice by the IC and IS routes, and the rise of antibodies. The same phenomenon of replacement by the Cox attenuated polioviruses was observed in the other patient with Type 3 poliovirus clinical disease (Table 9).

The Sabin vaccine used as a triple vaccine. Eight children aged from two months to 11 years and four months were fed at once with the Sabin vaccine combined in a mixture containing 1.0 ml. of each Type 1, 2, and 3 polioviruses with titers of $10^{5.9}$, $10^{5.7}$, and $10^{5.3}$ $TCID_{50}$ per ml., respectively. The virus isolation attempts were done from both throat swabs and stools one week after feeding, and blood was taken for antibody response three times monthly after feeding. As a result, coexistence of different types of polioviruses such as Types 1 and 2, Types 2 and 3, and Types 1, 2, and 3, were found in both throat swabs and stools without untoward side reaction. All of them showed a rise in antibody titers (Table 10).

The Cox trivalent vaccine. The Cox trivalent vaccine was given orally at once to 15 children and two adults. Blood was taken twice before, and once six weeks after, administration for antibody determination. One boy and one adult did not give positive antibody response, but the remaining 15 showed good response.

CF antibody response after oral administration with the Cox monovalent vaccine. Four infants, aged from four to 14 months, were fed with each type of the Cox monovalent vaccine, and blood was taken for CF 1, 3, 8, and 12 months after feeding. Very low titers, such as 1:4-1:16 of CF antibody, were found temporarily in three out of four vaccinees, one to three months after feeding.

TABLE 9. TYPE 3 POLIOVIRUS REPLACED BY THE COX ATTENUATED VIRUSES FED IN PARALYTIC POLIO CASE

Name of patient	Age
M.I.	2 years old

Vaccine fed		Type 2	Type 1		Type 3		
Weeks after feeding	Before	1	2	3	4	6	8
Weeks after onset of disease	1	2	3	4	5	7	9
Type of virus isolated from stool	3	2	2	1	-	-	
Inoculation of new isolates into mice — Intracerebrally		0/5	0/5	0/5			
Inoculation of new isolates into mice — Intraspinally	0/5	5/5*	1/5	0/5			
Neutralizing antibody response — Type 1 virus	0						<50x
Neutralizing antibody response — Type 2 virus	0						0
Neutralizing antibody response — Type 3 virus	0						1<50x

* Numerator: No. of deaths.
Denominator: No. of inoculated mice.

(Nishizawa)

TABLE 10. NEUTRALIZING ANTIBODY RESPONSE AFTER ORAL ADMINISTRATION WITH THE SABIN VACCINE COMBINED WITH TYPE 1, 2 AND 3 VIRUSES

No.	Name	Sex	Age	Virus isolation 1 week after feeding		Status at Prefeeding			Postfeeding								
				Stool	Throat Swab	Type 1	2	3	Type 1 antibody Month 1	2	3	Type 2 antibody Month 1	2	3	Type 3 antibody Month 1	2	3
1	M.H.	m	1.6	1,2		< 4	< 4	< 4		256	178		256	178		< 4	< 4
2	M.K.	m	4.9			< 4	>2048	< 4	>12	355		>2048	>2048		< 4	16	
3	M.T.	f	2.10	2,3	2	256	< 4	< 4		128	512		355	89		45	45
4	S.T.	f	0.9	3	2	355	< 4	< 4		1024	512		256	128		32	64
5	M.M.	f	5.10	1,2,3	1,2	64	45	< 4		89	178		65	89		6	45
6	M.K.	f	11.4						1024	178		1024	>2048		6	6	
7	H.I.	m	0.7	2	1,2	< 4	< 4	< 4									
8	Y.K.	m	0.2	1,2	1,2	8	10	8									

Date of feeding: Since May, 1959.
$TCID_{50}$ of vaccine: type 1 $10^{6.9}$ /ml, type 2 $10^{6.4}$ /ml and type 3 $10^{6.2}$ /ml.
Dosis: They were mixed up and 3 ml were given orally.
Blank indicates that test samples could not be collected.

Field trial in Nagaoka City, Niigata Prefecture, with the Sabin vaccine. Epidemics of poliomyelitis, small and large, have been reported in Nagaoka in the past few years. The health authorities attempted to carry out prevention against polio with the Salk vaccine and the vaccination was carried out subcutaneously or intradermally in children, particularly in those under three years, in the early spring of 1959. However, two paralytic cases occurred among the vaccinees last year and the parents of newborn children were very anxious to receive the oral live poliovirus vaccine. Originally, we had planned to give the Sabin vaccine orally to approximately 3,000 newborns aged three to 12 months. But, owing to the limited amount of the vaccine available, only 262 newborns were selected in four areas, since the spread of virus to contacts was expected. The vaccine was diluted with pH 7.4 PBS and mixed with syrup simplex to 10 per cent until the final dilution contained 10^5 $TCID_{50}$ per ml. The Types 1 and 3 monovalent vaccines were given successively last April and May, four weeks apart, and the Type 2 vaccine will be given in June. Blood was taken from all newborns for the neutralization test, and stools were collected for virus isolation; identification of the new isolates are to be carried out by screening for *d*+, *T*+ and *MS* markers. The field trial is still under way. On the contrary, the field trial with the Cox trivalent vaccine in Kobe was suspended for the time being because of the results so far obtained by Dr. Nishizawa.

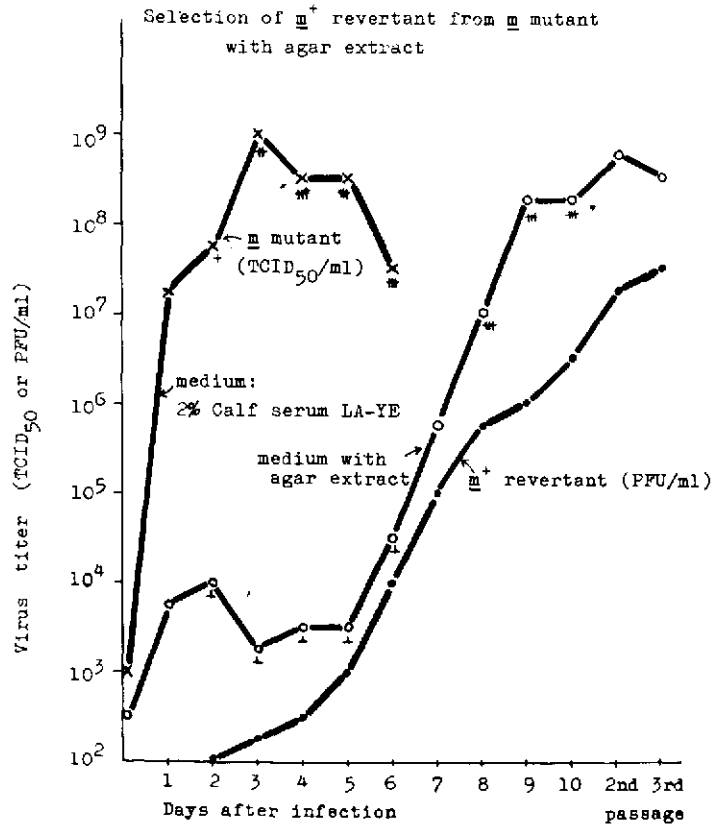
SUMMARY

The report emphasized how the attenuated live poliovirus vaccine did match epidemiological features of polio in Japan. Our results so far obtained from such a vaccine were limited to confirm the data[5, 6] published by Dr. Sabin[2], Dr. Cox[1], and Dr. Koprowski[3]. As Dr. Melnick[7] pointed out, however, the genetic stability and the neurovirulence of such attenuated virus are still the key points for such vaccines. Keeping our previous work in mind[8], that is, the appearance of *M*+ revertants from an *M* mutant, either by mixing with agar extract or by plating in dishes (Fig. 1 and Table 11), as well as the data showing that the Type 3 virus of the Cox vaccine is still *changeable* from *MS* to *MS*+ by passage through the vaccinee, and although the Sabin vaccine has been safe to date, a much more genetically stable strain, which might exist in nature as mentioned above, might be preferable for selection as a vaccine.

TABLE 11. APPEARANCE OF *m*+ REVERTANT IN THE DISHES INOCULATED WITH DILUTED *m* MUTANT

			Inoculum (0.1 ml)							
			Each 10 fold dilution							
m mutant			10^{-2}	10^{-3}	10^{-4}	10^{-5}	10^{-6}	10^{-7}	10^{-8}	10^{-9}
Name of strain (in tubes)										
E-453	m		4/4*	4/4	2/4	1/4	0/4			$TCID_{50}$/ml 1.6×10^8
		(in dishes)	17**	0						
MEF-1	mit+	m+	32	2						PFU/ml 2.7×10^4
			29	1						
			32	0						

* Numerator: No. of C.P. positive tubes.
Denominator: No. of test tubes.
** No. of *m*+ plaque in one dish.



* — to + + + = grade of cytopathogenic effect.

FIG. 1

REFERENCES

1. Kitaoka, M.: Epidemiology and control of poliomyelitis in Japan. Fifteenth General Meeting. *Jap. M. Sci.* **2**: 441-453, 1959.
2. Sabin, A. B.: Present position of immunization against poliomyelitis with live virus vaccine. *Brit. M. J.* **1**: 663-680, 1959.
3. Koprowski, H.: Live poliomyelitis vaccine. *Brit. M. J.* **1**: 1349-1350, 1959.
4. Cabasso, V. J., Jervis, G. A., Moyer, A. W., Roca-García, M., Orsi, E. V., and Cox, H. R.: Cumulative testing experience with consecutive lots of oral poliomyelitis vaccine. *Brit. M. J.* **1**: 373-387, 1960.
5. Pan American Health Organization: *Live Poliovirus Vaccine, First International Conference,* Washington, D. C., 1959. Scient. Pub. no. 44.
6. Koprowski, H., Norton, Thomas W., Wecker, Eberhard and Gard, Sven: Genetic markers and serological identity of wild and attenuated strains of Type 1 poliovirus, with special emphasis on strains of virus isolated from patients during an epidemic in the Belgian Congo. *Bull. World Health Org.* **22**: 243-253, 1960; Sabin, A. B.: Live poliovirus vaccine. *WHO Chron.* **14**: 137-142, 1960.
7. Melnick, J. L., Benyesh-Melnick, M., and Brennan, J. C.: Studies on live poliovirus vaccine. *J. Am. M. Assn.* **171**: 1165-1172, 1959.
8. Takemori, N.: Studies on the mutations of polioviruses in tissue culture. Fifteenth General Meeting. *Jap. M. Sci.* **1**: 156-160, 1959.

DISCUSSION

CHAIRMAN GEAR: Thank you, Dr. Kitaoka. The last four papers presented, including the last two of the third session, are now open for discussion. Dr. Dick.

DR. DICK: I should like to ask Dr. Gard and Dr. Kimball a question.

First, would Dr. Gard please give us more details of the monkey non-neurovirulent virus isolated from the paralytic patient? I should like to know whether the virus was recovered from cord, or from the stool of the patient.

Second, I should like to ask Dr. Kimball if she has obtained any titrations to support her statement that Salk vaccination does not affect virus excretion. "Salk vaccination" I take to mean the use of a potent vaccine given in three injections properly spaced.

DR. KIMBALL: These children had vaccine over a long period of time. We have no measure at all of the potency of the Salk vaccine used. We had numerous children in this study of Salk vaccination, who had no antibodies so I cannot speak at all for the potency of the vaccine. We only take the record of the vaccine that the children have had; then, if they do have antibodies, we feel that we cannot determine whether the antibodies are natural or Salk-induced.

The Salk vaccine was, in general, reasonably recent because this study was done in 1958 and the children were all quite young.

DR. GARD: With reference to the non-neurovirulent strains isolated from paralytic cases, my first personal experience was with virus isolated as early as 1935. It was a case described by Dr. Carl Kling, the first study published on the inapparent infection in monkeys. The material under study was lymph nodes. It was greatly disputed at the time, although I think his critics have come around later and have admitted that inapparent infections in monkeys may occur.

Be that as it may, later, on at least two occasion, we have had isolations of virus from one lethal case and one paralytic case, and isolations from stool, where virus identified as poliovirus in neutralization tests did grow in tissue culture but failed to bring down one monkey inoculated with each strain of virus.

DR. BELL: I should like to comment on Dr. Gard's observation on the spread of polio infection from children less than two years of age, as contrasted with the spread from older children. With respiratory spread diseases, namely, pertussis, rubeola, and varicella, we find that the spread in families tends to be toward the same age groups. For example, if a school-age child brings it into the household, then the exposed susceptible school-age children are most likely infected, whereas the child in the crib and other very young children are the least likely to be infected. Dr. Gard's data indicate a different pattern of spread for polio infection.

I wonder if Dr. Gard has made allowances for the age of the contacts who are exposed to the infected child?

DR. GARD: I should not say that we have made any observations of such large numbers that we should like to generalize. I shall merely mention one case where our index child was a boy 11 months old, who happened to be a twin. The two twin brothers spent the whole day together in a playpen. There were two older boys in the family, one aged three and the other five. During the observation period, which lasted about 12 weeks—that was the time the index child continued to excrete virus—we could never isolate any virus from the twin brother, but we did isolate virus from the two older children. It was first isolated from the three-year-old brother who had developed a special affection for that particular twin,—the index child—and who used to pick him up out of the pen and hug him. One week later, virus was isolated from the five-year-old brother.

On other occasions, however, we have observed, in connection with index children of about two,

that there has been spread to newborn infants in the same family.

DR. VAN ROOYEN: In connection with the question that Dr. Dick asked Dr. Kimball regarding the resistance of the human intestinal tract to poliovirus, Table 1 reveals the effect of repeated oral feeding with Cox trivalent vaccine in a group of highly immune adults. Some of these have received as many as eight doses of Salk vaccine over a period of five years. Furthermore, all have had contact with, and exposure to, cases of paralytic poliomyelitis during the conduct of their hospital duties over many years. It will be observed that it has not been possible to establish infection of gut in some, notwithstanding refeeding. In others, it will be noted that some excreted one or two types of virus for variable intervals of time. Seven months later, following a second oral dose of trivalent vaccine, some failed to excrete virus and others excreted homologous or heterologus virus. In the highly immune individual, virus was not excreted from the oropharynx, and the presence of intestinal infection sometimes occurred without corresponding elevation in antibody level.

DR. HAMMON: I should like to ask Dr. Paul a question regarding the data he presented at the third session, so as to enable me to interpret them better. After his reply, I shall probably wish to comment on it.

In connection with his discussion on the data on interference between enteroviruses, I should like to know whether the tests for an interfering virus were made after neutralization of the virus determined to be present first, or whether a single test was made each time, without attempting to neutralize any other virus that might have been present or known to be present in the stool.

DR. HORSTMANN: If I may answer for Dr. Paul on this point: In the beginning, in identifying the agents isolated, we did not use tissue-culture passages, but returned to the original rectal-swab suspensions for neutralization tests, which were done by the plaque reduction method in agar overlay bottles.

We found relatively small quantities of the viruses present, and no evidence of overgrowth of one or another strain. Subsequently, the neutralization tests were done in tubes on first tissue-culture passages, in the usual manner.

DR. HAMMON: I do not believe you understood my question, but I shall assume that the answer is what I believe it to be.

What I was referring to was whether or not, for example, after poliovirus was isolated from the stools of a child, polio antiserum of that type was added to the fecal suspension before the next test, to see if there was a second virus present that was being excreted in the intestinal tract of that individual.

The reason for this question is that data were presented indicating that there is probably some interference in the human intestinal tract between these enteroviruses. I merely wish to point out that the problem is considerably more complicated than that.

Dr. Paul indicated already that this is rather complicated, but there are two things happening here: There is a possible interference in the intestinal tract of man, and then there is possible interference in the tissue-culture tube in which one is attempting to make the isolation.

So I think these data are rather difficult to interpret without considering the possibility of interference between two viruses in the tissue-culture tube so that you come out with only one of them.

The reason I mention this is the fact that we ran across this in a longitudinal study of polio in the Philippines.

We made so many isolations of one ECHO virus, which we subsequently identified as ECHO-1, that we made an antiserum to this, and began adding this antiserum to all our fecal suspensions, before attempting to isolate poliovirus, for this was a study on poliovirus alone.

We went back and repeated our isolation attempts on many of the suspensions that we had tested before and isolated polioviruses which we had missed because we had isolated ECHO-1 virus, which was present apparently in larger quantity in the stool specimen than was the poliovirus. Then, in two, three, or four subsequent passages in tissue culture, we were ready to identify the agent. The smaller amount of poliovirus had been suppressed, interfered with, and we had identified only an ECHO-1 virus isolate.

TABLE 1. RESPONSE OF IMMUNE SUBJECTS TO REPEATED FEEDING OF TRIVALENT COX VACCINE

No.	Salk Doses	Feed	Virus Type—Days After Feeding: Fecal 0	Fecal 3	Fecal 6	Fecal 9	Fecal 16	Oral 0	Oral 2	Oral 4	Oral 6	Serum Antibody Level	Virus Type I	Virus Type II	Virus Type III
LT 1	0	First	Neg	III	–	III	–	–	–	–	–	Pre feed	512	64	<4
		Second	Neg	–	III	–	III	–	–	–	–	6 weeks	512	64	<4
LT 2	3	First	Neg	–	–	–	–	–	–	–	–	Pre feed	512	512	512
		Second	Neg	–	–	–		–	–	–	–	6 weeks	1024	512	512
LT 3	3	First	Neg	I	I	–	I	–	–	–	–	Pre feed	<4	32	128
		Second	Neg					–	–	–	–	6 weeks	<4	64	128
LT 4	0	First	Neg	I	I	–		–	–	–	–	Pre feed	<4	32	1024
		Second	Neg		+	–		–	–	–	–	6 weeks	<4	32	1024
LT 6	8	First	Neg	–	–	–	–	–	–	–	–	Pre feed	>1024	128	128
		Second	Neg	+	–	–	–	–	–	–	–	6 weeks	>1024	128	128
LT 8	3	First	Neg	–	–	–	–	–	–	–	–	Pre feed	128	512	128
		Second	Neg	–	–	–		–	–	–	–	6 weeks	128	256	128
LT 9	8	First	Neg	–	–	–	–	–	–	–	–	Pre feed	512	512	1024
		Second	Neg	–	II	II	–	–	–	–	–	6 weeks	512	512	1024
LT 10	8	First	Neg	I, III	III	–	–	–	–	–	–	Pre feed	256	512	512
		Second	Neg	III	III	III	–	–	–	–	–	6 weeks	256	512	512
LT 11	8	First	Neg	III	I	III		–	–	–	–	Pre feed	256	512	128
		Second	Neg	–	–	–		–	–	–	–	6 weeks	256	512	128
LT 13	8	First	Neg	–	–	–	–	–	–	–	–	Pre feed	256	512	128
		Second	Neg	–	–	–	–	–	–	–	–	6 weeks	256	512	128
LT 14	3	First	Neg	–	I	I	–	–	–	–	–	Pre feed	64	1024	512
		Second	Neg	–	–	I	+	–	–	–	–	6 weeks	64	1024	512
LT 15	3	First	Neg	I	I	I	–	–	–	–	–	Pre feed	8	128	128
		Second	Neg	–	–	–	–	–	–	–	–	6 weeks	128	512	128
LT 16	3	First	Neg	III	III	–	–	–	–	–	–	Pre feed	1024	138	512
		Second	Neg	II	II	–	–	–	–	–	–	6 weeks			
LT 21	8	First	Neg	–	–	–		–	–	–	–	Pre feed	512	64	512
		Second	Neg	–	–	–		–	–	–	–	6 weeks	512	256	512

– = no virus isolated; + = CPE present, typing in progress.

Then we tried mixing the viruses experimentally, i.e., ECHO-1, and polio 1. If we put in a little more poliovirus than ECHO-1, we ended up by getting only poliovirus after two or three passages. If we put in a little more poliovirus than ECHO-1, after two or three passages in tissue culture, we again got only the one that was originally there in larger quantity.

I therefore believe that this should be kept in mind in interpreting, or attempting to interpret, the data represented by possible virus interference.

The persons who were not found to excrete poliovirus Type 1, let us say, after being fed Type 1, might have been found excreting poliovirus had the other enterovirus that was found been neutralized and a search then made for poliovirus.

DR. HORSTMANN: I did understand Dr. Hammon's questions. I merely wished to point out that we were aware of the problem outlined by Dr. Hammon, but attempted to overcome it by using another technique. By means of the plaque method applied to the original fecal suspension, we were able to pick up small amounts of poliovirus or other enteroviruses which might have been overgrown in tissue-culture tubes.

Apparently, with the particular ECHO viruses which were prevalent during our trial, this problem did not occur in the same way that it occurred with Dr. Hammon's Type 1 ECHO virus. We found both ECHO and polioviruses to be present in only small quantities. So that, after extensive tests, we concluded that overgrowth of one virus by another was not a significant problem with our material, and returned, therefore, to tube cultures.

I believe it is also worth mentioning that subsequently we did isolate a number of ECHO and polioviruses in the same specimen grown in tissue-culture tubes. The other point which gives us confidence in the methods used is that antibody development and the isolation of viruses correlated so extraordinarily well, within 1 or 2 per cent of one another; therefore, we feel it is very unlikely that we missed any polioviruses in the specimens under the circumstances under which Dr. Hammon has suggested this might have occurred.

DR. SABIN: With reference to the question that Dr. Dick asked of Dr. Kimball, about the evidence for multiplication of poliovirus in children that may have been adequately immunized with properly spaced doses of Salk vaccine of proper potency, I carried out a rather extensive study in association with Dr. Krugman and others. This study was reported only in abstract form in the *British Medical Journal* and also in another review article.

We selected, first of all, children without demonstrable antibody before Salk vaccine. Three doses of Salk vaccine were then given over a one-year period, with the proper intervals, and then a summer period went by and they were tested at frequent intervals during that time. Because in some of the children there was a drop in antibody which had appeared after the three doses, we obtained a particularly potent Salk vaccine, that was not a commercially-distributed one, and gave them all a fourth dose.

We thus accumulated a group of about 25 children, most of whom had rather high antibody titers—many of them 256 to 512 for Types 1 and 3.

They were then fed either Type 1 or Type 3 vaccine, and the multiplication of viruses was followed quantitatively over a period of more than eight weeks. The multiplication of Type 1 and Type 3 virus in them was no different from that in children without any demonstrable antibody. Furthermore, the antibody response, the booster response to this multiplication, did not occur any faster than in children who had not previously had any Salk vaccine.

We came to the conclusion that with potent vaccine and four doses, and the antibody produced at the level indicated, we found no interference with multiplication of virus.

DR. BODIAN: Since the subject at this time is the spread of polioviruses, I think it important to contribute to this discussion something which I believe has been missing. I believe that a few years ago Dr. Howe came closest to presenting what I consider a rational approach to the problem of inhibition of virus excretion, namely an assessment of the role of antibody. Dr. Howe attempted to find the level of antibody which would inhibit this phenomenon of infection.

We know that it is possible to inhibit paralysis with a certain level of antibody. Is there a level of serum antibody which can be correlated with the inhibition of virus excretion in feces?

As some of you may know, Dr. Howe found a level which was inhibitory, namely a titer of about 1:500.

Recently, I have attempted to analyse this point, using passive antibody, in order to supplement experiments I had begun about 10 years ago.

In chimpanzees with a passive antibody level of 1:500, one sees the first signs of inhibition of fecal virus excretion. But this could only be detected by doing daily titrations of the virus in the feces.

Inhibition of virus in the throat occurs at much lower levels, and at levels of 1:500, we completely eliminated the virus in the oropharynx in animals which were nevertheless excreting virus in the feces. It therefore seems that there is one level of antibody which will inhibit paralysis, and this is a very low level, presumably very close to our ability to detect another level of anywhere from 1:8 to 1:256, in which range the throat excretion is dampened sharply, but in order to show any effect on excretion in feces, we have to get up to at least 1:500.

This does not mean that live poliovirus vaccine does not have another type of effect on virus excretion. It merely means that there may also be an effect due to antibody alone.

DR. GARD: I should like to remark on what Dr. Bodian just said. Actually, I reported last year at the First Conference that we had found an effect of excessively high titers produced by vaccination with inactivated virus.

We had to come up to titers of 1,000 or above to find evidence of inhibition of excretion through the intestines. This year, we planned an experiment to try to confirm that observation, by immunizing infants with inactivated vaccine, trying to produce a wide enough range of antibody titers to get any significant results.

These results are not yet complete. I hesitate to be too certain about their outcome. As things look now, we will probably have the confirmation of the previous observation that high titers tend to restrict intestinal excretion of the virus.

CHAIRMAN GEAR: We now come to the paper by Dr. Prem on "Vaccination of Pregnant Women and Young Infants with Trivalent Oral Attenuated Live Poliomyelitis Vaccine." This presentation will be followed by Dr. Vonka's paper on "The Development and Persistence of Polio Antibodies, Measured by Different Methods of Neutralization Test, in Young Adults Fed with 100,000 TCD_{50} of Type 3 Attenuated Virus." After that we shall have Dr. Voroshilova's paper on "Virologic and Serologic Investigations of Children Immunized with Trivalent Live Vaccine from A. B. Sabin's Strains."

NOTE: The Spanish translation of this paper was published in the Boletín OSP 50 (6): 525-549, Junio de 1961.

NG INFANTS
E

POLIOMYELITIS VACCINE

KONALD A. PREM, JAMES W. FERGUS, JOHN E. MATHERS, AND JOHN L. MCKELVEY
Department of Obstetrics and Gynecology, University of Minnesota School of Medicine, Minneapolis, Minnesota

DR. PREM *(presenting the paper):* This report describes the work currently being done by the Department of Obstetrics and Gynecology of the University of Minnesota Medical School on certain aspects of pregnant women and young infant immunization with live attenuated oral poliomyelitis viruses. The reasons for our interest in these fields have been stated previously.[1, 14]

Preliminary reports of this work were presented to this Conference one year ago. Since that Conference new ideas, observations, speculations, and conclusions have appeared for consideration. Some of the old have been modified or discarded. This report brings the results of our studies up to date. It is divided into two parts.

The first part concerns a study designed to:

(1) determine the immunologic response of the pregnant woman to trivalent oral poliomyelitis vaccine when administered in different dosages and at different time intervals; and

(2) determine if the fetus she carries *in utero* is significantly affected by this vaccination.

The second part concerns studies designed to:

(1) observe and compare the immunologic responses of young infants to trivalent oral poliomyclitis vaccine when fed soon after birth and at age four months;

(2) observe the virus excretion patterns of the infant and its family contacts when the infant is fed this vaccine;

(3) determine the relationship, if possible, of the passively transferred maternal antibody to the immunologic response and the virus excretion pattern of the young infant fed this vaccine;

(4) determine the duration of antibody persistence; and the proper time for and the immunologic response to revaccination with trivalent oral vaccine;

(5) determine the proper time for and the immunologic response to revaccination with trivalent oral vaccine if it becomes necessary.

Part 1. Immunization of Pregnant Women with Live Attenuated Oral Poliomyelitis Vaccine

MATERIAL AND METHODS

Participants. Three hundred and ten pregnant women who attended prenatal clinics under the supervision of the Department of Obstetrics and Gynecology of the University of Minnesota participated in this study. Of these, 152 received their care at Booth Memorial Hospital (BMH) and 105 at Catholic Infants' Home (CIH) in St. Paul, Minnesota. These groups of women included all patients registered for prenatal care at these institutions during the period of study. All were unwed. The remaining 53 were private and clinic patients who received obstetrical care in the clinics of University of Minnesota Hospitals. These participants were not consecutively registered patients. Participation in the program was voluntary.

Among the BMH and CIH women, the ages, previous Salk experience, and trimester of pregnancy in which the vaccine was fed, is similar to that previously reported.[1] Because they report earlier for prenatal care than either indigent or unwed women, the majority of the first trimester vaccinations were done in the private patient group.

Vaccine. The vaccines used in this study were liquid trivalent preparations developed by Dr. Herald R. Cox and produced and provided to the authors by Lederle Laboratories. Each cubic centimeter of vaccine contained $10^{5.8}$ TCD_{50}* of each of the SM (Type 1), MEF_1 (Type 2), and Fox (Type 3) strains of attenuated poliovirus. The attenuation history of these strains has previously been reported by Cabasso *et al.*[2] The following lots of vaccine were used: a mixture of lots No. 7-1231-121, No. 7-1232-216, and No. 7-1233-318; lots No. 7-1238-800; No. 7-1238-801; No. 7-1238-801-2, and No. 7-1238-804A. Vaccine was dispensed in individual two cc. vials or by calibrated dropper from a 25 cc. multiple dose vial. All 0.5 cc. and 1.0 cc. doses were given by the latter technique. All vaccine was stored at constant temperature of 4° C. until used to insure uniform potency.

Method of Administration. The vaccine was poured from the individual 2.0 cc. dose vial or squirted by dropper in the measured quantity directly into the mouth of each participant and followed immediately by a sip of water. Vaccination at BMH and CIH was usually in the late forenoon. All other feedings were at random.

Laboratory. At the time of vaccination, ten cubic centimeters of whole blood was collected with sterile vacumatic tube by antecubital venipuncture. A second identical sample was obtained about four to eight weeks after feeding. About 7 per cent of those whose results are reported here had the post-feeding specimen taken before four or after eight weeks.

After clot retraction at room temperature the specimens were centrifuged and the serum removed by sterile pipette, placed in sterile serum tube, frozen, and stored at —20° C. Any blood specimens that did not have the serum separated immediately were refrigerated at 4° C. until such time as this separation could be done. When sufficient numbers of paired serum specimens accumulated they were shipped without refrigeration by air express to the Viral and Rickettsial Research Section of Lederle Laboratories, Pearl River, New York for serological testing.

The method of antibody determination used was the pH or color test according to the procedure of Salk and Youngner.[3] All sera were first inactivated for 30 minutes at 56° C. in a constant water bath. The serum samples were then prepared in four-fold dilutions in duplicate, 1:4 through 1:1024. Approximately 100 to 300 TCD_{50} of the representative strains of virus were added to the respective serum dilutions and the mixtures held at room temperatures for three hours. Trypsinized monkey-kidney tissue cell suspensions were added to each of the serum-virus mixtures, and to appropriate controls. The tubes were kept at constant temperature of 37° C. and read on the sixth or seventh day. Antibody titers were calculated by the method of Reed and Muench.[4] By this technique a four-fold rise in antibody titer is significant. Any titer of less than 1:4 is considered unmeasurable.

* Tissue-culture doses.

RESULTS

Pre-vaccination and post-vaccination antibody titers were completed on 262 of the 310 participants. The remaining 48 participants did not have a post-vaccination specimen taken because of discontinuance of care or delivery before a three week interval had passed to allow antibody response to the vaccine. Tables 1, 2, and 3 summarize the antibody titer responses of the 262 to the dosages of vaccine used. These tables show a 39.1 per cent four-fold rise in titer to Type 1, 30.9 per cent to Type 2, and 42.7 per cent to Type 3. The two-fold or booster response for each type is about 20 per cent higher. One Type 1 and two Type 3 antibody determinations were not done.

The per cent of significant response to each immunotype for each of the three dosages of vaccine used is shown in Fig. 1. Although these data fail to demonstrate the superiority of the largest over the smaller dosages, those participants with unmeasurable antibody titers who received 2.0 cc. of vaccine responded better (92 per cent) than did those who received 1.0 cc. (87 per cent) or 0.5 cc. (82 per cent). Although these differences may indicate a trend, they were not considered significant when subjected to statistical analysis.

Of the 262 women with paired sera available for serologic testing there were 72 (27.5 per cent) with unmeasurable antibody titers to one or more poliomyelitis immunotypes before vaccination. These, together with their antibody response to trivalent oral vaccine, are tabulated

TABLE 1. POLIOVIRUS TYPE I ANTIBODY RESPONSE OF 261 PREGNANT WOMEN FED TRIVALENT ORAL LIVE ATTENUATED VIRUS POLIOMYELITIS VACCINE*

PRE-FEEDING	ANTIBODY TITERS POST FEEDING											TOTAL NUMBER FED	NUMBER WITH POSITIVE RESPONSE	
	<1:4	1:4	1:8	1:16	1:32	1:64	1:128	1:256	1:512	1:1024	>1:1024		4 FOLD OR>	2 FOLD OR>
<1:4	1		2	1	6	4	9	4	8	2		37	36	36
1:4					1			1	2			4	4	4
1:8				1	1	2	1		2			7	6	7
1:16				2	1		2	1	2		1	9	6	7
1:32					3	5	4	2	3			17	9	14
1:64				1		6	12	8	4	1		32	13	25
1:128					1		15	7	15			38	15	22
1:256							2	10	13	3	2	30	5	18
1:512							5	6	30	6	8	55	8	14
1:1024									5	8	7	20		7
>1:1024										3	9	12		
TOTALS	1		2	5	13	17	50	39	84	23	27	261	102	154
													39.1%	59%

* 32 were fed 0.5 cc.
72 were fed 1.0 cc.
157 were fed 2.0 cc.
Each cc contained $10^{5.8}$ TCD_{50} of each virus.

TABLE 2. POLIOVIRUS TYPE II ANTIBODY RESPONSE OF 262 PREGNANT WOMEN FED TRIVALENT ORAL LIVE ATTENUATED VIRUS POLIOMYELITIS VACCINE*

Pre-Feeding	Antibody Titers Post Feeding											Total Number Fed	Number With Positive Response 4 Fold Or >	Number With Positive Response 2 Fold Or >
	<1:4	1:4	1:8	1:16	1:32	1:64	1:128	1:256	1:512	1:1024	>1:1024			
<1:4	4		3	3	1	1	2		2	1		17	13	13
1:4		1	1			1						3	1	2
1:8			2		2	1	1		1	1		8	6	6
1:16				3				1	1			5	2	2
1:32					8	2	6	2	4		2	24	14	16
1:64			1			3	5	1	4	1	1	16	7	12
1:128					1	2	13	12	6	4	9	47	19	31
1:256				1			1	3	9	2	6	22	8	17
1:512							2	3	25	11	11	52	11	22
1:1024								2	5	5	17	29		17
>1:1024									3	4	32	39		
TOTALS	4	1	7	7	12	10	30	24	60	29	78	262	81	138
													30.9%	52.7%

* 32 were fed 0.5 cc.
73 were fed 1.0 cc.
157 were fed 2.0 cc.
Each cc contained $10^{5.8}$ TCD_{50} of each virus.

TABLE 3. POLIOVIRUS TYPE III ANTIBODY RESPONSE OF 260 PREGNANT WOMEN FED TRIVALENT ORAL LIVE ATTENUATED VIRUS POLIOMYELITIS VACCINE*

Pre-Feeding	Antibody Titers Post Feeding											Total Number Fed	Number With Positive Response 4 Fold Or >	Number With Positive Response 2 Fold Or >
	<1:4	1:4	1:8	1:16	1:32	1:64	1:128	1:256	1:512	1:1024	>1:1024			
<1:4	3	3	7	2	18	4	11	4			1	53	47	50
1:4					1		3	1	1			6	6	6
1:8					1	2	3	2	4			12	12	12
1:16				2	3		3					8	3	6
1:32				1	7	4	10	3	5		1	31	19	23
1:64						1	3	2	2	1		9	5	8
1:128							17	13	9		2	41	11	24
1:256						1	2	3	13		3	22	3	16
1:512								3	32	8	5	48	5	13
1:1024									2	4	6	12		6
>1:1024									2	3	13	18		
TOTALS	3	3	7	5	30	12	52	31	70	16	31	260	111	164
													42.7%	63.1%

* 32 were fed 0.5 cc.
71 were fed 1.0 cc.
157 were fed 2.0 cc.
Each cc contained $10^{5.8}$ TCD_{50} of each virus.

in Table 4. Of these 72 women, 12 did not respond to vaccination with a significant antibody titer rise to a single immunotype. No triple or double negatives remained after vaccination. One woman remained negative to Type 1, five to Type 2, and five to Type 3. The twelfth who was considered a vaccination failure did have an increase in titer for Type 3 to 1:4. Of the four triple negatives before vaccination with oral vaccine, three had not received Salk vaccine.

A tabulation of unmeasurable antibody titers among these 262 women to the three poliomyelitis immunotypes according to the number of Salk injections prior to oral vaccination is shown in Table 5. As expected, more immunotype negatives were present in the group that had not received Salk vaccine before feeding trivalent oral vaccine than in the groups that had received one or more injections. There is also a tendency displayed for the number of negative immunotypes

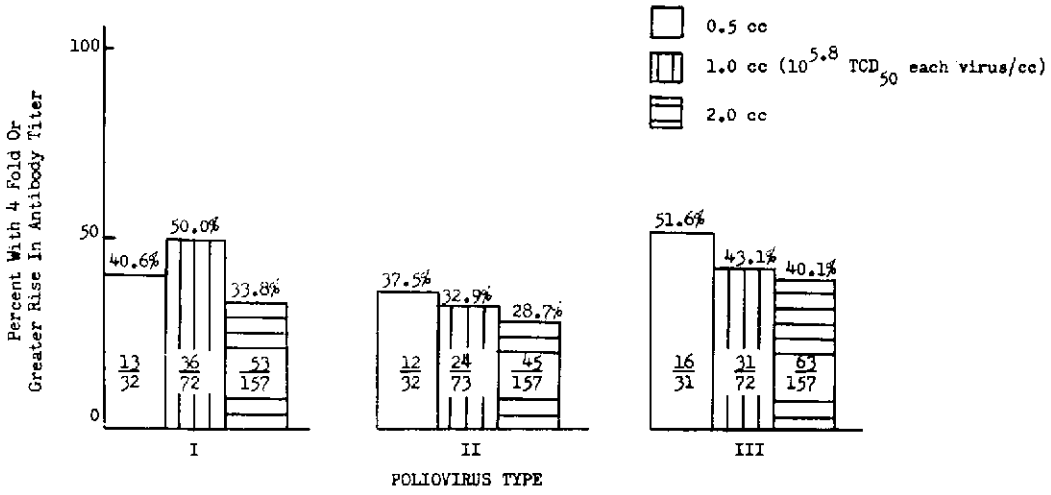


FIG. 1. Comparison of percentages of significant antibody titer rise by type among 262 pregnant women fed three different dosages of trivalent oral live attenuated poliomyelitis vaccine.

TABLE 4. DISTRIBUTION OF IMMUNOTYPE NEGATIVES AMONG 262 PREGNANT WOMEN BEFORE AND AFTER FEEDING OF TRIVALENT ORAL POLIOMYELITIS VACCINE

		Numbers with Unmeasurable (<1:4) Antibody Titer							
		BMH		GIH		UH		TOTALS	
	Polio Immunotypes	Before	After	Before	After	Before	After	Before	After*
Triple negative	1-2-3	4	1 (T_2)	2	0	0	0	6	1
Double negative	1-2	1	0	2	0	0	0	3	0
	1-3	6	1 (T_2)	3	0	6	1 (T_1)	15	2
	2-3	5	1 (T_2)	0	0	0	0	5	1
Single negative	1	5	0	3	0	4	0	12	0
	2	1	0	1	1	2	2	4	3
	3	11	3	9	0	7	2	27	5
Total with one or more negative immunotypes		33	6	20	1	19	5	72	12
Total pregnant women		109	109	100	100	53	53	262	262
Percent with one or more negative immunotypes		30.3	5.6	20.0	1.0	35.9	9.4	27.5	4.6

*All single negatives.

to decrease with successive injections of Salk vaccine. The per cent of successful responses after feeding trivalent oral vaccine in each group is the same. Although the numbers are small this suggests that Salk vaccine does not influence the antibody conversion rate of the oral vaccine by anamnestic action. Among the total number of unmeasurable titers to all immunotypes, 89.7 per cent responded to feeding by a four-fold or greater increase in antibody titer.

TABLE 5. RESPONSE TO TRIVALENT ORAL LIVE ATTENUATED POLIOMYELITIS VACCINE AMONG 72 PREGNANT WOMEN WITH ONE OR MORE UNMEASURABLE (<1:4) PRE-VACCINATION ANTIBODY TITERS AND CLASSIFIED ACCORDING TO IMMUNOTYPE AND NUMBER OF PREVIOUS SALK VACCINE INJECTIONS

Number of Previous Salk Injections	Number of Pregnant Women		Antibodies, Type: Before Vaccination — Number with Antibody Titer <1:4			After Vaccination — Number with Four-Fold or > Increase		
	Total	With 1 or More Antibody Titers <1:4	1	2	3	1	2	3
None	64	35	17	13	25	17	11	22
One	28	6	3	0	5	3	0	4
Two	58	16	9	1	11	9	0	10
Three or More	112	15	7	4	12	6	3	11
Totals	262	72	36	18	53	35	14	47

Number unmeasurable (<1:4) titers to all immunotypes.....107.
Number and per cent with four-fold or greater increase.....96 (89.7%).

The antibody titer response of three pregnant women to trivalent oral polio vaccine within 11 to 15 days after feeding is shown in Table 6. Two of these, P.M. and K.E., had fourfold or greater responses to all three immunotypes. The third had a two-fold response to Type 1 in addition to a significant titer rise to Types 2 and 3. These responses were the best three among 20 women who had antibody titers determined between six to 20 days after feeding.

TABLE 6. IMMUNOLOGIC RESPONSE OF THREE PREGNANT WOMEN TO TRIVALENT ORAL LIVE ATTENUATED POLIOMYELITIS VACCINE 11 TO 15 DAYS AFTER FEEDING

		E.H.	P.M.	K.E.
Age		20	19	17
Previous Salk Injections		2	0	2
Dose		2.0 cc*	1.0 cc*	0.5 cc*
Pre-Post Interval		11 days	15 days	14 days
Poliovirus Type	Pre	1:4	1:4	1:128
I	Post	1:128	1:512	1:512
	Pre	1:512	1:128	1:128
II	Post	>1:1024	>1:1024	>1:1024
	Pre	1:128	<1:4	1:32
III	Post	1:256	1:8	1:512

* $10^{5.8}$ TCD_{50} each virus/cc.

It has been suggested by Cox[8] that a repeat feeding of trivalent oral vaccine four to six weeks after initial feeding might produce better over-all results than a single feeding. To determine if this could be done, a number of pregnant women were revaccinated with slightly different schedules and in different dosages. Of this group complete antibody titer results are available for 39. Five of these were re-fed 0.5 cc. of trivalent oral vaccine twice at four-week intervals; three were given a single repeat feeding of one cc. at six-week intervals, and 31 were fed a second two-cc. dose six weeks after the first. The result of this program is shown on Table 7.

The upper half of this table shows the number of two and four-fold increases and decreases for all 39 women after comparing the pre-feeding and the post-feeding titers for each successive dose of vaccine fed. For each immunotype the number of antibody titer decreases after each revaccination was the same or greater than the number of antibody titer increases.

Because the antibody response of an individual with a low antibody titer is usually better

TABLE 7. SUMMARY OF IMMUNOLOGIC RESPONSE OF 39 PREGNANT WOMEN WHO WERE FED TRIVALENT ORAL ATTENUATED POLIOMYELITIS VACCINE[1] TWO OR THREE TIMES AT 4-6 WEEK INTERVALS

		POLIOVIRUS											
		Type I			Type II			Type III			Totals		
	Antibody Titer Change	Post 1st feeding	Post 2nd feeding	Post 3rd feeding	Post 1st feeding	Post 2nd feeding	Post 3rd feeding	Post 1st feeding	Post 2nd feeding	Post 3rd feeding	Post 1st feeding	Post 2nd feeding	Post 3rd feeding
Entire Group	4-fold or greater increase	18	2	0	12	2	1	17	4	1	47	8	2
	Same	11	13	2	15	21	3	14	14	0	40	48	5
	2-fold increase	6	10	0	8	6	0	6	5	0	20	21	0
	2-fold decrease	4	10	3	4	8	0	1	14	2	9	32	5
	4-fold or greater decrease	0	4	0	0	2	1	1	2	2	1	8	3
		39	39	5	39	39	5	39	39	5	117	117	15
	% 4-fold or greater increase	46.2	5.1	0	31.0	5.1	20.0	43.6	10.2	20.0	40.1	6.8	13.3
Prefeeding Antibody Titer 1:64 or Less	4-fold or greater increase	13	1	0	6	1	0	14	1	1	33	3	1
	Same	2	1	1	1	1	1	1	2	0	4	4	2
	2-fold increase	1	0	0	3	2	0	1	1	0	5	3	0
	2-fold decrease	0	1	2	0	2	0	0	3	1	0	6	3
	4-fold or greater decrease	0	0	0	0	1	0	0	0	0	0	0	0
		16	3	3	10	7	1	16	7	2	42	16	6
	% 4-fold or greater increase	81.3	33.3	0	60.0	14.3	0	87.5	14.3	50.0	78.6	18.8	16.7

[1] 5 fed 0.5 cc × 3 at monthly intervals.
3 fed 1.0 cc × 2 at 4-6 week interval.
31 fed 2.0 cc × 2 at 6 week interval.
($10^{7.8}$ TCD_{50} each type virus/cc)

to vaccination than one with a high titer, all prevaccination titers of 1:64 or less were separated from the total group and analyzed separately. The lower one half of Table 7 shows the responses to vaccination and revaccination of this low-titer group. Although the numbers are small the results from revaccination in this low titer group are no better than those for the larger group. In general, those women with poor antibody responses to the first feeding were the same who failed to respond to revaccination.

To determine if there is a teratogenic risk to the fetus when the pregnant woman is fed oral live attenuated viruses during early pregnancy, a follow-up of infants born of women vaccinated in this study and an earlier study by two of the authors [8] was done. Hospital newborn examination records were available and examined for all infants born in BMH, CIH, or at University of Minnesota Hospitals. In addition to the infants born of women vaccinated in this and the other study referred to, those born of women who were known to have been pregnant and fed oral poliomyelitis vaccine in the studies conducted by Barr and associates [9] were followed. The status of most of these infants was determined by a questionnaire sent to the physician attending the delivery. Some of these infants were delivered at University of Minnesota Hospitals or known personally by one of the authors. There were 69 women fed trivalent or one or more monovalent strains of oral poliomyelitis vaccine prior to the 20th week of gestation as measured from the last menstrual period and in whom the outcome of the pregnancy was known. Of these 69 women, 58 had a significant antibody titer response to one or more immunotypes after feeding of the vaccine. In this group there were five spontaneous abortions. Four occurred among women who were pregnant for ten weeks or less at the time of vaccination. One of these, a patient of one of the authors (KAP) was fed the vaccine at a time when

she was threatening to abort. She had no significant antibody titer rise after the feeding. The other three who aborted had significant antibody titer rises to one or more immunotypes. The details of their abortions are not known. One young woman vaccinated during the 17th week of gestation had a missed abortion. She had experienced no antibody titer rise after 2.0 cc. of trivalent oral vaccine. There is no evidence that the feeding of the vaccine played any part in her abortion.

Among these 69 women there were three who produced infants with congenital abnormalities. One of these was vaccinated sequentially at three-week intervals with monovalent strains of attenuated virus beginning two weeks before her last menstrual period. There was no antibody response to vaccination. The infant was normal except for a unilateral talipes equinovarus—a positional defect not related to administration of the oral vaccine. The other two infants with congenital abnormalities had more severe defects. One infant had a bilateral cleft palate, the other a spina bifida and renal abnormalities. The mothers of both of these had been vaccinated during the 16th week of gestation with 2.0 cc. of trivalent oral vaccine. The abnormalities exhibited by these infants are developmental and arise about the fourth to seventh week ovulation age or the sixth to ninth week of gestation as measured from the last menstrual period. The oral vaccine was given too late in pregnancy to be associated with these defects and therefore can be dismissed as an etiologic factor.

DISCUSSION AND CONCLUSIONS

Superficial analysis of the first three tables presented gives the impression that the response of pregnant women to trivalent oral vaccine is not very good. An evaluation of these tables shows that an antibody titer of 1:128 or higher was present before feeding for more than one-half of the women studied for each immunotype. This reflects a heavier natural antibody protection, more Salk injections or a combination of the two. A higher frequency of high antibody titers among pregnant women is to be expected. Probably no adult group has been more heavily vaccinated with Salk vaccine than these women selected by pregnancy. Among any group with antibody titers in this high range a poorer overall response to vaccination as measured by significant antibody titer rise is to be expected. Measurements other than total response, therefore, are necessary to measure the effectiveness of the vaccine. If the significant responses to oral vaccination of all women in this group with antibody titers of 1:64 and below are considered, the per cent of four-fold antibody titer increases to about 70 per cent for Type 1, about 50 per cent for Type 2, and about 75 per cent for Type 3.

The real worth of an immunizing agent however, is measured by its ability to produce significant antibody titer rises among those who are antibody negative at the time of vaccination. When this measurement is made among the pregnant women in this study a significant antibody titer response was found to have occurred in 89.7 per cent of all immunotypes. This response almost is identical to that reported by Cox [5] among 392 antibody negatives who were fed two cc. of vaccine. Two fifths of the individuals in this series however, received only 0.5 or 1.0 cc. of vaccine. This suggests that the larger dose used by Cox may not be necessary. The results of this study and those reported by Cox demonstrate the uniformity of results that can be obtained with this vaccine among widely separated groups of people. In this study, better results as measured by significant antibody titer responses were not obtained among the immunotype negatives that had previously received one or more Salk injections than among those negatives who had not received Salk vaccine.

Significant antibody responses were occasionally seen to one, two, or all three immunotypes as early as 11 days after injection of the trivalent vaccine. Plotkin [6] and associates have reported a significant antibody titer elevation in an infant 14 days after ingestion of CHAT (Type 1) strain.

Re-feeding of pregnant women with trivalent oral vaccine one or two times at four to six week intervals does not produce results comparable to the initial feeding of the vaccine. Analyses of those with low initial antibody titers show that those that fail after the first feeding also fail after the second and third doses when given at these intervals. These results indicate that an attempt to revaccinate with trivalent oral vaccine six or fewer weeks after the first feeding is not a worthwhile undertaking. One of the au-

thors [10] has unpublished data showing that refeedings of trivalent oral vaccine at a six to 12 month interval to adults other than pregnant women produce a better over-all immunologic response.

The definite association of rubella, when contracted by the pregnant woman during the first trimester of gestation, with the production of developmental effects in the fetus has sensitized obstetricians to all viral diseases occurring during pregnancy. Recently it has been shown [10] that certain attenuated viruses injected into pregnant animals produce teratogenic effects in the fetuses without any observable sign of illness in the mother. Although no association between wild poliomyelitis virus and congenital abnormalities has been made, several authors have reported a possible increase in abortion rate in even mild cases of poliomyelitis.[11, 12, 13]

Although data has been collected from the infants of 69 women vaccinated before the twentieth week of gestation we are especially interested in those 26 who were fed live attenuated poliomyelitis vaccine during the first 13 weeks of their pregnancy. Of these, 20 showed a significant antibody titer rise to one or more immunotypes. No congenital abnormalities attributable to the feeding of the trivalent oral vaccine were seen. Of the four abortions in this group vaccinated during the first trimester one was definitely not related to the administration of the vaccine and can be removed from consideration. Of the 25 patients that remain three aborted and could conceivably be related to the administration of the vaccine. Since the expected rate of abortion is 10 to 12 per cent for all pregnancies, these three represent an incidence that is no higher than that which would be expected to occur by chance alone.

SUMMARY

Trivalent oral live attenuated poliomyelitis vaccine in three different dosages were given to 310 pregnant women in all trimesters of pregnancy.

The immunologic response among these women was comparable to that achieved by others in non-pregnant individuals.

No statistically significant differences could be detected between the results achieved with the three different dosages used.

Although the number of observations is small no increase in abortions above that expected by chance alone was observed among those women vaccinated during the first trimester of pregnancy. No teratogenic effects attributable to the administration of the vaccine were seen.

Part 2. Immunization of Young Infants Under Six Months with Live Attenuated Oral Poliomyelitis Vaccine

Methods and Materials

Participants. Infants of six months of age or younger who were born at University of Minnesota Hospitals participated in the study. Some of the parents and siblings of these infants had participated in earlier studies of oral and Salk poliomyelitis vaccine conducted by the authors. Both clinic and private patients are included in the group.

For this study the infants were divided into two groups. One group composed of newborns was vaccinated at a median age of five days. The other group composed of infants two and one-half to six months old was vaccinated at a median age of four months.

Some of the infants in both groups were lost to the study when parents objected to blood drawing or moved from the area. About 90 infants in the newborn group and 47 in the older group returned one or more times to allow the taking of blood for post-vaccination antibody titer determinations. With a few exceptions a cord blood was obtained at the time of delivery from each infant studied to determine the level of passively transferred maternal antibody titer at birth.

No newborn infant included in any of the results tabulated in this report received Salk vaccine prior to the determination of the result of the oral vaccine feeding. Two infants in the group fed at age four months received a single injection of Salk vaccine between birth and the time of vaccination with oral vaccine. Neither of these infants had a measurable antibody titer to immunotypes 1 or 3 at the time of feeding.

Because cord blood had not been obtained from either of these infants, it could not be determined whether the pre-feeding antibody titer present for Type 2 was Salk-induced or the result of passive transfer from the mother.

Vaccine. Refer to preceding section dealing with the vaccination responses of pregnant women.

Administration of the Vaccine. Each of the infants was fed several drops of the vaccine at a time from an eye dropper until 0.5, 1.0, or 2.0 cc. were given. The vaccine was placed on the back of the tongue and followed in most instances by a feeding at the breast or bottle. With few exceptions, all newborns were vaccinated just prior to discharge from the newborn nursery.

Of the 90 newborn infants who returned one or more times for blood drawing after vaccination, nine had received 0.5 cc. of lot No. 800 and three 0.5 cc. of a mixture of lots No. 7-1231-121, No. 7-1232-216, and No. 7-1233-318. Thirty-four had received 1.0 cc. of lot No. 801-2; nine, 1.0 cc. lot No. 800; and 15, 1.0 cc. of lot No. 801. Of the 20 newborns receiving 2.0 cc. of vaccine, nine received lot No. 804-A; one, lot No. 801; and 10, lot No. 801-2.

Of the 47 older infants with follow-up antibody studies completed, 11 received 0.5 cc. of vaccine. Of these, six received lot No. 800 and five a mixture of lots No. 7-1231-121, No. 7-1232-216, and No. 7-1233-318. Five received 1.0 cc. of vaccine. Of these, four received lot No. 801-2 and one, lot No. 800. Thirty-one infants were fed 2.0 cc. of lot No. 804A.

Blood Collection. Cord blood was collected at the time of delivery from the infants in both study groups to determine the titer of the passively transferred maternal antibody. All other blood samples were obtained by jugular or antecubital venipuncture. In the newborn group blood for antibody determinations was drawn at the median ages of 45, 186, and 375 days. In the older age group, a blood sample for antibody determination was taken at the time of feeding of the vaccine and again at about age six months and age 12-18 months. All blood samples were aseptically collected without an anticoagulant.

Laboratory. Refer to preceding section dealing with the vaccination responses of pregnant women.

Calculation of Antibody Titer Response to Vaccination in the Young Infant. Because techniques for antibody titer determinations do not differentiate between the sources of antibody, it is necessary, if the effect of vaccination in the young infant is to be known, to be able to determine at any age the amount of circulating passively transferred maternal antibody that remains.

A previous study [11] of antibody transfer from mother to newborn infant and follow-up of the infant during the first year of life has shown that the poliomyelitis antibody disappears from the newborn at a uniform rate with a half-life value of 37 days. This is shown graphically in Fig. 2. This half-life value was found to be the same whether the maternal antibodies were induced by injections of Salk vaccine during pregnancy or whether they were naturally occurring. Other substantially different half-lives for passively transferred poliomyelitis antibodies have been reported.[6, 12] To determine if the 37 day half-life was sufficiently accurate to apply to our studies a second blood specimen was taken at the time of vaccination in the older infant group. The antibody titer of this blood specimen paired with the cord blood was determined and plotted on a correlation square against the residual passively transferred maternal antibody titer as calculated from the cord blood titer using the

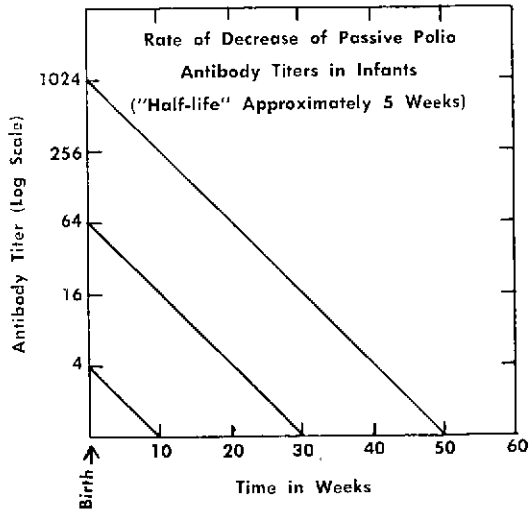


FIG. 2

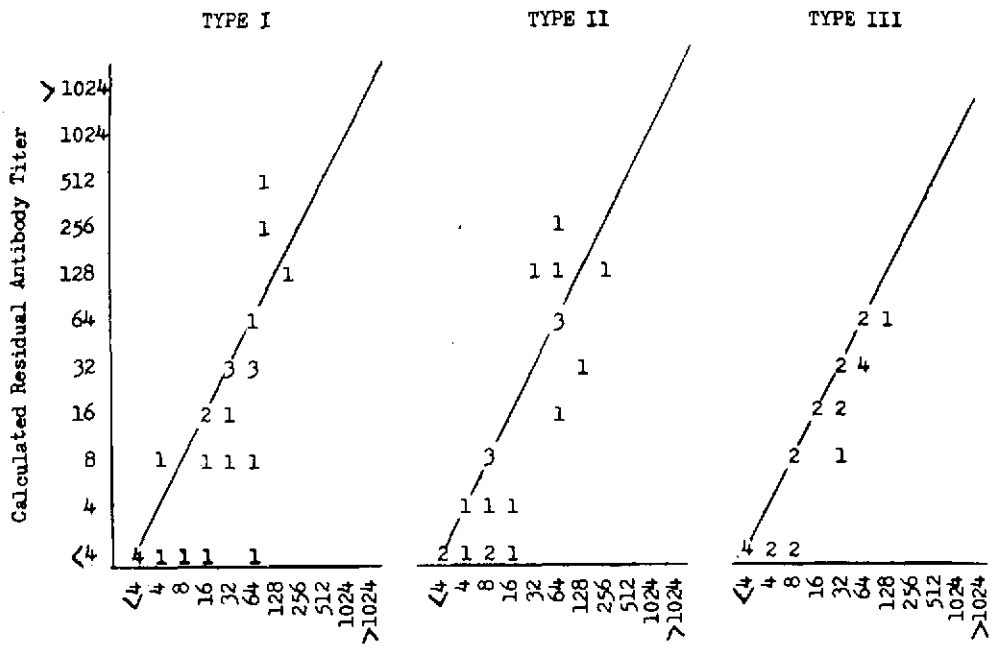


FIG. 3. Residual passively transferred maternal antibody titer of 47 infants at median age of four months plotted against measured titer at time of vaccination with trivalent oral live attenuated polio vaccine (residual titer calculated from cord titer using antibody half-life of 37 days.)

37 day half-life. The results of this comparison are shown in Fig. 3. If the 37 day half-life is an accurate ruler with which to evaluate the decay rate of the passively obtained antibody, comparisons made should fall on the diagonal line. Although many comparisons are clustered about the diagonal line, Fig. 3 shows that the half-life of those studied is probably slightly but not substantially less than 37 days. From these data it is concluded that the 37 day half-life can be used for the calculations proposed without favorably influencing the results of vaccination.

To determine the residual passively transferred maternal antibody titer quickly, one half of the titer at birth was assumed to be present at 19 to 56 days (one half to one and one half times the half-life), one fourth from age 57 to 94 days (one and one half to two and one half times the half-life), etc. In all cases if the post-vaccination antibody titer of an immunotype was fourfold greater than the calculated remaining maternal antibody titer the response was considered significant and the vaccination successful for that type. This rule of thumb was applied where appropriate to both groups of infants.

RESULTS

Newborn Infants. Paired cord and post-vaccination blood samples were available for comparison at median age of 45 days for 83 infants; of 186 days for 80 infants; and 375 days for 31 infants who were fed the vaccine. Sixteen infants were born lacking measurable antibody titers to one or more immunotypes. Two lacked antibody titers to Types 1 and 3. Two others lacked antibody titer to Types 2 and 3. All of these experienced a significant titer rise to all types except one Type 2. Twelve of the infants had single negative immunotypes—three for Type 1, two for Type 2, and seven for Type 3. Each of these single negatives was converted by a significant antibody titer rise after vaccination. The only failure in the entire group had received 2.0 cc. of vaccine. The per cent of infants with significant responses as previously defined at median ages 45, 186, and 375 days is shown in Fig. 4. This figure displays a definite lag in antibody titer response of the newborn infant to the oral vaccine. The delay in maximum antibody response demonstrated is probably not the result of reinfection of the infant fed. Many of the

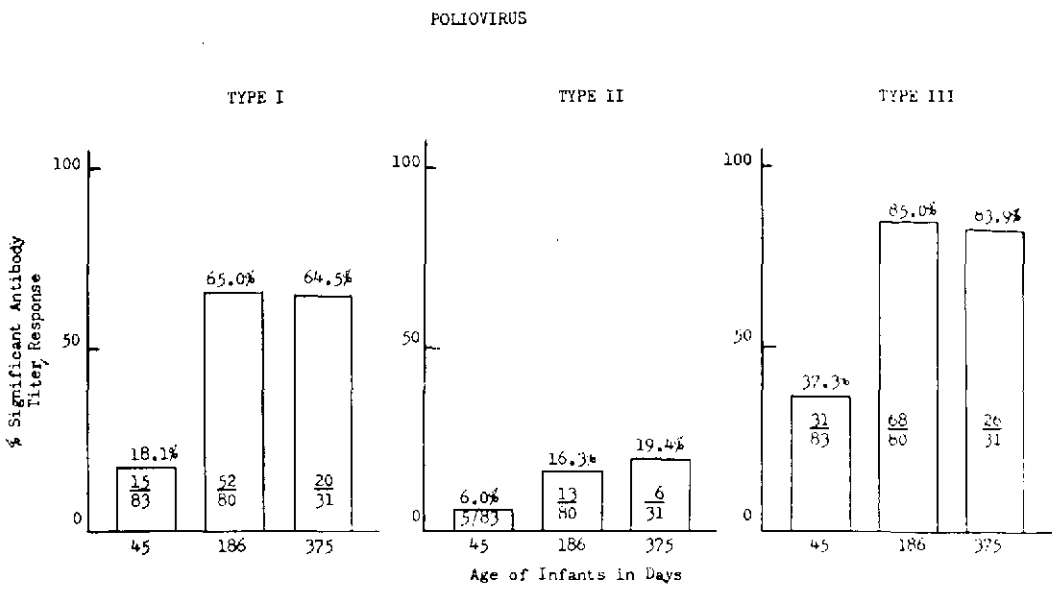


FIG. 4. Percent significant antibody titer response[1] at 45, 186, and 375 days of age among infants fed trivalent oral attenuated live poliomyelitis vaccine on the 5th day of life.[2]

[1] Four-fold or greater rise above the residual level of passively transferred maternal antibody titer at the same age as calculated by half-life value. Half-life of placentally transferred poliomyelitis antibody is 37 days.
[2] All ages are median ages.

infants who showed this delayed response had no siblings from which a reinfection could be obtained. It is much less probable that the source of reinfection is a parent. There is no significant difference between the per cent showing response at six months and those showing response at 12 months.

There is stability of the antibody titer between six and 12 months if a successful vaccination has occurred. The titer remains at about the same level plus or minus one tube. These observations confirm the prediction made in a preliminary report [1] that an interference or a masking of antibody response by a high cord blood titer might be present.

The influence of high and low cord antibody titers on success of vaccination among newborn infants as measured at median age of 186 days is shown in Table 8. Successful vaccination was achieved among infants with cord titers of 1:256 to 1:1024 in 47 per cent for Type 1; 12 per cent for Type 2; and 73 per cent for Type 3. This compares with the per cent of successful vaccination when the cord titer is 1:128 or less of 89 per cent for Type 1, 25 per cent for Type 2, and 91 per cent for Type 3. The differences between the numbers of successful vaccinations at the high and low cord titer levels for Types 1 and 3 are statistically significant. Infants for whom a cord blood was not available or the cord antibody titer was $> 1:1024$, are not included in Table 8. The antibody responses of these infants could not be determined as previously defined.

The per cent of successful vaccination to Type 1 among newborn infants according to dosage of vaccine used and age of the infant at the time the vaccination was evaluated is shown in Fig. 5. There is no striking correlation between the dosage fed and the success of the vaccination. This same evaluation for Types 2 and 3 is shown in Figs. 6 and 7. Again there is no correlation between success of vaccination and dosage fed.

Infants Age Four Months of Age. The antibody responses of the 47 infants vaccinated at median age four months for all three types of poliovirus are shown in Tables 9, 10, and 11. The antibody titer four to eight weeks after vaccination is compared to the residual passively trans-

TABLE 8. INFLUENCE OF PASSIVELY TRANSFERRED MATERNAL ANTIBODY ON VACCINATION RESPONSE OF 80 INFANTS FED TRIVALENT ORAL LIVE ATTENUATED POLIOMYELITIS VACCINE AT AGE 5-7 DAYS

CORD BLOOD ANTIBODY TITER	VACCINATION RESULT AT SIX MONTHS	TYPE I		TYPE II		TYPE III		TOTALS	
		#	%	#	%	#	%	#	%
1:256 to 1:1024	Successful*	15	47	4	12	19	73	38	41
	Unsuccessful	17	53	30	88	7	27	54	49
<1:4 to 1:128	Successful*	33	89	7	25	43	91	83	74
	Unsuccessful	4	11	21	75	4	8	29	26
Cord blood missing or titer >1:1024		11		18		7		36	
Totals		80		80		80		240	

* Antibody titer at six months four-fold or greater than residual titer of passively transferred maternal antibodies at the same age as calculated by antibody half-life (37 days).

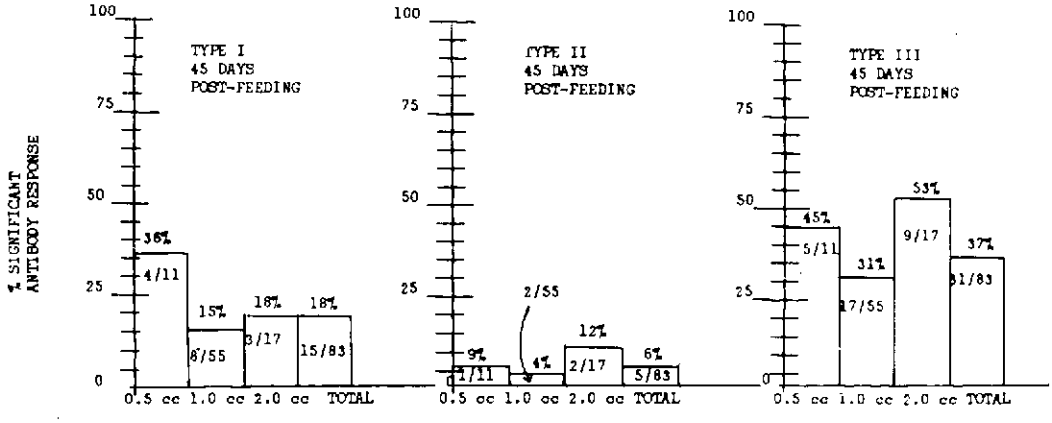


FIG. 5. Percent significant antibody titer response at age 45 days of 83 infants fed three different dosages[1] of trivalent oral attenuated live poliomyelitis vaccine on the 5th day of life.[2]

[1] Each cc of vaccine contains $10^{5.8}$ TCD_{50} of each virus.
[2] All ages are median ages.

ferred maternal antibody titer as calculated for the same time by the half-life value of 37 days. At time of vaccination there were 25 with unmeasurable titers (< 1:4) to Type 1, 20 to Type 2, and 26 to Type 3. With one exception all of those with an unmeasurable antibody titer to Types 1 and 3 responded with a significant rise in titer. The lone exception was a Type 1 that responded to vaccination with a booster response to the 1:4 level. The response to the vaccine of those with unmeasurable Type 2 antibody titers was of a low order. Only 20 per cent demonstrated a significant antibody titer rise after vaccination. These could be heterotypic responses. Successful vaccination, as previously defined, was achieved in 77 per cent for Type 1, 19 per cent for Type 2, and 94 per cent for Type 3.

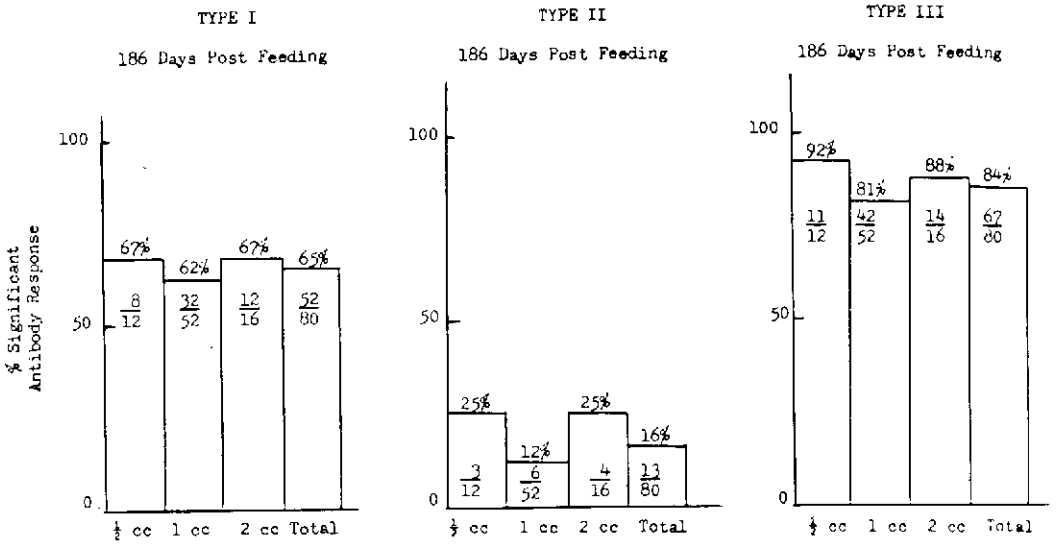


FIG. 6. Percent significant antibody titer response at age 186 days of 80 infants fed three different dosages[1] of trivalent oral attenuated live poliomyelitis vaccine on the 5th day of life.[2]

[1] Each cc of vaccine contains $10^{5.8}$ TCD_{50} of each virus.
[2] All ages are median ages.

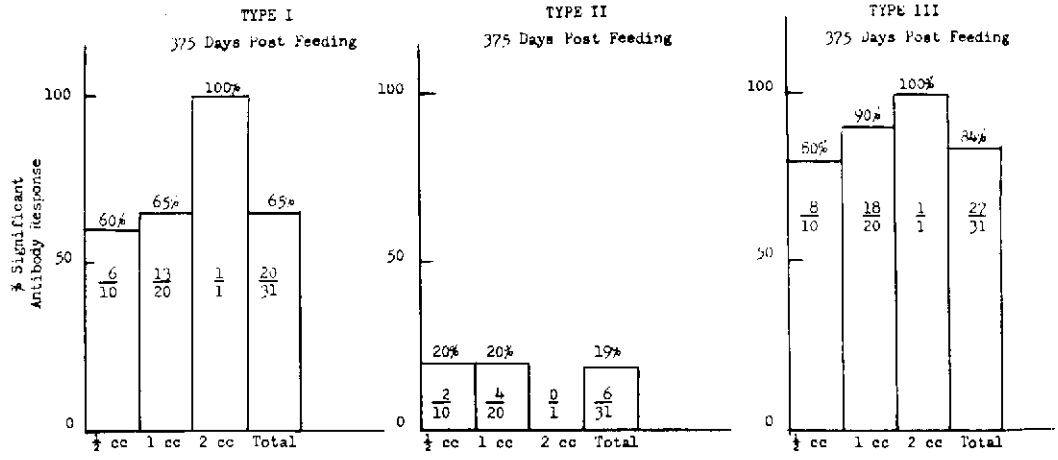


FIG. 7. Percent significant antibody titer response at age 375 days of 31 infants fed three different dosages[1] of trivalent oral attenuated live poliomyelitis vaccine on the 5th day of life.[2]

[1] Each cc of vaccine contains $10^{5.8}$ TCD_{50} of each virus.
[2] All ages are median ages.

A comparison of antibody titers by immunotype at birth, before and after vaccination at median age four months and according to dosage of vaccine fed, is shown in Fig. 8. The antibody titer responses to Types 1 and 3 are obvious. The antibody titers to Type 2 appear to decay at the half-life rate.

Revaccination. At the present time an infant revaccination program is under way. Each infant in the study will be revaccinated if the response to first feeding is poor or when two or three titers reach a low level. The usual blood controls are being obtained. To date the results of three such revaccinations are complete. One

TABLE 9. POLIOVIRUS TYPE I ANTIBODY RESPONSE OF 47 INFANTS FED TRIVALENT ORAL ATTENUATED LIVE POLIOMYELITIS VACCINE AT AGE ABOUT FOUR MONTHS

Calculated Residual Titer*	Antibody Titers Post Feeding											Total Number Fed	Number With 4-Fold Or Greater Response
	<1:4	1:4	1:8	1:16	1:32	1:64	1:128	1:256	1:512	1:1024	>1:1024		
<1:4		1			2	2	8	4	7	1		25	24
1:4						1	1					2	2
1:8				1	2	1		1	1	1		7	6
1:16						1	1	1				3	3
1:32			1	1					1			3	1
1:64					1	2	1					4	0
1:128								1				1	0
1:256						1						1	0
1:512													
1:1024													
>1:1024							1					1	0
TOTALS		1	1	2	5	8	12	7	9	2		47	36 (77%)

* Residual titer of passively transferred maternal antibodies at time of last post-vaccination blood drawing. Half-life value = 37 days.

TABLE 10. POLIOVIRUS TYPE II ANTIBODY RESPONSE OF 47 INFANTS FED TRIVALENT ORAL ATTENUATED LIVE POLIOMYELITIS VACCINE AT AGE ABOUT FOUR MONTHS

Calculated Residual Titer*	Antibody Titers Post Feeding											Total Number Fed	Number With 4-Fold Or Greater Response
	<1:4	1:4	1:8	1:16	1:32	1:64	1:128	1:256	1:512	1:1024	>1:1024		
<1:4	12	3	2		1	2						20	5
1:4	2	1	2		3							8	3
1:8	2	1										3	0
1:16		1		3			1					5	1
1:32			1		3	1						5	0
1:64					1							1	0
1:128					1		1	1				3	0
1:256						1			1			2	0
1:512													
1:1024													
>1:1024													
TOTALS	16	6	5	3	9	4	2	1	1	0	0	47	9 (19%)

* Residual titer of passively transferred maternal antibodies at time of last post-vaccination blood drawing. Half-life value = 37 days.

TABLE 11. POLIOVIRUS TYPE III ANTIBODY RESPONSE OF 47 INFANTS FED TRIVALENT ORAL ATTENUATED LIVE POLIOMYELITIS VACCINE AT AGE ABOUT FOUR MONTHS

Calculated Residual Titer*	Antibody Titers Post Feeding											Total Number Fed	Number With 4-Fold Or Greater Response
	<1:4	1:4	1:8	1:16	1:32	1:64	1:128	1:256	1:512	1:1024	>1:1024		
<1:4				2	1	3	7	5	5	2	1	26	26
1:4					2					1		3	3
1:8	2					1	1	1	2			7	5
1:16								2	1		2	5	5
1:32							1		1	1		3	3
1:64							1		1			2	1
1:128													
1:256											1	1	1
1:512													
1:1024													
>1:1024													
TOTALS	2			2	3	4	10	8	10	4	4	47	44 (94%)

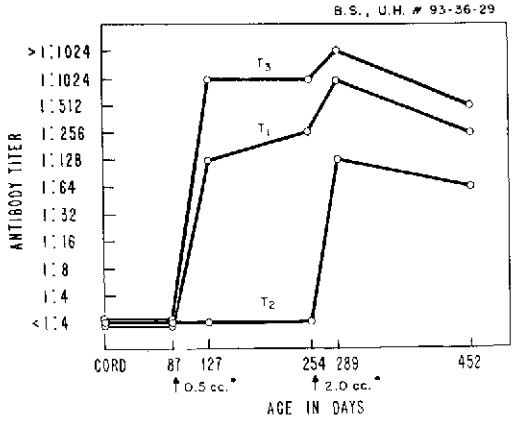
* Residual titer of passively transferred maternal antibodies at time of last post-vaccination blood drawing. Half-life value = 37 days.

	POLIOVIRUS TYPE I				POLIOVIRUS TYPE II				POLIOVIRUS TYPE III			
	Cord	Pre-Feeding	Post-Feeding		Cord	Pre-Feeding	Post-Feeding		Cord	Pre-Feeding	Post-Feeding	
Age	Birth	3-6 Mos.	4-7 Mos.	‡ 12 Mos.	Birth	3-6 Mos.	4-7 Mos.	‡ 12 Mos.	Birth	3-6 Mos.	4-7 Mos.	‡ 12 Mos.
>1024	xxxxxx				++ oo xxxx				o xxxx		o xxxxx	o
1024	o xx		o x		oo xxxx	+ o x	o		x	x	xxxx	
512	+ o xxxx xxxx	x	+ xxxxx	ooo	xxxx	o x	+ o x		xxxx xxxx		+ o xxxxxxx	oo
256	xxx	+ x	+ oo xx	+	o xx	xx			+ o xxx		o xxxxxx	+ x
128	oo xxxx	xxxx	oo xxxxxx	+ oooo xx	xxx xxx	oo xxxxx	o xxxxx		xxxxx	o xxx	+ ooo xxxx	oo x
64	+	xx	+ xxxxx xxxxxx		+ x	xxxx	+ xx	x	+ o xxx	o x	++ o xx	- o
32	+ x	+ oo * xxxxxxx	++ xxxx	+	x	++ xx	o xxxxxx	o x	x	o xxxxxxxx	- xx	+ o
16	o	xxxxx	o xx		x	+ xx	+ xx	oo		+++ xxx	x	
8	x	+ oo xx	o x		o	xxxxx	+ ooo xx	+ oo	o x	+ o xxx		
4	x	+ o			x	oo xxxx	xxxxx		o			
<4	o x	+ oo xxxxxxxxx				+ o xxx	++ xxx xxxxxx	++ oo	o xx	ooo + xxxxx xxxxx	x	

* 1.0 cc vaccine
° 0.5 cc vaccine
x 2.0 cc vaccine

FIG. 8. Comparison of antibody titers by immunotype at birth and before and after vaccination at age about four months with trivalent oral attenuated live poliomyelitis vaccine.

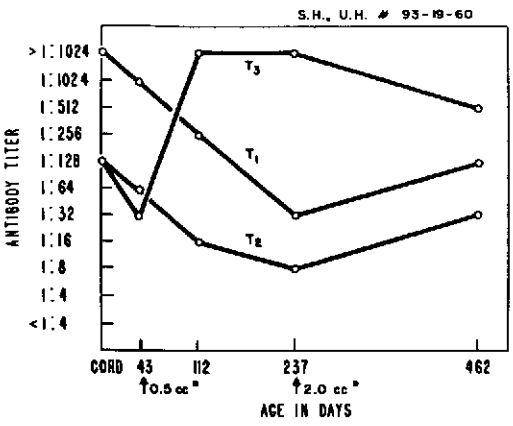
infant with high Type 1 and 3 titers after receiving 0.5 cc. of trivalent vaccine at age four months, was revaccinated with 1.0 cc. of vaccine to increase an unmeasurable titer for Type 2. This failed. The responses of the other two infants are shown graphically in Figs. 9 and 10. The antibody profile of an infant who was a triple negative at birth and at three months of age is shown in Fig. 9 before and after two doses of trivalent oral poliomyelitis vaccine given six months apart. After the first feeding of 0.5 cc., an excellent antibody response to Types 1 and 3 was obtained. The antibody titer to Type 2 remained unmeasurable. A 2.0 cc. feeding was given at age 254 days. At this point the Type 2 titer was still unmeasurable. Blood samples obtained five weeks later showed a booster response to Types 1 and 3 as well as an initial response to Type 2. Two hundred days after revaccination antibody titers to all three immunotypes were 1:64 or greater. The third infant was vaccinated at age 43 days with 0.5 cc. of vaccine. A low residual titer of 1:32 to Type 3 was increased to



* $10^{5.8}$ TCD_{50} each type virus/cc.

FIG. 9. Immunologic response of a triple negative 3 months old infant after feeding two doses of trivalent oral poliomyelitis vaccine at intervals of six months.

> 1:1024. The Types 1 and 2 titers in spite of vaccination continued to decay at a rate compatible with a half-life of 37 days. At 237 days of age a larger dose of vaccine (2.0 cc.) was fed.



* $10^{5.8}$ TCD_{50} each type virus/cc.

FIG. 10. Immunologic response of a six weeks old infant after feeding two doses of trivalent oral poliomyelitis vaccine at intervals of six months.

This resulted in a significant simultaneous four-fold antibody titer rise to Types 1 and 2 when blood was obtained six months after vaccination.

Stool Excretion of Viruses. A weekly stool specimen was obtained from 15 infants fed 2.0 cc. of trivalent oral vaccine. All members of the families of seven of these infants also collected weekly stools for ten weeks to determine the pattern of intrafamilial spread after vaccination with trivalent vaccine. Stools were frozen and kept in the deep freeze or winter outdoors until shipped to Lederle Laboratories by air express for virus isolation.

The types of viruses excreted and the duration of excretion among the infants fed are shown in Table 12. Maximum duration of excretion of a single type was eight weeks. Six of the seven infants in the older age group, but only two of the eight in the younger age group excreted Types 1 and 3 simultaneously. Duration of excretion for Types 1 and 3 viruses singly or together was longer in the older than in the younger age group. Each infant in the older age group had at least one type of virus in the stool at the end of the first week. Type 2 virus was not isolated from any of the stools of these 15 infants. An older sibling of one of the infants included in this table was also fed 2.0 cc. of vaccine. All three types of virus fed were isolated from her stool one week after feeding. No viruses were isolated from the infants fed after the eighth week.

Spread to Contacts. Evidence of spread to contacts by virtue of virus isolation from the stool is presented in Table 13. Three of six sibling contacts of infants fed at age five days excreted virus during the ten-week study. One of these had both Type 1 and 3 isolated from his stools. Two of three siblings of the two infants vaccinated at age four months excreted virus. Of the nine siblings exposed to contact infection four had been previously immunized by oral poliomyelitis vaccine and had never received Salk vaccine. Of these four, two had a single stool isolation of Type 3, one at one week, and one at eight weeks after vaccination of the infant studied. No isolations were made from the other two. The other five had been previously immunized with three or four injections of commercial Salk vaccine. Of these five, two had stools negative for poliovirus throughout the period of study. The other three excreted Type 3 virus—one for two, one for four, and one for five weeks. In addition one shed Type 1 virus along with a Type 3 for two weeks. These findings suggest that previous vaccination with oral poliomyelitis vaccine may prevent or shorten the period of excretion of a poliovirus obtained through contact infection. A Type 1 virus was isolated from one stool of the father of one infant.

DISCUSSIONS AND CONCLUSIONS

When a group of newborn infants is fed trivalent oral poliomyelitis vaccine at age five days, some of the infants respond with immediate antibody titer rises within 45 days. This response varies somewhat for the three immunotypes with the best immediate response made by Type 3. Fifty per cent of these infants, however, do not develop a significant response to Types 1 and 3 until after 45 days. Both the immediate and the delayed responses are poorer to Type 2.

Between birth and age four months, something happens in an immunological way to the young infant that promotes a better immediate response to an antigenic stimulus. This has been observed previously by Osborn.[15] Proof that this is also true for oral poliomyelitis vaccine in this study is shown in the figure on page 225. This figure compares the per cent of successful response at age 45 days among those infants vaccinated at age five days to the per cent of response four to six weeks after vaccination of infants at age four months.

TABLE 12. DISTRIBUTION OF TIME INTERVALS BETWEEN FEEDING AND LAST VIRUS ISOLATION FROM EIGHT INFANTS FED TRIVALENT ORAL LIVE ATTENUATED POLIOMYELITIS VACCINE* AT AGE 5-7 DAYS AND SEVEN INFANTS FED AT AGE 2-5 MONTHS

		NUMBER OF INFANTS (FED AGE 5-7 DAYS)				NUMBER OF INFANTS (FED AGE 2-5 MONTHS)			
		T_1	T_2	T_3	T_1T_3	T_1	T_2	T_3	T_1T_3
Time in Weeks	0**	5	8	1	6	0	7	0	1
	1	2	0	5	1	6	0	7	6
	2	3	0	6	2	5	0	5	4
	3	3	0	3	1	5	0	4	2
	4	1	0	1	0	3	0	5	2
	5	1	0	1	0	2	0	5	2
	6	1	0	0	0	0	0	4	0
	7	2	0	0	0	1	0	1	0
	8	1	0	0	0	0	0	1	0
	9	0	0	0	0	0	0	0	0
	10	0	0	0	0	0	0	0	0
Total Infants		8	8	8	8	7	7	7	7

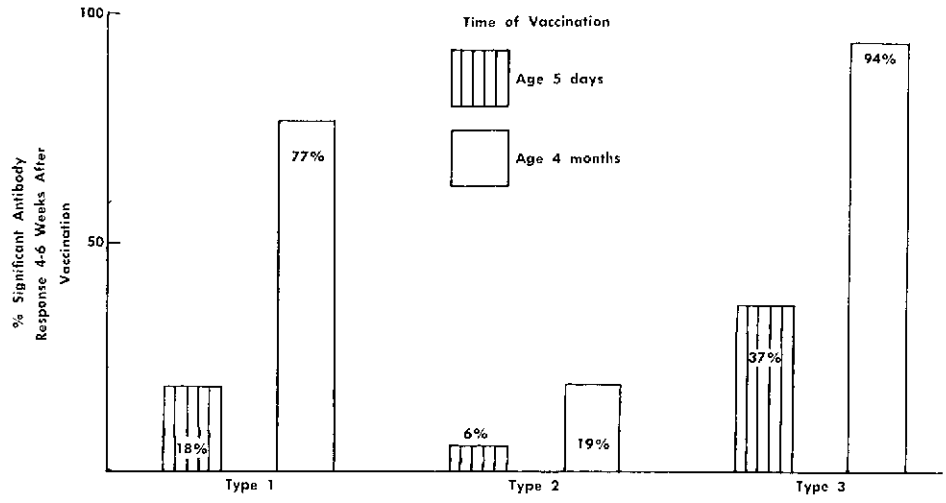
* 12 infants fed $10^{6.1}$ TCD_{50} each virus; 1 infant fed $10^{6.7}$ TCD_{50} each virus.
** Zero indicates no isolations.

TABLE 13. VIRUS ISOLATION AMONG FAMILY CONTACTS OF SEVEN INFANTS FED TRIVALENT ORAL LIVE ATTENUATED POLIOMYELITIS VACCINE*

	Sibling Contacts				Adult Contacts			
	Virus Isolated				Virus Isolated			
	T_1	T_2	T_3	Totals	T_1	T_2	T_3	Totals
Fed Age 5-7 Days (5 Infants)	1	0	3	6	1	0	0	10
Fed Age 2-5 Months (2 Infants)	0	0	2	3	0	0	0	4
Totals	1	0	5	9	1	0	0	14

* $10^{6.1}$ TCD_{50} each virus.

At least two changes are known to take place in the first four months of life that could explain this marked change in response to vaccination with attenuated oral polioviruses. Good [7] has shown that the newborn infant begins to produce gamma globulin at about age 40 days. This production increases rapidly until the infant is between 80-120 days of age. At this age the gamma globulin level is within normal range and the full immunologic capacity of the infant is nearly reached. The antibody titer passively transferred from mother to infant during intrauterine life declines rapidly after delivery and at age four months it is about one eighth (based on 37 day half-life) of the titer of the cord blood. This new ability of the infant to produce antibodies coupled with the gradual loss of the protective antibodies obtained from the mother makes the



four months infant ready for immunological response to ingested attenuated polioviruses.

Because there is considerable variation [7] in the age at which an infant reaches his full immunologic capacity, prompt responses to antigenic stimuli at any age after delivery are possible. The number of prompt responses to oral vaccine shown in this study for each immunotype confirms this.

The more frequent and higher per cent of successful responses to Type 3 is a reflection of antigenic potency. The immunologic responses among adults and children to the trivalent oral vaccine used in this study have proved consistently that this strain is more antigenic than either the Type 1 or 2. The lower cord titers for Type 3 when compared to Types 1 and 2 in the infants in this series may enhance the response to this type virus.

Stool excretion data from this study showed the Type 3 virus to have been present in the stool of 14 of the 15 infants fed. In contrast Type 1 virus was found in only 10 of 15 infants. Those infants that failed to excrete Type 1 usually had high homotypic antibody titer. The only infant that failed to excrete a Type 3 virus had an antibody titer of 1:1024. Type 3 virus was excreted from two infants with titers of greater than 1:1024. These results show that the Type 3 virus has a greater ability to establish itself in the intestinal tract than the Type 1. The Type 2 virus was not recovered from the stools of any of the 15 infants studied who were below the age of six months.

From the data presented, it can be concluded that neither vaccination at age five days nor at four months will give the infant protection to all three types of poliomyelitis viruses as measured by antibody titer response. The response to Type 2 is poor for each group. The responses to Type 1 and Type 3 are about 10 per cent better for the group vaccinated at age four months when compared to the per cent of successful delayed responses at six months for the group vaccinated at birth.

It is obvious from these data that the infants in both groups need revaccination. At age six months, the group vaccinated at age five days will respond to repeat feeding. This has been demonstrated. At age six months, however, only two months or less after previous feeding, this group vaccinated at age four months may be refractory to revaccination.

It is logical to conclude that two vaccinations with trivalent oral poliomyelitis vaccine at the suggested interval will give better over-all results than one vaccination at age four months. For this reason I favor routine vaccination at discharge from the newborn nursery followed by a repeat feeding at age six months. What per cent of failures in the over-all group will be salvaged by the second feeding is not known at this time. The answer, however, will be known in the near future.

The immunologic responses in both groups of children and the simultaneous isolation of Types 1 and 3 from the stools of 10 of 15 children fed, indicates that no serious degree of interference is

present between these two polioviruses. In those cases where simultaneous excretion of these two types was seen, Type 3 virus was excreted for a slightly longer period of time (over 4.9 weeks to 4.1 weeks). When simultaneous excretion was not seen among the newborn infants, the cord antibody titer to Type 1 was high.

The newborn is relatively free from enteroviruses at the age these feedings were carried out. This minimizes the possibility that interference between the polioviruses fed and non-polio enteroviruses accounted for the poorer response shown. When the newborn group is compared with the older group that is more likely to harbor interfering organisms, the excellent immunization and stool isolation results among the latter group again suggest that there was no interference of any kind, at least for Types 1 and 3.

The only conclusion that can be drawn from this study concerning the Type 2 strain is that it is a poor antigen in the young infant.

The ultimate answer, however, to poliomyelitis immunization may be three doses of trivalent vaccine—one given at birth, one at age six months, and another at age 12 months to every newborn infant. With this schedule the antibody titers of the infant and those of the older children in the family as well will have a continuous boosting effect each time a new addition to the family is brought home from the hospital. It seems reasonable to conclude that this kind of a vaccination program may produce a full protection for an entire lifetime against the dread effects of paralytic poliomyelitis.

SUMMARY

The antibody responses of 90 newborn infants and 47 infants four months old after feeding three different dosages of trivalent oral live attenuated poliomyelitis vaccine are reported. Virus excretion patterns of some of the infants and their intrafamilial contacts have been observed.

Although the response of the newborn infant to trivalent oral vaccination is good for Types 1 and 3, the response of the infants vaccinated at age four months is better. The response to Type 2 is poor in both groups. No difference in results dependent upon the dosages used were noted.

Revaccination is necessary for both groups. A suggested schedule for vaccination at birth, age six months and at age 12 months is favored.

ACKNOWLEDGMENTS

The authors wish to express their appreciation to Dr. Herald R. Cox and his associates at Lederle Laboratories for their valuable assistance in making this study possible. We also wish to thank Miss Margaret Giebenhain and Miss Nympha Altamarino, who did the serum separations; the nurses and aides in the North Clinic of University of Minnesota Hospitals, whose efficient assistance made the drawing of blood from infants easier; and the personnel and residents of Booth Memorial Hospital and Catholic Infants Home in St. Paul, Minnesota, for their help in distributing vaccine, keeping records, and collecting necessary blood samples. Special gratitude is due Mrs. Donna Henningsen, Dr. Robert N. Barr, Dr. Henry Bauer, and other personnel of the Minnesota State Board of Health without whose last minute help this manuscript would not have been prepared in time for distribution at this Conference.

REFERENCES

1. Prem, K. A. and McKelvey, J. L.: Immunologic response of pregnant women to oral trivalent poliomyelitis vaccine. In: *Live Poliovirus Vaccines, First International Conference.* Scient. Pub. no. 44 of the Pan American Health Organization, pp. 260-269, 1959.
2. Cabasso, V. J., Jervis, G. A., Moyer, A. W., Roca-García, M., Orsi, E. V., and Cox, H. R.: Cumulative testing experience with consecutive lots of oral poliomyelitis vaccine. In: *Live Poliovirus Vaccines, First International Conference.* Scient. Pub. no. 44 of the Pan American Health Organization, pp. 102-134, 1959.
3. Salk, J., Youngner, J. S., and Ward, E. N.: Use of color change of phenol red as an indicator in titrating poliomyelitis virus or its antibody in a tissue-culture system. *Am. J. Hyg.* **60**: 214-230, 1954.
4. Reed, L. J. and Muench, H.: A simple method of estimating 50 per cent end-points. *Am. J. Hyg.* **27**: 493-497, 1938.

5. Cox, H. R., Cabasso, V. J., Markham, F. S., Moses, M. J., Moyer, A. W., Roca-García, M., and Ruegsegger, J. M.: Immunologic response to trivalent oral poliomyelitis vaccine. In: *Live Poliovirus Vaccines, First International Conference.* Scient. Pub. no. 44 of the Pan American Health Organization, pp. 229-248, 1959.
6. Plotkin, S. A., Koprowski, H., and Stokes, J.: Clinical trials in infants of orally administered attenuated poliomyelitis viruses. *Pediatrics* **23**: 1041-1062, 1959.
7. Good, R.: Immunity and resistance to infection in early infancy. Report of 29th Ross Pediatric Research Conference. March, 1958.
8. Prem, K. A. and McKelvey, J. L.: Antibody response in adults and children following simultaneous oral administration of three type strains of live attenuated poliomyelitis virus. In: *Live Poliovirus Vaccines, First International Conference.* Scient. Pub. no. 44 of the Pan American Health Organization, pp. 249-253, 1959.
9. Barr, R. N., Bauer, H., Kleinman, H., Johnson, E. A., Martins da Silva, M., Kimball, A. C., and Cooney, M. K.: The use of orally administered live attenuated polioviruses as a vaccine in a community setting: A controlled study. In: *Live Poliovirus Vaccines, First International Conference.* Scient. Pub. no. 44 of the Pan American Health Organization, pp. 369-407, 1959.
10. Blattner, R. J., Koprowski, H.: Immunity and resistance to infection in early infancy. Report of 29th Ross Pediatric Research Conference, March, 1958.
11. Martins da Silva, M., Prem., K. A., Johnson, E. A., McKelvey, J. L., and Syverton, J. T.: Response of pregnant women and their infants to poliomyelitis vaccine. *J. Am. M. Assn.* **168**: 1-5, 1958.
12. Strean, G. J., Gelfand, M. M., Pavilanis, V., and Sternberg, J.: Maternal fetal relationships: Placental transmission of poliomyelitis antibodies in the newborn. *Canad. M. Assn. J.* **77**: 315-323, 1957.
13. Horn, P.: Poliomyelitis in pregnancy. *Obst. Gyn. N. Y.* **6**: 121-137, 1955.
14. Prem, K. A., McKelvey, J. L., and Fergus, J.: Immunologic response of infants under six months of age to oral trivalent poliomyelitis vaccine. In: *Live Poliovirus Vaccines, First International Conference,* Scient. pub. no. 44 of the Pan American Health Organization, pp. 254-259, 1959.
15. Osborn, J. J., Dancis, J., and Julia, J. J.: Studies of the immunology of the newborn infant. *Pediatrics* **9**: 736-744, 1952.
16. Prem, K. A. Unpublished data.

TOPIC III. EFFICACY. (A) LABORATORY EVIDENCE

1. THE DEVELOPMENT AND PERSISTENCE OF POLIO ANTIBODIES, MEASURED BY DIFFERENT METHODS OF NEUTRALIZATION TEST, IN YOUNG ADULTS FED WITH 100,000 TCD_{50} OF TYPE 3 ATTENUATED VIRUS

V. VONKA, E. SKŘÍDLOVSKÁ, J. JELÍNEK, AND J. DUBEN*

DR. VONKA *(presenting the paper):* Differences in the quality of antibodies in the course of poliomyelitic infection have been repeatedly discussed. Ward [1] reported on the different influence of the quantity of virus, included in the virus antibody assay, on the titer of antibodies in the acute and convalescent sera of patients with paralytic polio. Brunner and Ward [2] found later that the virus-acute-antibody complexes dissociated more readily at a low pH, than complexes of virus and antibody from the convalescent stage of infection. Sabin [3], using pH and CP tests in his studies on polio antibody development, suggested that, in the early stages of poliomyelitic infection, the antibodies of low avidity were formed, being converted in the further course of infection to high avidity antibodies. He expressed the view that the method of the pH test was the most suitable for low avidity antibody estimations. These antibodies could not be detected by the routine CP test, by means of which only high-avidity antibodies forming with virus complexes, more resistant to the influence of dilution, could be demonstrated. In persons possessing antibodies in their pre-immunization samples of sera, Sabin found earlier increases in high rather than in low avidity antibodies, and presumed a qualitative change in antibody character from low to high avidity as the first step in the antibody development in these individuals.

In our last paper,[4] we compared the results obtained with the simultaneous use of both the pH and CP tests in performing serological surveys and in studying the antibody response in persons vaccinated with inactivated and live attenuated poliovirus vaccines. On the basis of an analysis of the decrease of antibodies in the sera of infants in the first months of life, we draw the suggestion that the presence of pH antibodies only, did not necessarily signify antibodies of low avidity, but rather, low levels of antibodies not demonstrable in the less sensitive routine CP test.

In order to obtain more information about the antibody development and the changing nature of antibodies in the course of poliomyelitic infection, we vaccinated a group of young adults with Type 3 attenuated poliovirus and investigated the polio antibodies in a series of serum samples, taken at different intervals after virus was fed, using different methods of neutralizing antibody estimation. Types 1 and 2 antibody response was studied simultaneously.

MATERIALS

A group of 22 young adults were fed with about 100,000 TCD_{50} of Type 3 attenuated poliovirus (Sabin strain), Leon 12, a_1b. These subjects, between the ages 15 to 18, were selected on the basis of a preliminary serological investigation from the total number of about 60, all living in a nurses' quarters. The standard of hygiene

* Dr. Vonka and Dr. Skřídlovská (Department of Virology, Institute for Sera and Vaccines, Prague); Dr. Jelínek (Institute for Epidemiology and Microbiology, Prague); Dr. Duben (Hygiene and Epidemiology Station, Havlíckuv Brod.).

in this school was very high. None of the subjects investigated had been vaccinated previously by either Salk vaccine or by other attenuated strains.

Just before the vaccination, a new blood sample and a stool sample were taken. Further blood and stool specimens were sampled on the fourth, seventh, 10th, 14th, 21st, 28th, 35th, and 42nd day after feeding the virus. An additional blood sample was taken again on the 84th day.

The sera were inactivated at 56° C. for 30 minutes and antibiotics were added. Until investigation, the serum and stool specimens were kept at —20° C.

METHODS

1. The following methods of neutralization test were used:

(a) the routine CP test with one hour incubation of the virus-serum mixture at room temperature (CP/1);

(b) the cytopathogenic test with six hours incubation at 37° C. and overnight at +4° C. (CP/6);

(c) the pH test with one hour incubation of the virus-serum mixture at room temperature (pH/1);

(d) the pH test with six hours incubation of the virus-serum mixture at 37° C. and overnight at +4° C. (CP/6); and

(e) the immunoinactivation test according to S. Gard [5, 6] (IIT).

In tests (a) to (d), approximately 100 TCD_{50} of the virus strains Brunhilde (Type 1), MEF-1 (Type 2), and Leon 12, a_1b (Type 3) were used.

The sera from each subject were investigated simultaneously in all tests, using the same virus and serum dilutions as in Parker's medium with 0.3 per cent of glucose. The sera were diluted in fourfold steps from 1:4 to 1:1024, for IIT up to 4096. The same virus concentrations were used in CP and pH tests. In the investigation of each subject virus titrations were included, using per dilution five tissue-culture tubes in CP tests, 10 tubes in IIT and eight cups in pH tests. A standard human antiserum containing antibodies to all three types of polioviruses was tested simultaneously in all five tests performed.

Cytopathogenic tests. Tissue-culture tubes from versenated monkey-kidney cells with 1.3 ml. of Earle-LAH (0.3 per cent) medium without serum were inoculated with 0.2 ml. of the virus-serum mixture. Every dilution of serum was assayed on three tubes; when testing the standard serum four tubes were used. The results were read repeatedly in all but two cases after two, three, five, and seven days.

pH tests were carried out in polystyrene panels. The 0.4 ml. amounts of the virus serum mixtures were covered with paraffin oil, and 0.2 ml. of cell suspension containing 25,000 versenated monkey-kidney cells in the above medium, enriched by 15 per cent of monkey serum previously tested for the absence of polioviruses inhibitors, was added. The final concentration of monkey-kidney serum was then 5 per cent. Every dilution of serum was tested against every type of virus in two cups.

TABLE 1. THE MEAN TITERS ACHIEVED IN CONTROL VIRUS TITRATIONS PERFORMED SIMULTANEOUSLY WITH DIFFERENT TESTS USED

THE TITERS EXPRESSED AS LOG_{10}

TEST	TYPE 1	TYPE 2	TYPE 3
CP/1	6,70 ± 0,103	6,53 ± 0,107	6,51 ± 0,088
CP/6	6,71 ± 0,116	6,45 ± 0,060	6,37 ± 0,081
pH/1	6,83 ± 0,070	6,30 ± 0,114	6,43 ± 0,153
pH/6	6,65 ± 0,109	6,33 ± 0,117	6,31 ± 0,164
IIT	N D	N D	6,47 ± 0,097

TABLE 2. THE MEAN TITERS OF POLIO ANTIBODIES ACHIEVED IN THE REPEATED INVESTIGATION OF A REFERENCE SERUM BY DIFFERENT METHODS OF NEUTRALIZATION TEST
THE TITERS EXPRESSED AS LOG_2

TEST	TYPE 1	TYPE 2	TYPE 3
CP/1	7,37 ± 0,195	11,41 ± 0,340	4,83 ± 0,259
CP/6	10,08 ± 0,305	12,75 ± 0,164	6,50 ± 0,330
pH/1	9,27 ± 0,243	12,63 ± 0,420	8,00 ± 0,302
pH/6	10,50 ± 0,313	13,58 ± 0,248	9,00 ± 0,246
IIT	N D	N D	9,08 ± 0,268

The immuno-inactivation test was performed according to the method recommended by S. Gard. Ten tissue-culture tubes were used per one dilution. The test was read repeatedly after 3, 5, and 7 days. Only Type 3 antibodies were estimated by this technique.

The virus and antibody titers were computed according to Kärber's method.

2. The isolation and identification procedures and the virus titrations were performed as described previously.[7]

RESULTS

Results obtained in control virus and reference serum titrations. Table 1 presents the results achieved in repeated virus titrations. The mean titers of three types of polioviruses were very similar in all tests used, with only slight differences which were in no one case significant. These results suggest that only a very small proportion of virus was inactivated during the six-hour incubation period at 37° C.

The results obtained in repeated titrations of standard human serum are shown in Table 2. The mean titers, expressed as log_2 of the initial dilution of serum in the reaction mixture, show marked differences between the antibody titers estimated by means of different methods. The titers of antibodies to all three types are on the lowest level in CP/1. The prolongation of the incubation period of the virus-serum mixture had a much greater influence on the antibody titer in the CP than in the pH test.

Tables 1 and 2 show the results obtained from

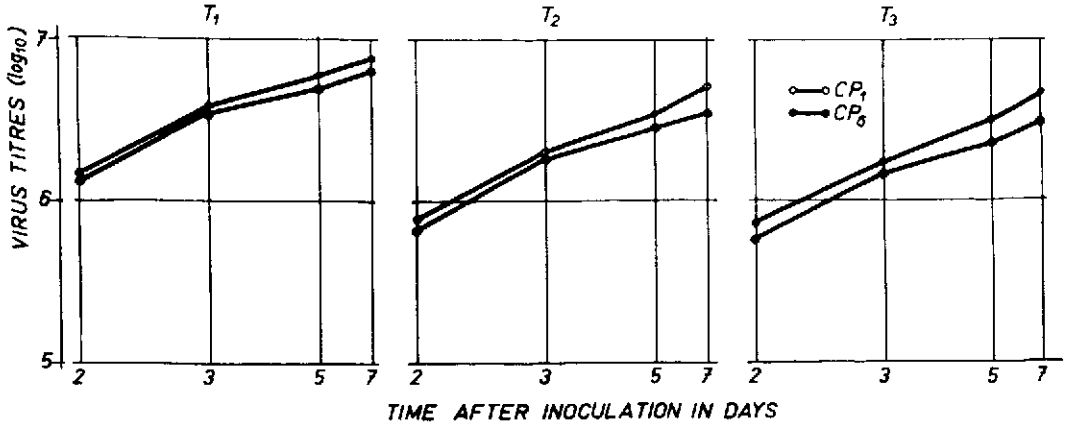


FIG. 1. The mean virus titers estimated in 2, 3, 5 and 7 day readings in repeated control titrations included in CP/1 and CP/6 tests.

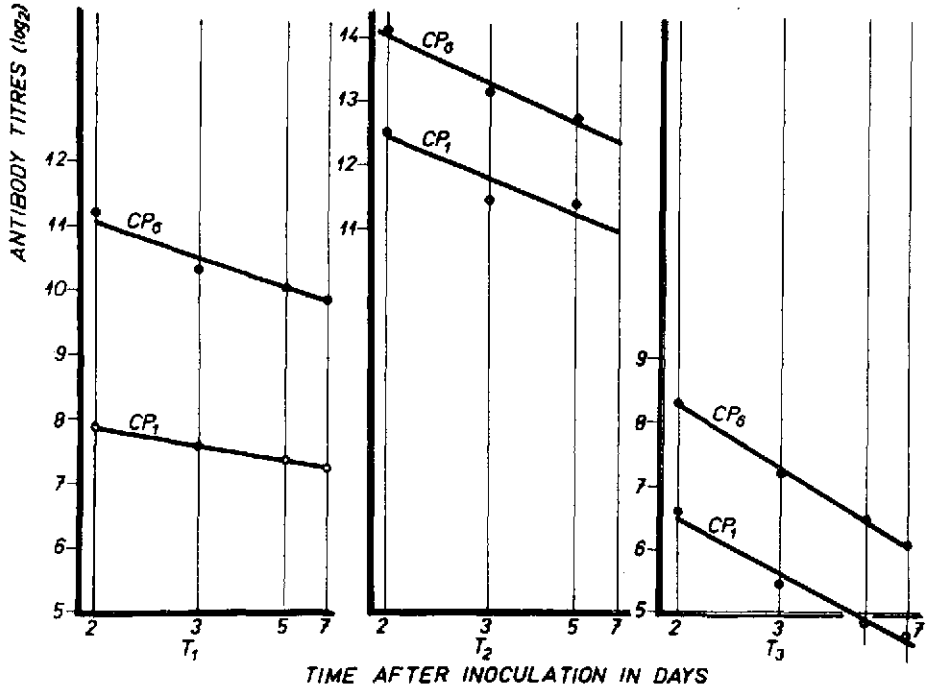


FIG. 2. The mean antibody titers estimated in 2, 3, 5 and 7 day readings in the control titrations of reference serum in CP/1 and CP/6 tests.

the fifth-day reading. Figures 1 and 2 show the relation of the virus and antibody titers in CP/1 and CP/6 to the time of reading. The results demonstrate an increase in virus titers and a decrease in antibody titer in the course of seven days. In a log/log plotting of time (day of reading) the relation is nearly linear for both the virus and antibody titers. The decrease in antibody titer proceeds equally in CP/1 and CP/6, as can be observed from the parallelism of the lines.

Results of the investigation of virus excretion in vaccinated individuals. Table 3 indicates that in all eight individuals lacking homologous antibodies, Type 3 virus was excreted for up to six weeks after the administration of the virus. Seven out of 14 cases possessing Type 3 antibodies excreted Type 3 virus, mostly in lower titers and for a shorter period of time than the non-immunes. In one subject excreting no Type 3 virus, Type 2 virus was isolated from a stool sample taken on the 35th day after virus was fed.

Results of the serological investigation performed in subjects excreting the virus.

(a) Homotypic antibody response.

Figure 3 shows that the homotypic antibodies to Type 3 were detected seven to 14 days after feeding of the virus. In six out of eight investigated persons without pre-existing homologous seroimmunity it was possible to demonstrate antibodies sooner in the CP/6, pH, and IIT tests than in the conventional CP/1 test. In one of the two remaining cases, the seven and 10-day samples were not available; in the second one, pH tests were not performed. The titers of antibodies achieved in CP/1 were in all cases on the lowest level.

Antibodies in CP/6 appeared simultaneously or later than the antibodies in pH tests and their levels, in most cases, were significantly lower than in pH tests. Titers achieved in the IIT were in all cases higher than in the CP/6, but usually on the same level as in pH tests. In subject 18/HB, in which the most considerable differences between CP and pH tests were demonstrated, the antibody titers in IIT were significantly lower than in pH tests.

In some cases, the highest differences in antibody titers achieved by different tests were found in the first phases of antibody development, and

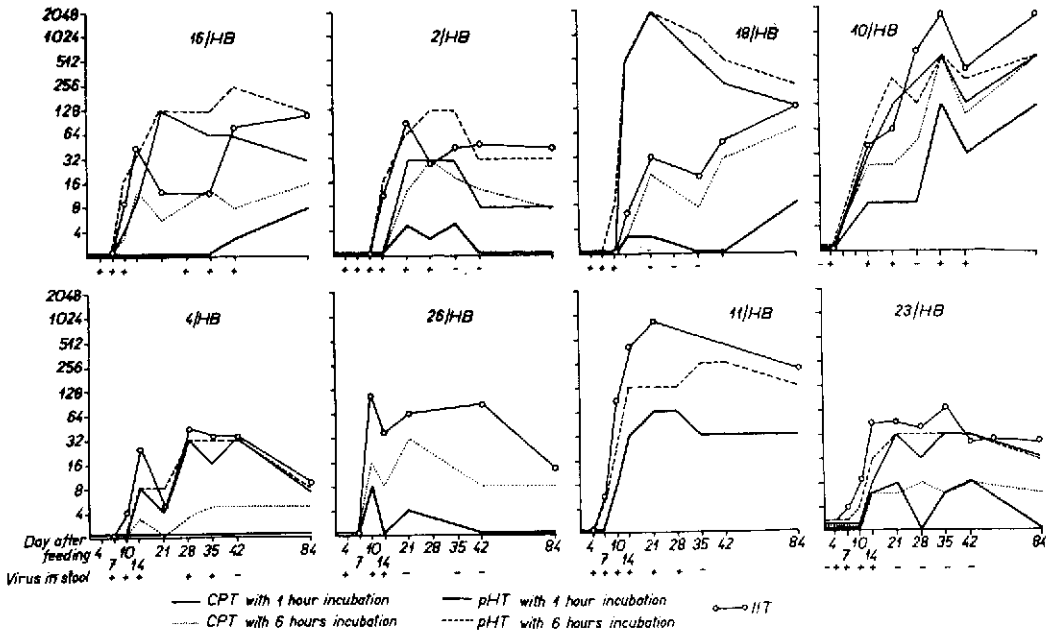


FIG. 3. The development of Type 3 antibody measured by different neutralization tests in young adults without preexisting serological immunity, fed with 100,000 TCD_{50} of Type 3 attenuated virus.

in the further course became lower. After the fourth week, when the pH antibodies reached their highest levels, they began to drop, while still later a certain increase in CP antibodies could be observed (subjects 18/HB, 16/HB, 4/HB). On the other hand, in three other subjects, antibodies detectable in CP/1 persisted for a relatively short period and in one subject no antibodies could be demonstrated by means of this test at all, in spite of a good virus multiplication in the alimentary tract.

In all cases investigated, antibodies to Type 3, at least in some of the tests used, were demonstrable after the 12-week period.

In subjects possessing homologous serological immunity the antibody response was not faster than in non-immunes (Fig. 4). The antibody increases usually appeared between the 10th to 14th day after feeding of the virus. However, when comparing the results obtained in these individuals to those obtained in non-immune persons, some other marked differences can be demonstrated.

In four out of seven subjects investigated, only a transient increase in homologous antibody could be detected. Only in one subject (22/HB) did the antibody increase in pH tests and CP/6 precede the antibody increase in CP/1. In subject 1/HB, antibody increase was demonstrated only in CP/1 and IIT (1/HB), and in another subject the antibody increase in CP/1 preceded the antibody increase demonstrable in other tests. In the remaining subjects, a simultaneous increase of antibody titers was observed in all tests used. Furthermore, the antibody titers estimated in CP/6 in these subjects—except case 22/HB—were usually on the same level as in pH tests and the titers in IIT were in all cases higher than in any one test performed with 100 TCD_{50}.

(b) Heterotypic antibody response.

The results obtained in studying the heterotypic antibody response are presented in Fig. 5.

The antibody response to Type 2 was found in 11 out of 15 cases, and persisted in eight after the 12-week period. Type 1 antibody increase was observed in 11 cases too. Only in six cases, however, was it detectable at the end of the observation period.

As for the time of appearance, in one case the increase in Type 2 antibody preceded the homotypic antibody response. In two cases, a simultaneous rise against all three types was observed; in two other cases it was observed simul-

TABLE 3. THE VIRUS EXCRETION IN STOOLS OF 22 INDIVIDUALS AGED 15-18 FED WITH 100,000 OF TYPE 3 ATTENUATED POLIOVIRUS

No.	Case	Age	pH/1 Antibodies Before Vaccination			Log_{10} of Virus per Gram of Stool								
						No. of Days After Feeding of Virus								
			1	2	3	0	4	7	10	14	21	28	35	42
1	F. 1/HB	18	–	–	+	–	–	2, 5	2, 5	–	–	–	–	–
2	CH. 2/HB	18	+	–	–	–	2, 5	5, 5	2, 5	3, 5	4, 5	4, 0	–	–
3	D. 3/HB	18	–	+	+	–	–	–	–	–		–		
4	H. 4/HB	18	+	–	–	–		4, 0	4, 5	2, 5	–	3, 5	(tr†)	
5	V. 5/HB	18	–	+	+		–	–	–	–	–	–	–	–
6	S. 6/HB	18	–	–	+	–	–			–	–	–	–	–
7	K. 7/HB	18	+	–	+	–		2, 5	–	–	tr	–	–	–
8	Ž. 8/HB	18	+	–	+	–	–	–	–		–	–	5, 5*)	–
9	P. 9/HB	18	+	+	+	–	2, 5		–	2, 5	–	–	–	–
10	K.10/HB	17	–	–	–	–	3, 5			3, 5	3, 0	–	tr	tr
11	Z.11/HB	17	+	+	–	–	5, 5	5, 5	3, 5	5, 5	3, 5	4, 0	–	
12	S.15/HB	18	+	–	+	–				–	–	–		
13	H.16/HB	17	+	–	–	–	4, 5	5, 5	5, 0	3, 5		3, 5	3, 5	3, 5
14	P.17/HB	16	+	+	+	–	2, 5	–	3, 5	–	–	–	–	–
15	Š.18/HB	16	–	–	–	–	3, 5	5, 0	5, 0		–	–	–	
16	Š.20/HB	16	–	+	+	–	tr	4, 5	–	–		–	–	–
17	K.21/HB	18	+	+	+	–	–	–	–		–	–	–	
18	F.22/HB	15	+	+	=	–	tr	5, 0	3, 0	3, 5	3, 5	–	–	–
19	S.23/HB	15	+	+	–	–	3, 5	3, 5	3, 5	2, 5	–	–	–	–
20	J.25/HB	18	–	+	+		3, 5	–	–	–		–	–	
21	ˇ26/HB	18	–	+	–	–	tr		tr	4, 5	–		–	–
22	M.27/HB	18	–	–	+						–		–	–

† Traces of virus in stool.
* In this one case Type 2 virus was isolated.
NOTE: The antibodies were estimated in pH test with one-hour incubation of the virus-serum mixture at room temperature.

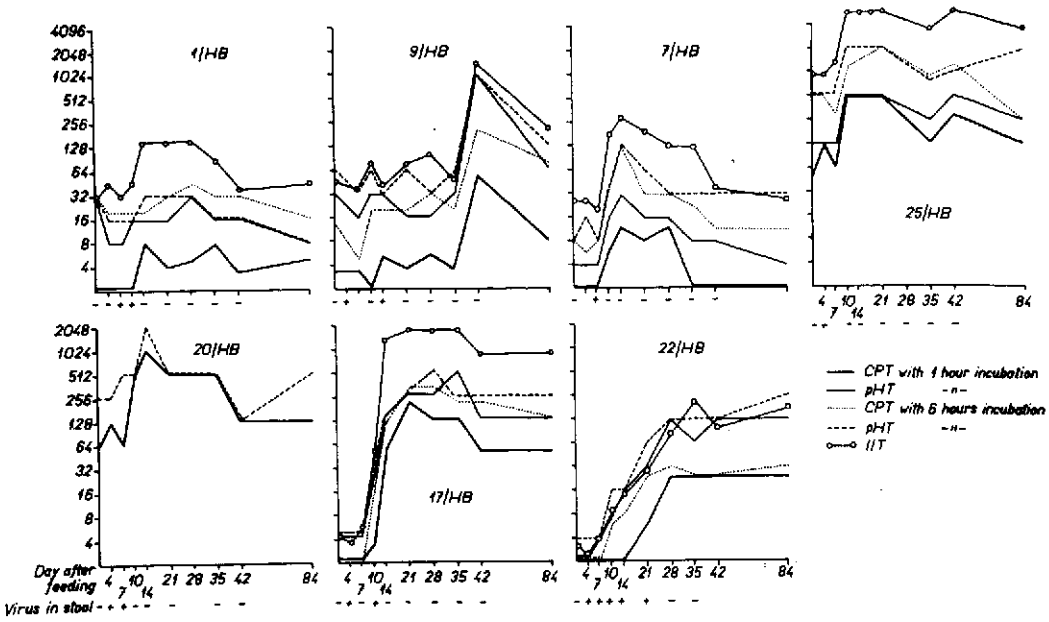


FIG. 4. The development of Type 3 antibody measured by different neutralization tests in young adults with preexisting serological immunity, fed with 100,000 TCD_{50} of Type 3 attenuated virus.

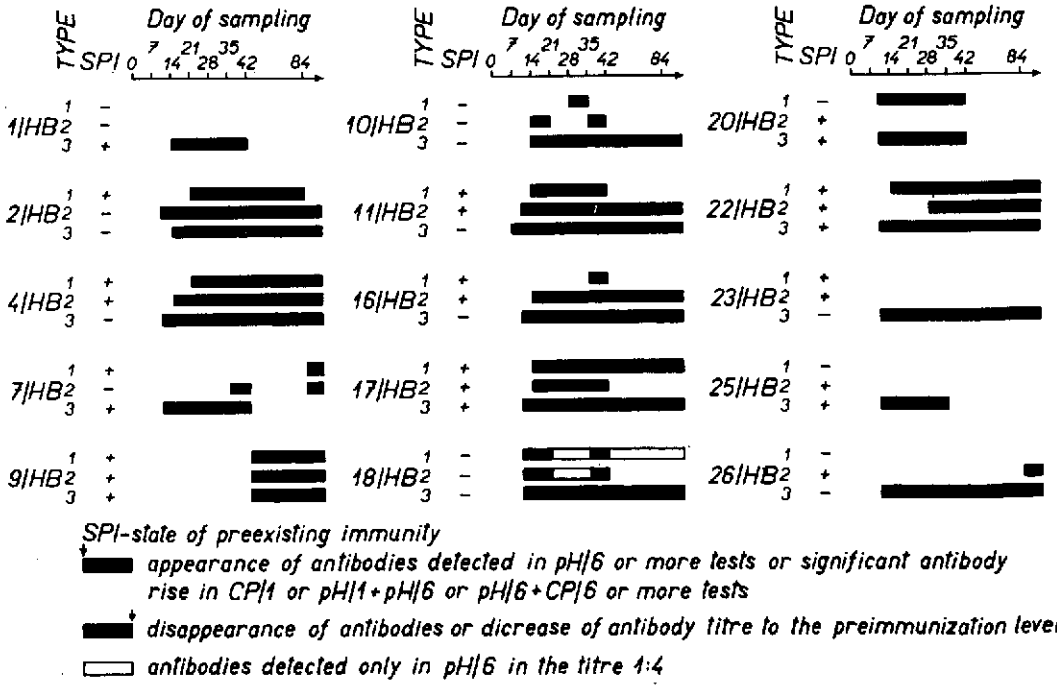


FIG. 5

tancously against Types 2 and 3, and once simultaneously against Types 1 and 3. In most cases, the antibody rises to Type 1 were observed later than to Type 2.

The appearance of heterotypic antibodies in subjects lacking detectable levels of Type 1 and Type 2 antibodies in the pre-immunization sample of sera was of special interest to us. Type 1 antibodies appeared in three out of six cases without homologous serological immunity, persisting in two cases for a short period of time; in the remaining case they were demonstrated in a very low titer only in pH/6 after 12 weeks. A Type 2 antibody increase was observed in four out of five subjects lacking Type 2 antibodies prior to immunization. In two of these subjects, the antibodies persisted after 12 weeks. The appearance of heterotypic antibodies repeatedly had the character of biphasic process.

Differences in the titers of Type 1 and Type 2 antibodies, estimated by means of different tests, were similar to those observed in Type 3 antibodies.

Some preliminary results concerning the possible differences in antibody avidity.

(a) As already mentioned, the results of CP/1 and CP/6 were read on the second, third, fifth, and seventh day after inoculation, the IIT on the third, fifth, and seventh day. Between the titers estimated in the second and seventh-day readings considerable differences were observed, varying from case to case. We tried to establish the following: (1) whether the magnitude of such differences in antibody titers was in any relation to the pre-immunization immunity state; and (2) whether these differences changed in the course of poliomyelitic infection.

For this purpose we divided the subjects investigated in two groups according to their prevaccination state of immunity. Geometric mean titers were computed from the antibody titers estimated in corresponding serum specimens.

The results presented in Fig. 6 show: (1) In all three CP tests used, a more considerable difference between the antibody titers estimated in the first and the last readings was observed in subjects lacking pre-existing antibodies, being usually three to fourfold in CP/1 and CP/6 and threefold in IIT. In subjects possessing pre-existing antibodies it was twofold or less in all three tests. The analogical decrease of GMT of Type 3 antibodies found in the standard human serum tested simultaneously with non-immune subjects, was 4.5-fold for CP/1, 3.5-fold for CP/6 and 2.4-fold for IIT. The corresponding data obtained when subjects with pre-existing immunity were investigated were 4.3, 3.7, and 2.5, respectively.

(2) In the course of the infection the differences between the second and seventh and the third and seventh-day readings, respectively, remained nearly unchanged. Only in the group of non-immunes a slight decrease of the differences can be found in samples of sera taken at the end of the observation period in CP/1 and IIT, but not in CP/6 tests.

(b) The most pronounced difference between the antibody titers in pH and CP tests was estimated in subject 9/HB.

The serum samples taken on the 14th day (S-5) and on the 84th day (S-10) were tested against virus dilution 10^{-1} to 10^{-5} in CP/1 and pH/1, using four tubes (cups) per every dilution. The results of this experiment are given in Table 4.

In order to obtain more knowledge of this interesting discrepancy we followed the residual virus activity (RVA) in mixtures containing approximately $10^{3.5}$ TCD_{50} of virus per 0.1 ml. and the final dilution 1:100 of S-5 and S-10, respectively. The mixtures were incubated at 37° C. and after one, three, six, and 24 hours, samples were drawn and diluted in several tenfold steps in pre-cooled medium. Immediately after it, 10 tissue-culture tubes were inoculated with 0.1 ml. from each dilution. The five-day readings of two consecutive experiments are presented in Table 5.

The results indicate that in the test system used almost no neutralizing effect by S-5 in the dilution tested could be demonstrated even after 24-hour incubation, although, as previously demonstrated, the same serum in the dilution 1:256 "neutralized" about $10^{3.5}$ TCD_{50} of virus in the pH test.

On the other hand, S-10, having a lesser neutralizing potency in pH test, reduced the virus titer during the incubation period of 24 hours by 1.4 $\log_{10}$.

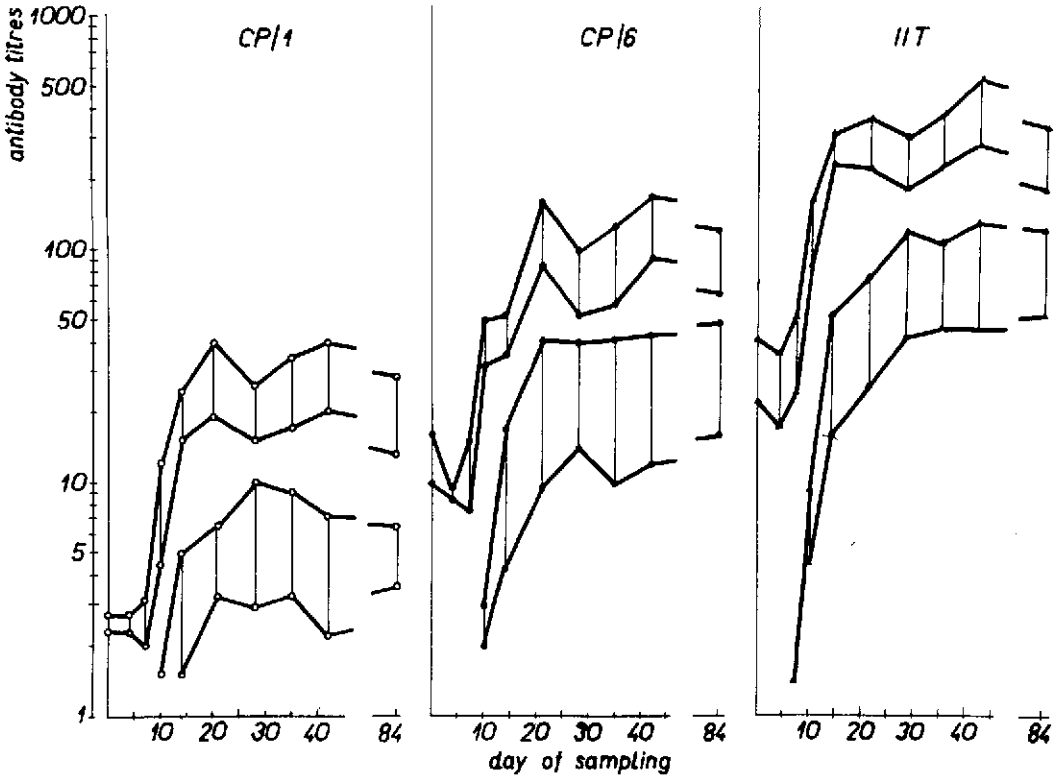


FIG. 6. The decrease of the GMT of Type 3 antibodies estimated in the 2 and 7 day reading in CP/1 and CP/6 tests and in the 3 and 7 day reading in IIT, in the sera of individuals without and with homologous preexisting immunity.

TABLE 4. THE DEPENDANCE OF TYPE 3 ANTIBODY TITER IN THE SERA TAKEN IN 18/HB 14 DAYS AFTER TYPE 3 ATTENUATED VIRUS WAS FED (S-5) AND 10 WEEKS LATER (S-10), ON THE DIFFERENT QUANTITIES OF VIRUS IN CP/1 AND pH/1

SERUM SAMPLE	CP/1					pH/1				
	THE DILUTION OF VIRUS					THE DILUTION OF VIRUS				
	10^{-1}	10^{-2}	10^{-3}	10^{-4}	10^{-5}	10^{-1}	10^{-2}	10^{-3}	10^{-4}	10^{-5}
S-5	<4	<4	<4	11	90	16	64	256	512	2048
S-10	<4	<4	6	16	64	16	22	64	180	512

(c) Subject 1/HB attracted our special attention. In this subject a short-time virus excretion was followed by the appearance of Type 3 antibodies in CP/1, without any increase in preexisting antibody titers already demonstrable in the pre-immunization serum specimen in pH and CP/6 tests. These results could serve as an example of transformation of low to high avidity according to Sabin's concept. On the other hand, an antibody rise was demonstrable in IIT. On the basis of this finding, an experiment was carried out in order to obtain the necessary informa-

TABLE 5. RESIDUAL VIRUS ACTIVITY IN SAMPLES DRAWN IN DIFFERENT TIME PERIODS FROM VIRUS-SERUM MIXTURES CONTAINING 1:100 DILUTIONS OF SERA TAKEN IN 18/HB 14 DAYS AFTER TYPE 3 ATTENUATED VIRUS WAS FED (S-5) AND 10 WEEKS LATER (S-10)

INCUBATION OF THE VIRUS-SERUM MIXTURE	FIRST EXPERIMENT			SECOND EXPERIMENT		
	VIRUS CONTROL	S-5	S-10	VIRUS CONTROL	S-5	S-10
1 hour	3,3	3,1	3,1	3,7	3,3	3,3
3 hours	3,5	3,2	2,7	ND	ND	ND
6 hours	3,2	3,0	2,3	3,3	3,1	2,3
24 hours	3,2	3,0	1,7	2,9	2,8	1,5

NOTE: The samples were diluted immediately after drawing in tenfold steps and ten tissue-culture tubes were inoculated from each dilution.

tion of whether the nature of the change was a quantitative, rather than a qualitative one.

We followed the RVA in mixtures containing approximately $10^{4.5}$ TCD_{50} of virus per 0.1 ml. and the final dilution 1:40 of serum taken before the feeding of virus (S-1) and the 14th day after feeding (S-5). After one, three, and six hours of incubation at 37° C. samples were drawn and diluted as described previously. The results of two repeated experiments are presented in Table 6.

The results suggest that there is a difference in the neutralizing capacity of both sera. The first serum specimen reduced the virus titer by 90 per cent with the second reducing the titer by 99 per cent after six hours incubation. The differences of this order are large enough to be demonstrated by HT, where the final concentration of virus in the inoculum is 10 TCD_{50}, but too small to be demonstrated by the end-point technique with 100 TCD_{50} in the CP/6 or in the pH tests.

DISCUSSION

Our results indicate marked variations in antibody response after feeding of Type 3 attenuated poliovirus in different subjects and different tests. CP/1 was demonstrated as the least sensitive and in some cases was quite inadequate for detecting the antibody response. The results achieved show how misleading any conclusions based on this one test may be.

TABLE 6. RESIDUAL VIRUS ACTIVITY IN SAMPLES DRAWN IN DIFFERENT TIME PERIODS FROM VIRUS-SERUM MIXTURES CONTAINING 1:40 DILUTION OF SERA TAKEN IN 1/HB BEFORE TYPE 3 ATTENUATED VIRUS WAS FED (S-1) AND ON THE 14TH DAY AFTER FEEDING (S-5)

INCUBATION OF THE VIRUS-SERUM	FIRST EXPERIMENT			SECOND EXPERIMENT		
	VIRUS CONTROL	S-1	S-5	VIRUS CONTROL	S-1	S-5
1 hour	3,8	3,6	3,8	3,3	3,1	3,1
3 hours	3,9	3,1	2,8	ND	ND	ND
6 hours	3,7	2,8	2,0	3,4	2,8	1,7

NOTE: The samples were diluted immediately after drawing in tenfold steps and 10 tissue-culture tubes were inoculated from each dilution.

The data presented in this paper support the evidence by Gelfand *et al.*[8] and Black and Melnick [9] that there exists an antigenic relationship not only between Types 1+2 and 2+3, but also between Type 1+3. The results achieved in studying the heterotypic antibodies incite a certain caution when evaluating the antibody response in vaccinated persons in only one sample taken after all three types of virus were fed. The character of heterotypic response in persons lacking pre-existing immunity is unclear. Two possible explanations must not be omitted. At first, a very low state of immunity, undetectable even by the most sensitive tests used but conditioning a booster effect, might be present. Second, the possibility of Type 2 infections must be considered owing to the demonstrated presence of Type 2 poliovirus in the investigated community. On the other hand, however, Type 2 virus was not isolated from any of the serologically investigated subjects and also the character of antibody response was usually not typical for Type 2 infections.

There is no doubt that the question of avidity and its changing character may play an important role in many aspects of the immunology of poliomyelitis. The quality of antibodies and its estimation could be very valuable in evaluating the effect of different vaccines, and for both epidemiological and diagnostic purposes.

The concept of changing the character of antibodies during the establishment of serological immunity is not generally accepted. We think that our results give certain support to the existence of differences in the quality of antibodies in different stages of poliomyelitis infection. It seems that in non-immunes antibodies are produced in the first period of antibody development, characterized by a considerable lack of ability to combine irreversibly with viruses. As is shown in case 18/HB, the prolongation of the incubation of the virus-serum mixture or the use of IIT may be without essential effect on the antibody titer. In the further course, antibodies more readily combining with viruses developed, while the level of antibodies forming only unstable complexes with viruses, was reduced.

A question of special importance is whether the existence of low avidity antibody is limited during a short period after the infection, or whether the low avidity antibody persists for a longer time. Sabin presumes that low avidity antibody can persist and that in such individuals the first step of antibody development, after new antigenic stimulus, is the transformation of low to high avidity antibodies. The results achieved in subject 1/HB strongly suggest that at least in this one, the presumed change in antibody character was rather a small quantitative change, undetectable by the serum dilution and point determination method with CP/6 or pH tests with 100 TCD_{50} of virus.

It is necessary to emphasize that the avidity of antibodies, as this term is used in serological studies with polioviruses, is expressed mainly as a discrepancy between the results achieved in two different tests, usually in pH and in CP/1 tests. It was demonstrated [4] that the latter test may be unable to detect low levels of antibodies, without any regard to their possible avidity. We think that the pH test should be compared to the CPT with 3-6 hours of incubation, rather than to the CP/1 test. The results presented in this paper show that titers obtained in CP/6 were comparable to those achieved in pH tests only in cases with pre-existing antibodies. Therefore, the relation of titers achieved in pH tests to titers in CP/6 test may be of some value. Titers on approximately the same level could indicate high avidity antibody; the magnitude of differences might be indirectly proportional to the antibody avidity.

The varying decreases in antibody titers between the second and seventh-day readings, associated with pre-existing immunity state, bring some evidence that the magnitude of this decrease may also be helpful in estimating the quality of antibodies.

When summarizing the general experience about the changing quality of antibodies, it is evident that the main problem is still unsolved. It must be taken into consideration that all our conclusions and presumptions are based on the experiments performed with only one strain of one type in a rather exceptional group of subjects. Only further experience, utilizing all the new knowledge about the virus-antibody-cell interaction, will bring more progress in the study of antibody avidity, its nature and persistence and its possible role in the immunity to virus dis-

eases. The analysis of all potential non-specific factors should be the first step in this investigation.

REFERENCES

1. Ward, R., Chang, C., and Rader, D.: Importance of optimum amounts of virus to demonstrate neutralizing antibody rise in convalescent poliomyelitis sera. *Proc. Soc. Exp. Biol.* **90**: 162-165, 1955.
2. Brunner, K. T., and Ward, R.: Differences in stability of antigen-antibody complexes formed with poliovirus and acute and convalescent phase human sera. *J. Immun.* **83**: 405-410, 1959.
3. Sabin, A. B.: Properties of attenuated polioviruses and their behavior in human beings. New York Academy of Sciences, Monograph no. *5:* 113-127, 1957.
4. Vonka, V., Zácek, K., and Duben, J.: Paper presented at the International Symposium on Live Poliovirus Vaccine, Moscow, May 1960.
5. Gard, S.: Neutralization of Theiler's virus. *Acta Path. Microb. Scand.* **37**: 21-30, 1955.
6. ————: Immuno-inactivation of poliovirus. *Arch. ges. Virusforsch.* **7**: 449-460, 1957.
7. Skovránek, V., Zácek, K., Vonka, V., Adam, E., Adamová, V., Burian V., and Vojtová, H.: Field trial with Sabin's live poliovirus vaccine in Czechoslovakia—1958-1959. In: *Live Poliovirus Vaccines, First International Conference.* Scient. Pub. no. 44 of the Pan American Health Organization, pp. 530-571, 1959.
8. Gelfand, H. M., LeBlanc, D., Fox, J. P., and Potash, L.: Studies on the development of natural immunity to poliomyelitis in Louisiana. III. The serological response to commercially produced "Salk vaccine" of children totally or partially susceptible to poliovirus infection. *Am. J. Hyg.* **70**: 303-311, 1959.
9. Black, F. L., and Melnick, J. L.: Appearance of soluble and cross-reactive complement-fixing antigens on treatment of poliovirus with formalin. *Proc. Soc. Exp. Biol.* **89**: 353-355, 1955.

2. VIROLOGIC AND SEROLOGIC INVESTIGATIONS OF CHILDREN IMMUNIZED WITH TRIVALENT LIVE VACCINE FROM A.B. SABIN'S STRAINS

M. K. VOROSHILOVA, V. I. ZHEVANDROVA, E. A. TOLSKAYA, G. A. KOROLEVA, AND G. P. TARANOVA

DR. VOROSHILOVA *(presenting the paper)*: Simultaneous use of three immunologic types of attenuated strains for mass oral poliomyelitis immunization allows the vaccination of large population groups against all three types of poliovirus within a short time, with most complete coverage.

It is believed, however, that serologic response to trivalent vaccine may be lower than that obtained with individual monovaccines because of interference between vaccine strains. Therefore, further accumulation and examination of serological data is necessary.

The serologic survey of children vaccinated during the winter of 1959 with trivalent live vaccine from Sabin strains, in the Estonian and Lithuanian SSR (Dobrova, Yankevich, Chumakov *et al.*, 1959), demonstrated sufficiently regular antibody response to the three poliovirus types in from 66.7 to 88.6-89.0 per cent of children without pre-vaccination antibody to one or more types of poliomyelitis virus. The results varied, however, in different observations. It would be of interest to determine factors which affect serologic response.

In May, June, and July 1959, trivalent live vaccine was used for mass immunization of children in the Karaganda and Moscow regions. This paper presents the results of virologic and serologic surveys of children vaccinated in the summer in towns and settlements of the Moscow region and in three towns of the Karaganda region, and in Moscow in the winter of 1960.

Surveys consisted of studying the dynamics of vaccine virus excretion by vaccinees and their contacts and in determining serological changes. A number of children was tested for the resistance of the intestinal tract on additional administration of vaccine strains one to six months after primary immunization.

At the same time, we studied multiplication of vaccine strains in poliomyelitis convalescents who were treated in a Moscow regional hospital for poliomyelitis sequelae, in a sanatorium for poliomyelitis convalescents in Karaganda, and in the clinic of the Institute for Poliomyelitis Research.

Materials and methods. Children aged two months to 15 years were immunized orally with a mixture of monovaccines, prepared at the Institute for Poliomyelitis Research, from Sabin attenuated strains of the three poliovirus types. The vaccine was diluted immediately before use so that two drops contained 100,000 TCD_{50} of each strain. Practically, of the diluent one ml. to seven ml. of monovaccine of each type were added. The vaccine was given to children in a spoonful of water or tea, followed by the ingestion of water. The vaccine was fed once or twice with 1-1½ month interval.

Before vaccination, stool specimens (one or two) from all children were tested for enteric viruses, and blood specimens were tested for poliomyelitis antibody.

In order to study the dynamics of vaccine virus excretion after oral immunization with trivalent live-poliovirus vaccine, important investigations were carried out in four children's homes: in two (L. and R.) in the summer of 1959, and in two (No. 5 and No. 10) in the winter of 1960.

Table 1 presents data on the age and number of vaccinees, dates of vaccination, and vaccine lot numbers.

During the first month after vaccination four to five stool specimens were tested, and during the second month two to three stool specimens from each child were tested (Table 2). Stool suspensions (10 per cent by weight) were prepared and after centrifugation were treated with antibiotics.

TABLE 1. INFORMATION ON CHILDREN VACCINATED WITH TRIVALENT LIVE VACCINE WHO WERE TESTED FOR VACCINE STRAIN EXCRETION

CHILDREN'S HOMES	DATE OF VACCINATION	NUMBER OF CHILDREN TESTED	VACCINATED					CONTACT	VACCINE LOT NO. USED FOR IMMUNIZ.
			TOTAL	0–1 YR.	1–2 YRS.	2–3 YRS.	3–7 YRS.		
L.	22.VII.59	93	72	14	22	10	26	21	Type 1 Lot 1 Type 2 Lot 4 Type 3 Lot 3
R.	4.VII.59	86	65	9	22	23	11	21	—"—
No. 5	30.XII.59	76	76	34	25	14	3		Type 1, 2 Lot 0 Type 3 Lot 3
No. 10	28.XII.59	42	42	—	8	18	16		—"—
	Total	297	255	57	77	65	56	42	

TABLE 2. NUMBER OF STOOL SPECIMENS TESTED AFTER VACCINATION

CHILDREN'S HOMES	TESTED DURING THE FIRST MONTH			TESTED DURING THE SECOND MONTH		
	NUMBER OF CHILDREN	NUMBER OF STOOLS	NUMBER OF STOOLS PER 1 CHILD	NUMBER OF CHILDREN	NUMBER OF STOOLS	NUMBER OF STOOLS PER 1 CHILD
L. and R.	137	626	4–5			
Nos. 5 and 10	118	534	4–5	104	309	2–3
Total	255	1160	4–5	104	309	2–3

Virus isolation was performed by inoculation of three tube cultures of trypsinized monkey-kidney cells (0.5 ml. of stool suspension per one tube culture). The medium for virus isolation consisted of 0.5 per cent lactalbumin hydrolysate in Earle's or Hanks' solution with 2 per cent normal bovine serum. The addition of the serum decreased toxic effect of stools. For the first three days, inoculated cultures were observed daily for detection of cytopathogenic effect or non-specific toxic effect of stools. Cultures with specific degeneration on the second to tenth day were frozen and tissue-culture fluid typed in the neutralization test with mixtures of poliomyelitis antisera.

Cytopathogenic agents which were found to be non-polioviruses, were then identified in extensive neutralization tests with antisera against ECHO viruses and Coxsackie B-1-5 and A-9 viruses. Cultures in which cytopathogenic effect failed to be detected for 10 days were considered negative, as were corresponding stools containing no viruses, at least vaccine ones.

For serologic studies venous blood was collected before one and one half months after the first, and one and one half, three, four, and six months after the second feeding. In February 1960, children in children's homes L. and R. received Type 1 monovaccine and one month later trivalent vaccine was incorporated into dragée-

candy. The children were bled two weeks later. Altogether, sera from 841 children, particularly those whose ages ranged from six months to four years, were tested.

In the Moscow region (see Table 3) serum specimens were collected from 425 children; of those, 225 lived in the districts of Ukhtomsk, Kuntsevo, Reutovo, Balashikha, and Khimki, and in the towns of Lyublino, Perovo, Electrostal, Babushkin, and Volokolamsk. The children had not been vaccinated with killed vaccine. One hundred and seventy serum specimens were obtained from children in three children's homes. Ninety-four serum specimens were collected from children under two years of age.

In the Karaganda region, 416 children from the towns of Karaganda, Balkhash, and Osokarovka were examined before vaccination.

negative, and 160 (37.6 per cent) of the children had antibody to all three poliovirus types. Of other antibody patterns, the one showing antibodies to Type 2 alone was found most frequently (14.1 per cent); to Types 1 and 2 (10.8 per cent). Very seldom (only in 1.7 per cent) was only Type 3 antibody present. In children under one year, 20 sera of 33 tested had no antibody to any poliovirus type, and only three sera neutralized Type 2 poliovirus. Among children one to two years of age there were 20.6 per cent triple negatives, two to three years, 12.8 per cent, and three to seven years, 5.9 per cent. All 39 children from seven to 10 years of age had poliomyclitis antibody. Of those, 25 had antibody to all three types of poliovirus.

Of 416 children in the Karaganda region (see Table 6) 97 (23.6 per cent) had no Type 1 anti-

TABLE 3. AGE-DISTRIBUTION OF CHILDREN FROM WHOM SERUM SPECIMENS WERE COLLECTED IN THE MOSCOW REGION

	Total Number of Children	Age					
		0–11 Mos.	1 Yr.	2 Yrs.	3–6 Yrs.	7–9 Yrs.	10–15 Yrs.
Towns and districts	255	5	15	24	130	32	42
Children's home Lu.	78	15	24	9	30		
Children's home R.	65	3	24	30	15		
Children's home Lyt.	27		8	2	10	7	
Total	425	23	71	65	185	39	42
Per cent	100.0	5.4	16.7	15.3	43.5	9.2	9.9

Tables 4-6 present data on the lack of antibody to polioviruses and the number of children with different antibody patterns to the three poliovirus types in the Moscow and Karaganda regions.

As seen in the tables, of 425 children in the Moscow region before vaccination, 188 (44.2 per cent) had no Type 1 antibody, 133 (31.3 per cent) had no Type 2 antibody, and 208 (48.8 per cent) had no Type 3 antibody to poliovirus. Eighty-five children (20.0 per cent) were triple

body, 71 (17.1 per cent) no Type 2 antibody, and 99 (23.8 per cent) Type 3 antibody. Twenty-four (5.8 per cent) children were triple-negative. In the age-group under one year, 46.6 per cent of the children had no antibody to any type of poliovirus. Both in the Moscow and Karaganda regions percentage of children possessing Type 2 antibody increased with age. The highest per cent of sera with Type 1 and Type 3 antibody was found in the age group of seven to 10 years. It may probably be connected with the first epi-

TABLE 4. ABSENCE OF POLIOMYELITIS ANTIBODY IN CHILDREN OF DIFFERENT AGES BEFORE VACCINATION (MOSCOW REGION, 1959)

AGE-GROUP (YEARS)	NUMBER OF SERA TESTED	NUMBER OF SERA WITHOUT ANTIBODY TO						TRIPLE-NEGATIVE SERA	
		TYPE 1		TYPE 2		TYPE 3			
		No.	%	No.	%	No.	%	No.	%
0–1	23	23	100.0	20	86.0	23	100.0	20	86.0
1–2	71	59	83.1	50	70.4	63	88.7	43	60.6
2–3	65	51	78.6	17	26.2	50	76.9	9	13.8
3–7	185	41	22.2	34	18.4	58	31.4	11	5.9
7–10	39	2	5.1	7	17.9	6	15.4	–	–
10–15	42	12	28.6	5	11.9	6	19.0	2	4.8
Total	425	188	44.2	133	31.3	208	48.8	85	20.0

demic season of 1955 when children, at that time one to three years old, were infected to a greater extent than older children. In the age group of 10 to 15 years, 4.8 per cent of the children were triple negative, 28.6 per cent had no Type 1 antibody, 11.9 per cent no Type 2, and 19.0 per cent no Type 3 antibody (Moscow region, Table 4).

Serological investigations were carried out mainly by pH neutralization tests in tubes or insulin vials. A portion of sera was tested by the cytopathogenic neutralization test. Besides, some sera were tested in parallel by complement-fixation tests and precipitation tests in agar gel on slides. Serum specimens from each child (two to six specimens) were always examined simultaneously in one test.

RESULTS

Examination of pre-vaccination stool specimens revealed the presence of extensive virus carriage of nonpoliomyelitis enteric viruses in children from children's homes L. and R. in the summer (about 50 per cent of the children, Table 7).

Virologic examination of stool specimens collected after immunization with live trivalent vaccine demonstrated vaccine virus excretion in children's homes L. and R. (summer) during the first month after vaccination in 70 per cent of the children, and in children's homes No. 5 and No. 10 (winter) in 96 per cent of the vaccinated children (Table 8).

During the first month after vaccination, gradual decrease of excretion rate was observed from the first to the fourth week (Table 9). During the second post-vaccination month vaccine virus excretion continued to decrease, but more than half of the vaccinees were still excreting vaccine viruses (Table 8).

Establishment of vaccine viruses depended also on the age of the vaccinees. In children under three years, vaccine viruses were found to establish with greater frequency and for longer periods than in older children (three to seven years, Table 10).

By frequency of vaccine virus excretion according to types, the first place was occupied by Type 2 vaccine virus, the second by Type 1 vaccine virus, and the third by Type 3 vaccine virus, observations being made in children vaccinated with trivalent live vaccine. Frequency of Types 1 and 2 vaccine virus excretion was approximately the same, while Type 3 vaccine virus was excreted much less frequently (Tables 11-14).

TABLE 5. ANTIBODY PATTERNS IN CHILDREN BEFORE IMMUNIZATION WITH TRIVALENT LIVE VACCINE (MOSCOW REGION)

AGE-GROUP (YEARS)	NUMBER OF SERA TESTED	ANTIBODY TO THREE TYPES OF POLIOVIRUS															
		000		00+		0+0		0++		+00		+0+		++0		+++	
		No.	%	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%
0–1	23	20	86.0	–	–	3	14.0	–	–	–	–	–	–	–	–	–	–
1–2	71	43	60.6	1	1.4	14	19.7	1	1.4	4	5.6	2	2.8	2	2.8	4	5.6
2–3	65	9	13.8	1	1.5	28	43.1	3	4.6	6	9.2	1	1.5	7	10.7	10	15.4
3–7	185	11	5.9	4	2.2	10	5.4	16	8.6	7	3.8	12	6.5	30	16.2	95	51.3
7–10	39	–	–	–	–	1	2.6	1	2.6	–	–	7	17.9	5	12.8	25	64.1
10–15	42	2	4.8	1	2.4	4	9.5	5	11.9	–	–	2	4.8	2	4.8	26	61.9
Total	425	85	20.0	7	1.6	60	14.1	26	6.1	17	4.0	24	5.6	46	10.8	160	37.6

Table 6. Absence of Poliomyelitis Antibody in Children of Different Ages Before Vaccination (Karaganda, 1959)

Age-Group (Years)	Number of Sera Tested	Number of Sera Without Antibody to						Triple-Negative Sera	
		Type 1		Type 2		Type 3			
		No.	%	No.	%	No.	%	No.	%
0–1	15	8	53.3	7	46.6	9	60.0	7	46.6
1–4	236	66	28.0	43	18.2	75	31.8	17	7.2
5–7	120	18	15.0	19	15.8	14	11.7		
8–10	22	2	9.1	1	4.6				
11–14	20	3	15.0	1	5.0	1	5.0		
15+	3								
Total	416	97	23.3	71	17.1	99	23.8	24	5.8

Table 7. Examination of Stool Specimens from Children for Intestinal Virus Carriage Before Immunization

Children's Homes	Number of Children			Isolated Cytopathogenic Agents:																
	Tested	Excreted CPA	%	ECHO Group												Coxs.		Cox. B_1+ $ECHO_8$	Untyp. CPA
				1	3	8	11	12	14	17	18	19	11+ 12			B_1	A_9		
L. and R.	131	59	45	1	1	7			1	1	1	1				7		2	37
Nos. 5 and 10	116	29	13				9	6					2				1		9

Table 8. Frequency of Excretion of Vaccine Viruses After Immunization of Children

Children's Homes	Number of Children During the First Month			Number of Children During the Second Month		
	Tested	Excreted Virus	Virus Isolation Rate	Tested	Excreted Virus	Virus Isolation Rate
L. and R.	136	96	70			
Nos. 5 and 10	118	114	96	104	69	66

TABLE 9. FREQUENCY OF VACCINE VIRUS EXCRETION BY CHILDREN DURING THE FIRST MONTH AFTER IMMUNIZATION BY WEEKS (CHILDREN'S HOMES NO. 5 AND NO. 10)

WEEK AFTER IMMUNIZATION	NUMBER OF CHILDREN TESTED	NUMBER EXCRETING VIRUS	VIRUS ISOLATION RATE
1	105	98	93
2	58	53	91
3	102	84	82
4	86	62	72

Maximum excretion of Types 1 and 2 vaccine viruses was observed in the first post-vaccination week, and of Type 3 virus in the second week (Fig. 1). During the first month after vaccination Types 1, 2, and 3 vaccine viruses are excreted almost twice as frequently as during the second month (Table 14). Examples of simultaneous multiplication of vaccine strains Types 1, 2, and 3 are presented in Figures 2 and 3.

Special investigation was devoted to the problem of establishment of vaccine viruses in immunized children in relation to the presence or absence of poliomyelitis antibody. It was shown that the establishment of Types 1 and 2 vaccine viruses was almost twice as frequent in children without antibody than in those with them. On the contrary, Type 3 vaccine virus was much more frequently excreted by children possessing homologous antibody (Tables 15-17).

Examination of sera collected at random from 223 children with pre-vaccination lack of antibody to some types by pH neutralization test, showed that one month after feeding with trivalent live vaccine some children responded with antibody development to all three types, and others to two or one type of poliovirus. Of 142 children without pre-vaccination Type 1 antibody, 92 (64.8 per cent) acquired antibody to this type. Of 106 children without Type 2 antibody before vaccination, 85 (80.2 per cent) developed it. Smaller number of children responded with Type 3 antibody development (86 of 151, or 56.9 per cent). Of 60 triple-negative children, 27 (45.0 per cent) developed each Type 1 and Type 3 antibody, and 43 (71.7 per cent) Type 2 antibody.

Percentage of children with pre-vaccination antibody increased by 39.9 per cent for Type 1, 35.4 per cent for Type 2, and 35.9 per cent for Type 3.

These results are definitely lower compared to those obtained by I. H. Dobrova in the Estonian SSR after immunization with trivalent vaccine in the winter-spring season (see Table 18).

In the Moscow and Karaganda regions, where vaccinations had been carried out in the summer, establishment of vaccine strains was prevented by extremely wide dissemination of non-poliomyelitis enteric viruses during this period. In favor of possible inhibiting influence of enterovirus season is the fact that the least satisfactory results were obtained in children's homes L. and R. where considerable extent of ECHO and Coxsackie virus carriage had been found (Table 18).

And yet, despite the unfavorable effect of enteric virus carriage, one feeding of trivalent live vaccine resulted in significant changes in antibody patterns compared with pre-vaccination status in that 128 children of 223 seronegatives converted to triple positives. Fifty-one of 60 triple-negative children developed antibody to one or more types of poliomyelitis viruses, and only nine children remained triple negative (Table 19 and Fig. 4).

Best results in regard to increase in the percentage of children with antibody to all the three types were observed in children from different towns and settlements of the Moscow region (68.4 per cent) not vaccinated previously with killed vaccine (Table 20). In the Karaganda region

TABLE 10. ESTABLISHMENT OF VACCINE VIRUSES IN RELATION TO THE AGE OF THE VACCINATED (CHILDREN'S HOMES NO. 5 AND NO. 10)

AGE YRS.	WEEKS AFTER IMMUNIZATION														
	1			2			3			4			5—8		
	NO. TESTED	EXCRETED VIRUS	%	NO. TESTED	EXCRETED VIRUS	%	NO. TESTED	EXCRETED VIRUS	%	NO. TESTED	EXCRETED VIRUS	%	NO. TESTED	EXCRETED VIRUS	%
0–1	27	24	88	4	4	100	32	30	94	21	18	86	29	20	70
1–2	31	29	94	16	13	81	29	24	82	25	14	56	28	19	68
2–3	30	28	93	21	20	95	26	20	77	23	19	82	28	20	72
3–7	17	17	100	17	16	94	15	10	66	17	11	60	19	10	53
0–7	105	98	93	58	53	91	102	84	82	86	62	72	104	69	66

TABLE 11. FREQUENCY OF TYPE 1 VIRUS EXCRETION BY WEEKS (CHILDREN'S HOMES NO. 5 AND NO. 10)

	Week					
Number of Children	1	2	3	4	1–4	5–8
Tested	99	61	101	86	118	101
Excreted virus	70	29	47	31	97	41
Per cent	70	47	46	36	82	40

TABLE 12. FREQUENCY OF TYPE 2 VACCINE VIRUS EXCRETION BY WEEKS

	Week					
Number of Children	1	2	3	4	1–4	5–8
Tested	99	61	101	86	118	101
Excreted virus	76	34	61	40	103	46
Per cent of excretion	77	56	60	45	87	45

TABLE 13. FREQUENCY OF TYPE 3 VACCINE VIRUS EXCRETION BY WEEKS

	Week					
Number of Children	1	2	3	4	1–4	5–8
Tested	99	61	101	96	118	101
Excreted virus	47	36	34	29	76	36
Per cent of excretion	47	60	33	30	64	35

TABLE 14. SUMMARY TABLE ON THE FREQUENCY OF TYPES 1, 2, AND 3 VACCINE VIRUS EXCRETION BY WEEKS (IN PER CENT)

	Week					
Virus Type	1	2	3	4	1–4	5–8
1	70	47	46	36	82	40
2	77	56	60	46	87	45
3	47	60	33	30	64	35

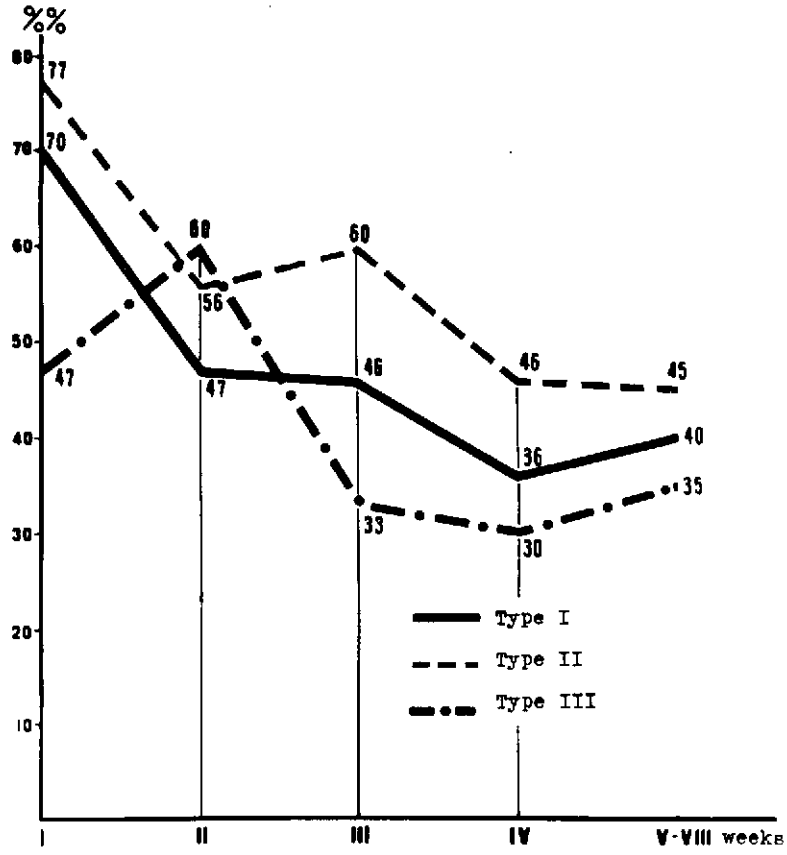


FIG 1. Excretion of Types 1, 2, 3 vaccine viruses by children immunized with trivalent live vaccine from Sabin's strains.

TABLE 15. ESTABLISHMENT OF TYPE 1 VACCINE VIRUS IN PERSONS IMMUNIZED WITH TRIVALENT LIVE VACCINE, IN RELATION TO THE PRESENCE OR ABSENCE OF POLIOMYELITIS ANTIBODY

	ANTIBODY			
CHILDREN	1°	1° 2° 3°	1+	1+2+3+
No. tested	86	24	40	33
Excreted virus	43	12	12	9
Per cent	50	50	30	27

the number of triple positive children increased by 56.5 per cent, while in children's home L. in the Moscow region only by 23.1 per cent. Summary data on the increase in the number of children with antibody to all the three types of poliovirus vaccinated during summer were only 4.6 per cent lower than those obtained in winter vaccination with trivalent vaccine. (Estonian, SSR data by I. H. Dobrova). The increase in the number of children developing Type 2 antibody differed from that observed in the winter only by 5.4 per cent. A greater difference between the

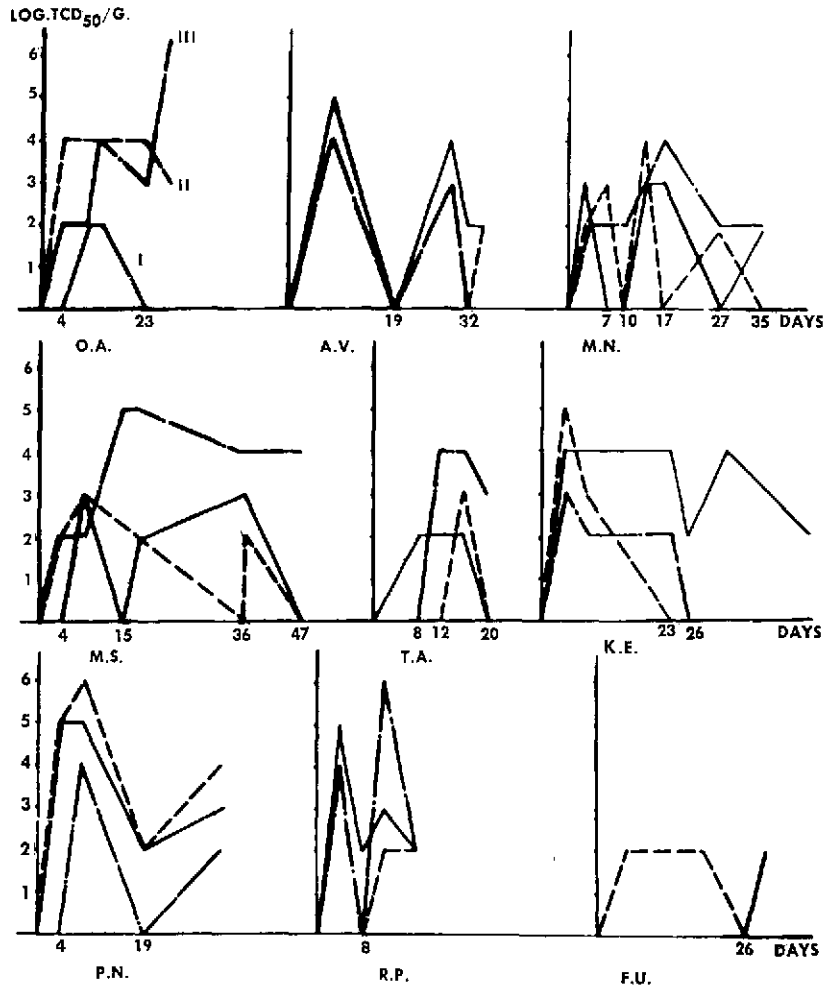


FIG. 2. Examples of simultaneous multiplication of vaccine strains on feeding live trivalent vaccine.

TABLE 16. ESTABLISHMENT OF TYPE 2 VACCINE VIRUS IN PERSONS IMMUNIZED WITH TRIVALENT LIVE VACCINE, IN RELATION TO THE PRESENCE OR ABSENCE OF POLIOMYELITIS ANTIBODY

CHILDREN	ANTIBODY			
	2°	1° 2° 3°	2+	1+2+3+
No. tested	50	24	86	33
Excreted virus	41	20	27	11
Per cent	82	71	31	30

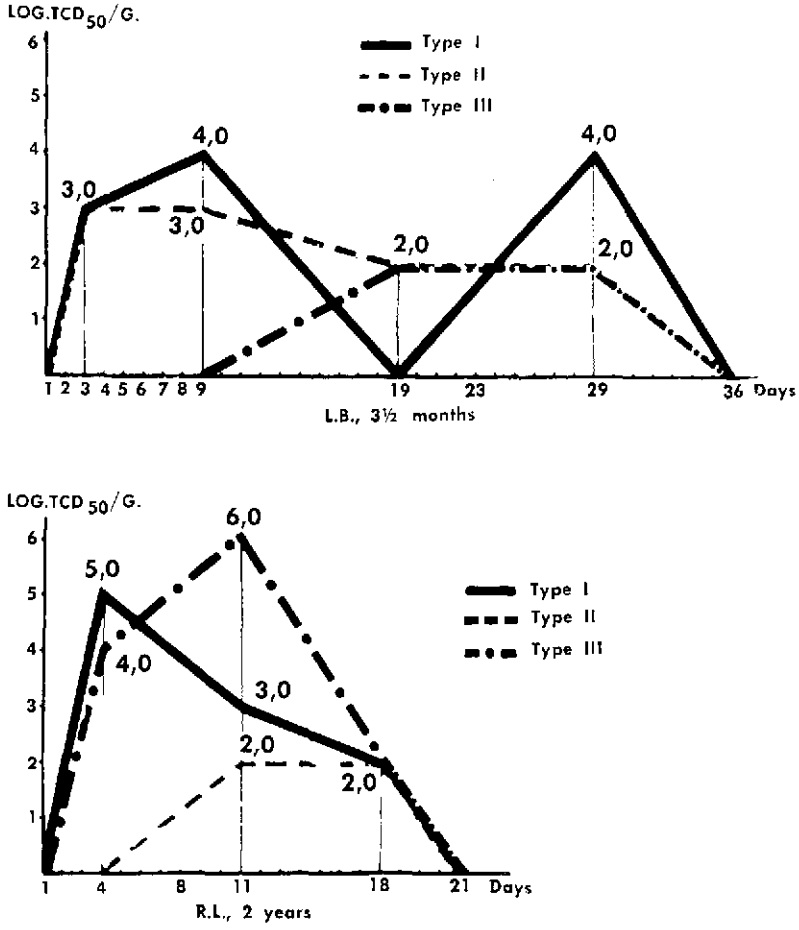


FIG. 3. Examples of simultaneous multiplication of vaccine strains on feeding live trivalent vaccine.

TABLE 17. ESTABLISHMENT OF TYPE 3 VACCINE VIRUS IN PERSONS IMMUNIZED WITH TRIVALENT LIVE VACCINE, IN RELATION TO THE PRESENCE OR ABSENCE OF POLIOMYELITIS ANTIBODY

CHILDREN	ANTIBODY			
	3°	1° 2° 3°	3+	1+2+3+
No. tested	96	24	40	33
Excreted virus	28	8	15	13
Per cent	29	33	37	39

number of children developing antibody after vaccination in the winter and in the summer was observed for Type 1 and Type 3 antibody (17.2 and 9.0 per cent, respectively).

Table 21 and Figures 5, 6, and 7 present antibody titers in vaccinated children. It may be seen, that Type 1 antibody developed most often in titers 1:16-1:64; 25 per cent of the children

TABLE 18. ANTIBODY RESPONSE IN SERONEGATIVE CHILDREN ONE MONTH AFTER ONE FEEDING WITH TRIVALENT LIVE VACCINE

	ANTIBODY DEVELOPMENT IN GROUPS OF "NEGATIVE" CHILDREN											
	1°		2°		3°		1°		2°		3°	
							TYPE 1		TYPE 2		TYPE 3	
	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%
Moscow region (towns and districts)	57* / 78	73.1	82 / 92	89.1	60 / 86	69.8	19 / 31	61.3	24 / 3	77.1	18 / 31	58.1
L. children's home	10 / 32	50.0	15 / 20	75.0	11 / 38	28.9	7 / 19	36.8	15 / 19	78.9	4 / 19	21.0
Karaganda region	19 / 32	59.4	15 / 22	68.2	15 / 27	55.5	1 / 10	10.0	4 / 10	40.0	5 / 10	50.0
Moscow and Karaganda regions (summary data)	92 / 142	64.8	85 / 106	80.2	86 / 151	56.9	27 / 60	45.0	43 / 60	71.7	27 / 60	45.0
Estonian SSR (data by I. N. Dobrova)	28 / 39	71.8	20 / 24	83.3	8 / 30	73.3	8 / 13	61.5	10 / 13	76.9	10 / 13	76.9

* 57/78—57 of 78 children formerly Type 1 negative, developed Type 1 antibody.

TABLE 19. ANTIBODY PATTERNS BEFORE VACCINATION AND ONE MONTH AFTER ONE FEEDING WITH TRIVALENT LIVE VACCINE FROM SABIN STRAINS IN 223 CHILDREN WITH ANTIBODY DEFICIENCY

	BEFORE VACCINATION		ONE MONTH AFTER	
	NO. OF CHILDREN	%	NO. OF CHILDREN	%
000	60	26.9	9	4.0
00+	12	5.4	5	2.2
0+0	37	16.6	25	11.2
0++	33	14.8	14	6.3
+00	7	3.1	8	3.6
+0+	27	12.1	5	2.2
++0	47	21.1	29	13.0
+++	0	0	128	57.3

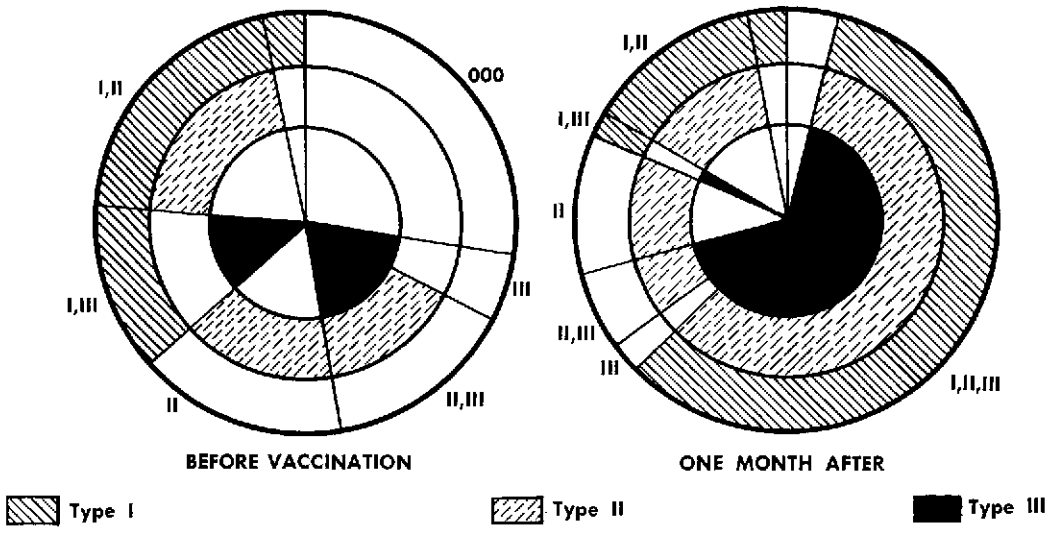


FIG. 4. Antibody patterns before vaccination and one month after one feeding with trivalent live vaccine from Sabin's strains (Moscow region).

TABLE 20. INCREASE IN THE NUMBER OF CHILDREN POSSESSING ANTIBODY TO THE THREE TYPES OF POLIOVIRUS ONE MONTH AFTER ONE FEEDING WITH TRIVALENT LIVE VACCINE

	Number of Children	Increase of Numbers of Children with Antibody to							
		3 types		Type 1		Type 2		Type 3	
		No.	%	No.	%	No.	%	No.	%
Moscow region (towns and districts)	133	91	68.4	57	42.9	78	58.7	55	41.4
L. children's home	39	9	23.1	16	41.1	16	41.0	12	28.1
Karaganda region	51	28	54.9	16	31.4	13	25.3	13	25.5
Moscow and Karaganda regions (summary data)	223	128	56.6	89	39.9	79	35.4	80	35.9
Estonian SSR (data by I. N. Dobrova)	49	30	61.2	28	57.1	20	40.8	22	44.9

developed Type 2 antibody titers of 1:256 or greater. The lowest titers after one feeding were observed with Type 3 antibody: half of the children had Type 3 antibody in titers 1:16.

Analysis of age distribution of antibody (Table 22) showed that one month after one feeding of trivalent live vaccine Types 1 and 3, antibodies were produced in a higher portion of

TABLE 21. ANTIBODY TITERS IN CHILDREN DEVELOPING ANTIBODY ONE MONTH AFTER ONE FEEDING WITH TRIVALENT LIVE VACCINE

SERUM DILUTION	ANTIBODY DEVELOPMENT TO					
	TYPE 1		TYPE 2		TYPE 3	
	NO. OF CHILDREN	%	NO. OF CHILDREN	%	NO. OF CHILDREN	%
1:4	7	8.4	1	1.2	8	10.4
1:8	9	10.8	12	14.3	7	9.1
1:16	16	19.3	13	15.5	24	31.1
1:32	18	21.7	14	16.7	15	19.5
1:64	9	10.8	17	20.2	7	9.1
1:128	17	20.5	6	7.1	11	14.3
1:256 and greater	7	8.4	21	25.0	5	6.5
Total:	83	100.0	84	100.0	77	100.0

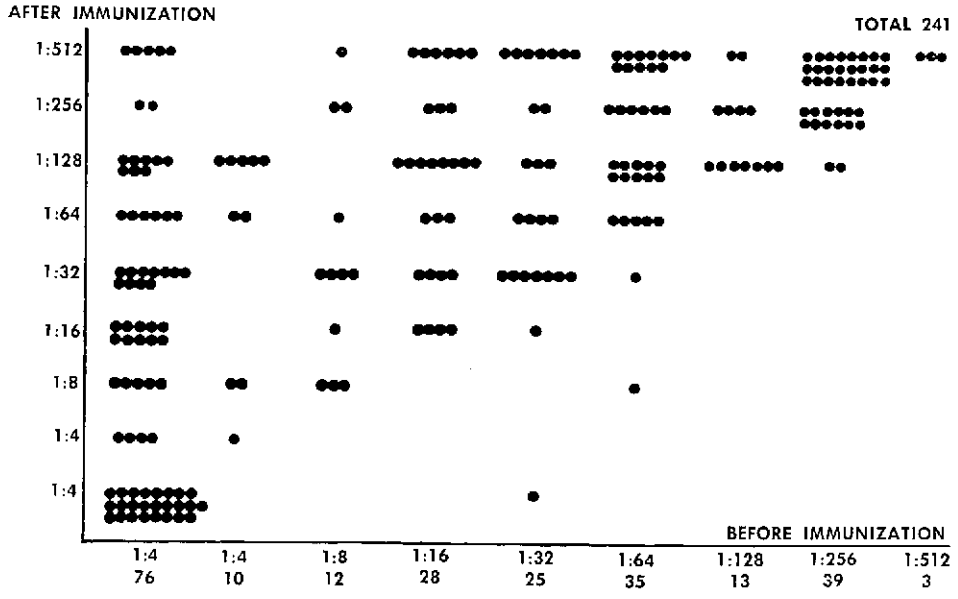


FIG. 5. Rise of Type 1 antibody titers one month after one feeding with live trivalent vaccine from Sabin's strains (Moscow region).

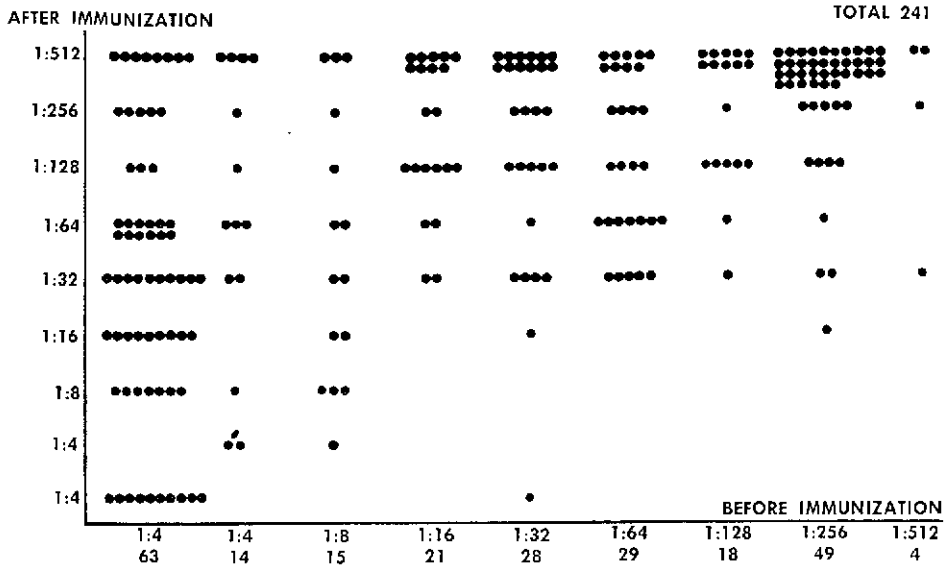


FIG. 6. Rise of Type 2 antibody titers one month after feeding with trivalent live vaccine from Sabin's strains (Moscow region).

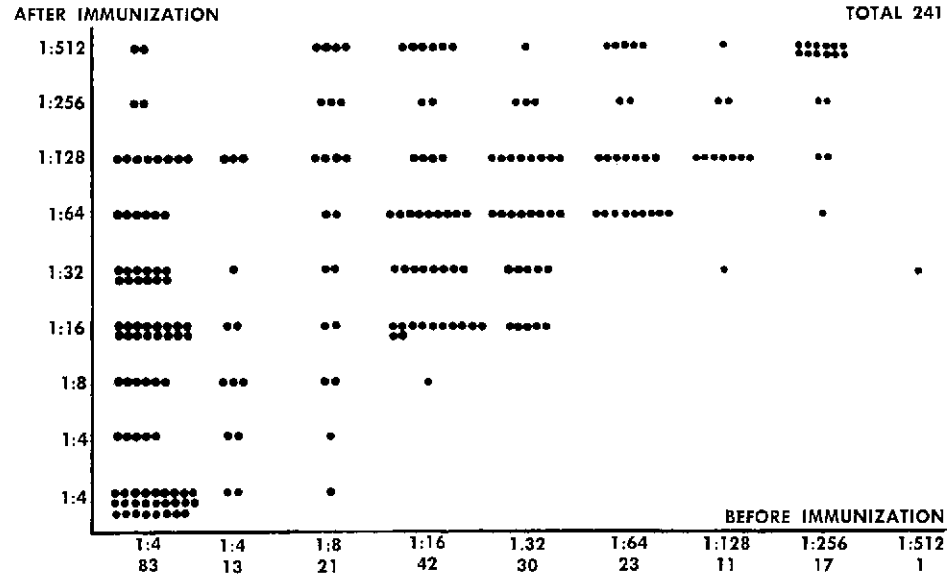


FIG. 7. Rise of Type 3 antibody titers one month after feeding with trivalent live vaccine from Sabin's strains (Moscow region).

older children: 33.3 per cent of Type 1 negative, and 27.8 per cent of Type 3—negative children acquired antibody. The corresponding figures for children one to three years of age were 50.0 per cent and 36.0 per cent, respectively, for children three to seven years 87.7 per cent and 75.5 per cent, respectively, and for children seven to 14 years, 94.1 per cent and 78.9 per

TABLE 22. ANTIBODY RESPONSE ONE MONTH AFTER ONE FEEDING WITH TRIVALENT LIVE VACCINE IN CHILDREN WITHOUT PREVACCINATION HOMOLOGOUS ANTIBODY BY AGES (MOSCOW AND KARAGANDA REGIONS)

AGE-GROUPS (YEARS)	TOTAL NUMBER OF CHILDREN	ANTIBODY RESPONSE TO								
		TYPE 1			TYPE 2			TYPE 3		
		NO. OF CHILDREN	ACQUIRED ANTIBODY	%	NO. OF CHILDREN	ACQUIRED ANTIBODY	%	NO. OF CHILDREN	ACQUIRED ANTIBODY	%
0–1	18	18	6	33.3	15	12	80.0	18	5	27.8
1–3	70	56	28	50.0	37	21	56.7	61	22	36.0
3–7	95	45	43	87.7	37	36	97.3	53	40	75.5
7–14	40	19	15	94.1	17	16	94.1	19	15	78.9
Total	223	142	92	64.8	106	85	80.2	151	86	56.9

cent, respectively. It is possible that children of older-age groups have had previous poliomyelitis infection experience which was not manifested by the development of antibody detectable by the pH test.

At the same time, one must take into account the fact that younger children are more exposed to infection with enteric viruses which evidently prevented good immunologic response to vaccine strains fed in summer.

In contrast, Type 2 antibody developed in 80.0 per cent of the children under one year of age. The impression is that younger children are more susceptible to Type 2 virus or that Type 2 virus is less inhibited by "wild" enteroviruses present in the intestinal tract. Type 2 antibody developed in 56.7 per cent of the children aged one to three years, in 97.3 per cent of the children three to seven years, and in 94.1 per cent of the children seven to 14 years.

Refeeding trivalent live vaccine one month later did not result in considerable improvement of the results obtained after first feeding. While in the L. children's home the percentage of children acquiring Type 1 antibody after one feeding did not change one month after refeeding, in Karaganda some decrease was even found in numbers of sera with low titers, which was reflected, though not considerably, in summary results. In groups of children without pre-vaccination Type 2 antibody both in the L. children's home and in the Karaganda region, there was some increase in the per cent of children developing Type 2 antibody (11.8 per cent). Greater changes were observed in increase of Type 3 antibody, especially in the L. children's home (from 28.9 per cent after first feeding to 48.5 per cent after refeeding). Probably, by the time of refeeding, the intestinal virus carriage preventing Type 3 vaccine strain multiplication had been reduced, which led to more extensive multiplication of vaccine strains and produced further rise in antibody levels, which was reflected in general antibody rise by 16.4 per cent (Table 23).

Comparison of the results observed one month and three to four months after two feedings with trivalent live vaccine from Sabin strains, reveals a certain reduction in the percentage of children acquiring Type 1 antibody (by 10.5 per cent) and a small increase to Type 2 (by 3.4 per cent) and Type 3 (by 2.6 per cent) poliomyelitis antibody (Table 24).

The lack of significant improvement of the results at later periods, and even a certain reduction of Type 1 antibody, is evidently related to a drop in titers in cases of incomplete immunity, as a result of interference by enteric viruses and of reduced opportunity for contact transmission of Type 1 and Type 3 viruses inhibited by Type 2 vaccine virus multiplication. As surveys of vaccine contacts showed, they had predominantly Type 2 virus carriage, accompanied by antibody production. Thus, the children were deprived of additional immunization with Types 1 and 3 poliovirus vaccine.

For investigation of contact transmission of vaccine polioviruses under conditions of vaccination with trivalent live vaccine in the summer, 21 of 93 children in children's home L. and 21 of 63 children in children's home R. were left unvaccinated and in contact with vaccinees.

Virologic investigation of stool specimens during the period of contact with vaccinees showed that all children under observation at one time or another were carriers of enteric viruses of non-poliomyelitis nature, belonging mainly to ECHO-8 viruses or Coxsackie B-1. Despite extensive dissemination of enteric viruses, many children in contact with vaccinees picked up vaccine viruses and began to excrete them.

Of 21 children, seven excreted Type 1 poliovirus, 13 Type 2, and 10 Type 3. About half of the children after contact with the vaccinees became carriers of all three types of poliovirus. The greatest frequency of excretion of three vaccine viruses in the younger children of from five to 16 months was observed before contact as possessing no antibody to any poliovirus types. Of nine such children, five excreted Type 1 virus, eight Type 2, and seven Type 3 virus. Smaller numbers of children developed antibody: three to Type 1, seven to Type 2, and three to Type 3. The other children possessing poliomyelitis antibody before contact developed rise in titers, particularly to Type 2 (Figures 10 and 11).

Under conditions of mass virus carriage of non-poliomyelitis enteroviruses (ECHO -8, 3, 14, 17, 19, and Coxsackie B-1) establishment of vaccine viruses and serologic response in those vacci-

TABLE 23. THE EFFECT OF REFEEDING WITH TRIVALENT LIVE VACCINE FROM SABIN STRAINS ON ANTIBODY RESPONSE

TIME AFTER VACCINATION	ANTIBODY RESPONSE TO								
	TYPE 1			TYPE 2			TYPE 3		
	TOTAL NO. OF CHILDREN 1°	ACQUIRED ANTIBODY	%	TOTAL NO. OF CHILDREN 2°	ACQUIRED ANTIBODY	%	TOTAL NO. OF CHILDREN 3°	ACQUIRED ANTIBODY	%
Lu. children's home									
1 month after first feeding	32	16	50.0	20	15	75.0	38	11	28.9
1 month after second feeding	30	15	50.0	22	18	81.8	33	16	48.5
Karaganda region									
1 month after first feeding	32	19	59.4	22	15	68.2	27	15	55.5
1 month after second feeding	27	14	51.8	18	14	77.8	29	19	65.5
Total									
1 month after first feeding	64	35	54.7	44	30	68.2	65	26	40.0
1 month after second feeding	57	29	50.9	40	32	80.0	62	35	56.4
Difference			−3.8			+11.8			+16.4

TABLE 24. ANTIBODY RESPONSE ONE AND THREE TO FOUR MONTHS AFTER TWO FEEDINGS WITH TRIVALENT LIVE VACCINE FROM SABIN STRAINS

TIME AFTER VACCINATION	ANTIBODY RESPONSE TO								
	TYPE 1			TYPE 2			TYPE 3		
	TOTAL NO. OF CHILDREN 1°	ACQUIRED ANTIBODY	%	TOTAL NO. OF CHILDREN 2°	ACQUIRED ANTIBODY	%	TOTAL NO. OF CHILDREN 3°	ACQUIRED ANTIBODY	%
R. children's home									
1 month	38	22	57.9	16	15	93.7	41	11	26.8
4 months	37	18	48.6	12	11	91.6	38	13	34.2
Karaganda region									
1 month	32	19	59.4	22	15	68.2	27	15	55.5
3 months	15	7	46.6	8	6	75.0	11	7	63.6
Total									
1 month	70	41	58.6	38	31	81.6	68	26	38.2
3–4 months	52	25	48.1	20	17	85.0	49	20	40.8
Difference			−10.5			+3.4			+2.6

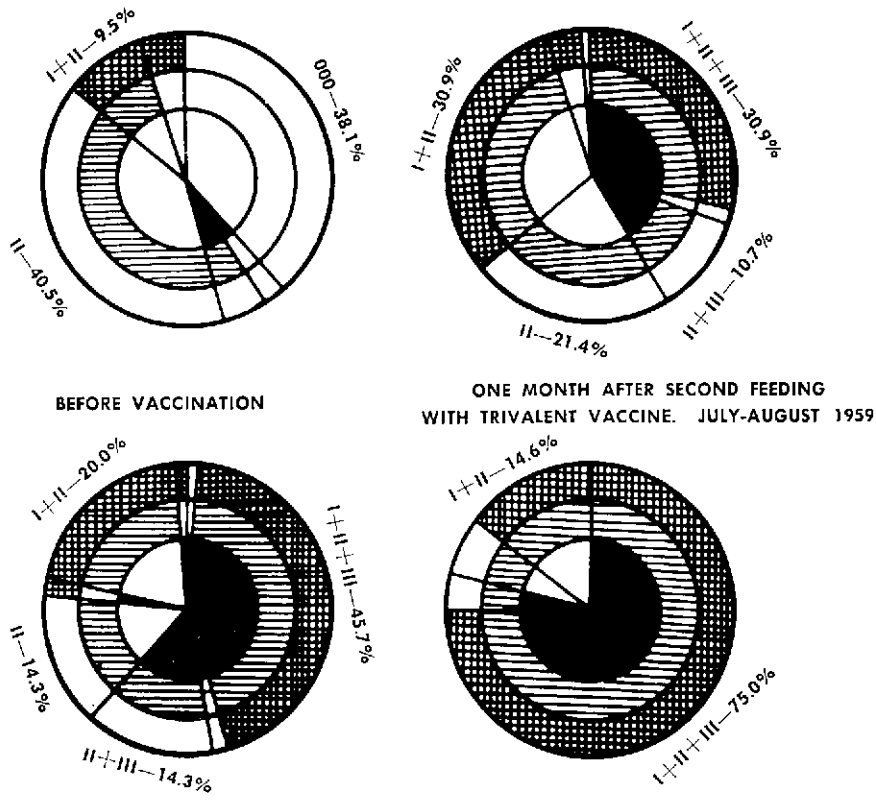


FIG. 8. Antibody patterns in children with antibody deficiency. Children's Homes L. and R.

TABLE 25. ISOLATION OF ATTENUATED VIRUS OF POLIOMYELITIS TYPE 1 FROM CHILDREN (0-3 YRS.) WITH CYTOPATHOGENIC AGENTS "CPA+" AND WITHOUT CYTOPATHOGENIC AGENTS "CPA—" DURING THE 14 DAYS AFTER IMMUNIZATION

CHILDREN	1+2 WEEKS		3+4 WEEKS	
	CPA−	CPA+	CPA−	CPA+
Investigated	107	70	107	70
Virus isolated	68	17	45	17
Per cent	63	24	41	24

nated with live trivalent vaccine from Sabin strains are much lower, especially as regards Types 1 and 3 viruses (Tables 25-28). At the same time simultaneous multiplication of three types of vaccine viruses and non-poliomyelitis enteroviruses was observed, with development of respective antibody (Figures 12 and 13).

Immunologic resistance of the intestinal tract to Types 1 and 2 polioviruses develops one to one and a half months after immunization with

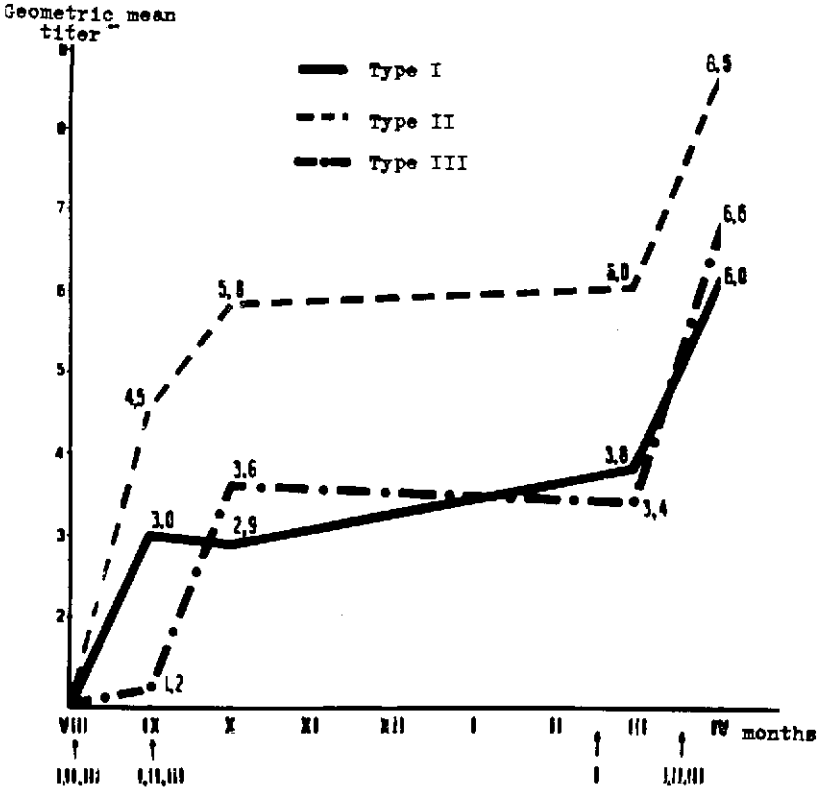


FIG. 9. Dynamics of antibody titers in seronegative children after two feedings with live liquid vaccine Types 1, 2, 3; candy Type 1; candy Types 1, 2, 3 (children's home in Lyubertsy).

TABLE 26. ISOLATION OF ATTENUATED VIRUS OF POLIOMYELITIS TYPE 2 FROM CHILDREN (0-3 YRS.) WITH CYTOPATHOGENIC AGENTS "CPA+" AND WITHOUT CYTOPATHOGENIC AGENTS "CPA—" DURING THE 14 DAYS AFTER IMMUNIZATION

CHILDREN	1+2 WEEKS		3+4 WEEKS	
	CPA−	CPA+	CPA−	CPA+
Investigated	107	70	107	70
Virus isolated	71	21	59	29
Per cent	66	30	55	24

live trivalent vaccine, despite the fact that by this time some children are still virus carriers after the first feeding. In contrast to this, Type 3 vaccine virus was excreted by a larger number of children in children's homes L. and R. after the second feeding than after the first feeding. One may suppose that, after the first feeding, extensive multiplication of this virus was prevented by the high extent of infection with non-poliomyelitis enteric viruses. Three months after

TABLE 27. ISOLATION OF ATTENUATED VIRUS OF POLIOMYELITIS TYPE 3 FROM CHILDREN (0-3 YRS.) WITH CYTOPATHOGENIC AGENTS "CPA+" AND WITHOUT CYTOPATHOGENIC AGENTS "CPA—" DURING THE 14 DAYS AFTER IMMUNIZATION

CHILDREN	1+2 WEEKS		3+4 WEEKS	
	CPA−	CPA+	CPA−	CPA+
Investigated	107	70	107	70
Virus isolated	40	2	34	14
Per cent	37	3	31	20

TABLE 28. ANTIBODY RESPONSE IN TRIPLE-NEGATIVE CHILDREN IMMUNIZED WITH TRIVALENT LIVE VACCINE UNDER CONDITIONS OF MASS LATENT INFECTION WITH NON-POLIOMYELITIS ENTERIC VIRUSES (CHILDREN'S HOMES L. AND R.)

	NUMBER OF CHILDREN	DEVELOPED ANTIBODY TO					
		TYPE 1		TYPE 2		TYPE 3	
		No.	%	No.	%	No.	%
One month after first feeding	21	7	33.3	17	80.9	3	14.3
One month after second feeding	18	7	38.9	15	83.3	7	38.9

the second feeding with trivalent live vaccine, immunologic resistance of the intestinal tract of the vaccinated was tested separately to Type 1, Type 2, and Type 3 vaccine viruses. Owing to considerable spread of non-poliomyelitis enteric viruses, the results of this test could be considered valid only in five cases for Type 1, in nine cases for Type 2, and in three cases for Type 3. In five children possessing no Type 1 antibody before vaccination, Type 1 vaccine virus multiplied after primary vaccination during four to five weeks, and Type 1 antibody developed in titers 1:32, 1:128 in four children only. On testing immunological resistance of the intestinal tract three months after the second feeding, one episode of Type 1 virus excretion was observed in one child, V.P., 10 months old ($10^{1.7}$ TCD_{50} per gram), who had previously developed Type 1 antibody to the titer 1:128. Child P.T., 23 months old, who had no antibody despite extensive multiplication of Type 1 virus after first ingestion of the vaccine, exhibited immunological resistance of the intestinal tract to Type 1 virus. Evidently, in this case, immunological resistance of the intestinal tract developed without concurrent production of serum antibody.

Eight out of nine children without pre-vaccination Type 2 antibody, excreting vaccine virus during four weeks after primary feeding, showed no multiplication of Type 2 vaccine virus. Child I.K., two years old, who had previously excreted Type 2 vaccine virus for one week and developed Type 2 antibody in the titer 1:16 after the test for the intestinal tract resistance, had one episode of Type 2 virus excretion with 10^8 TCD_{50} of virus per gram of feces.

In a special investigation of immunologic resistance in children who had had poliomyelitis, one episode of homologous Type 1 virus excre-

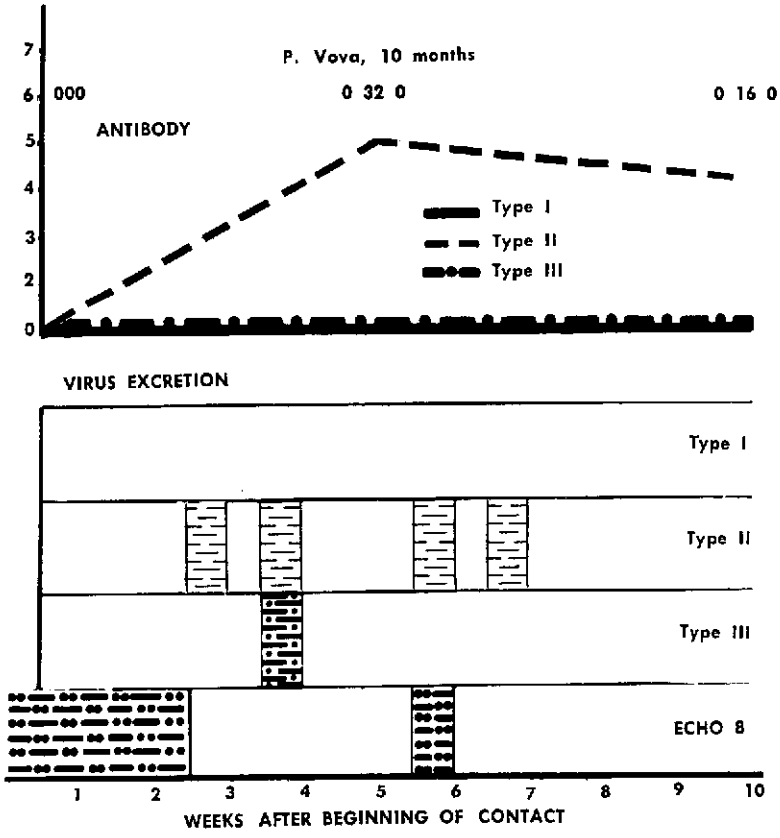


FIG. 10. The effect of ECHO-8 virus carriage on contact transmission of vaccine strains when trivalent live vaccine from Sabin's strains was administered twice.

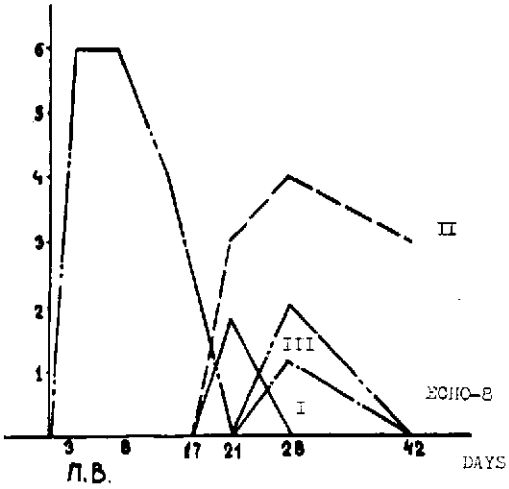


FIG. 11. The effect of ECHO-8 virus carriage on contact transmission of vaccine strains when trivalent live vaccine from Sabin's strains was administered twice.

tion was observed in seven out of 22 children. Type 3 vaccine virus did not multiply in one child who had had Type 2 poliomyelitis and in two children having had Type 3 poliomyelitis. It should be noted that Type 3 vaccine virus did not multiply also in the intestinal tract of four convalescents who had had Type 1 poliomyelitis, three of them possessing no Type 3 antibody. The same vaccine virus multiplied extensively in the intestinal tract of unvaccinated persons and in one child four months old, after two feedings of trivalent live vaccine. The possibility cannot be excluded that in these cases, there was non-specific resistance of the alimentary tract to Type 3 virus in convalescents after Type 1 poliomyelitis.

When heterologous immunologic resistance was tested in child A.B., three years old, who had had Type 1 poliomyelitis, extensive multiplication of Type 2 vaccine virus was observed.

The children who had been vaccinated twice with trivalent live vaccine in the summer of 1959, received Type 1 vaccine incorporated into dragée-candy in February 1960; and in March 1960, they received trivalent vaccine in dragée-candy. Virologic investigation of fecal specimens after these two additional feedings has not yet been completed. Serological investigation of children from chidren's homes L. and R. two weeks after final feeding showed that 75 per cent of children with antibody deficiency developed antibody to all three types of poliomyelitis virus. Type 1 antibody developed in 87.2 per cent, Type 2 antibody in 100 per cent and Type 3 antibody in 76.2 per cent of children without previous corresponding antibody (Fig. 8). At the same time, increase of titers to all three types was observed (Fig. 9).

Thus, in vaccination of younger children with trivalent vaccine, best results were obtained with regard to Type 2. Therefore, in order to avoid interfering effect of Type 2 virus, primary vaccination should be carried out with Types 1 and 3 viruses, and then trivalent mixture may be given. For revaccinations, trivalent live vaccine may be used preferably in the form of dragée-candy.

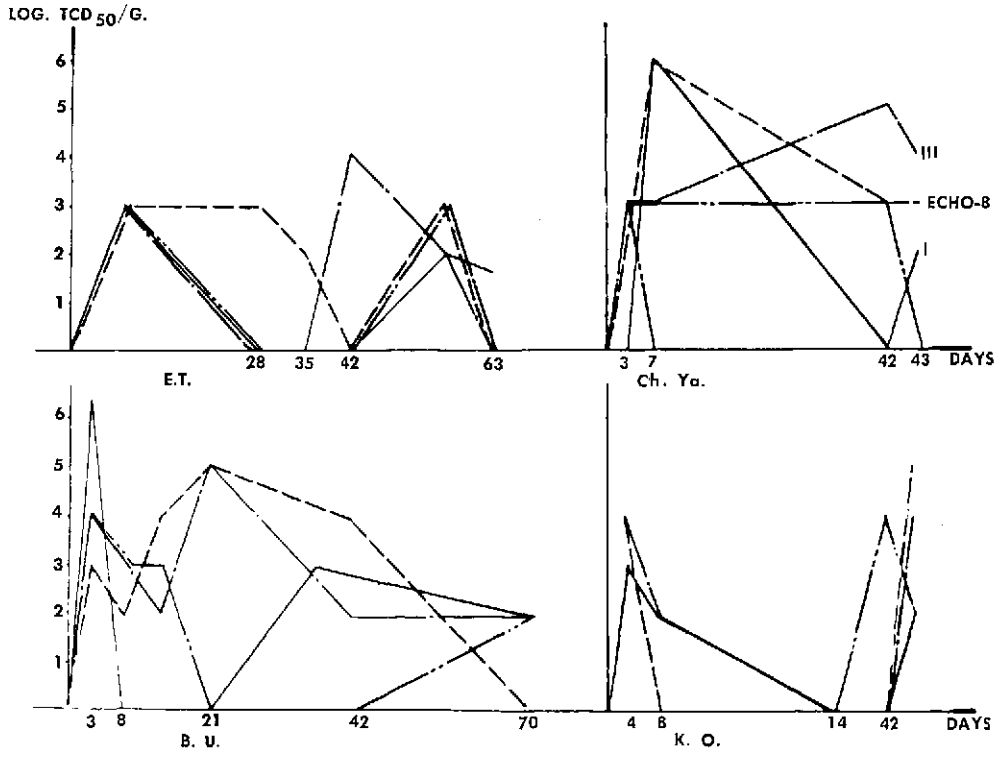


FIG. 12. Examples of interaction between vaccine strains and non-polio enteroviruses on two feedings with trivalent live vaccine.

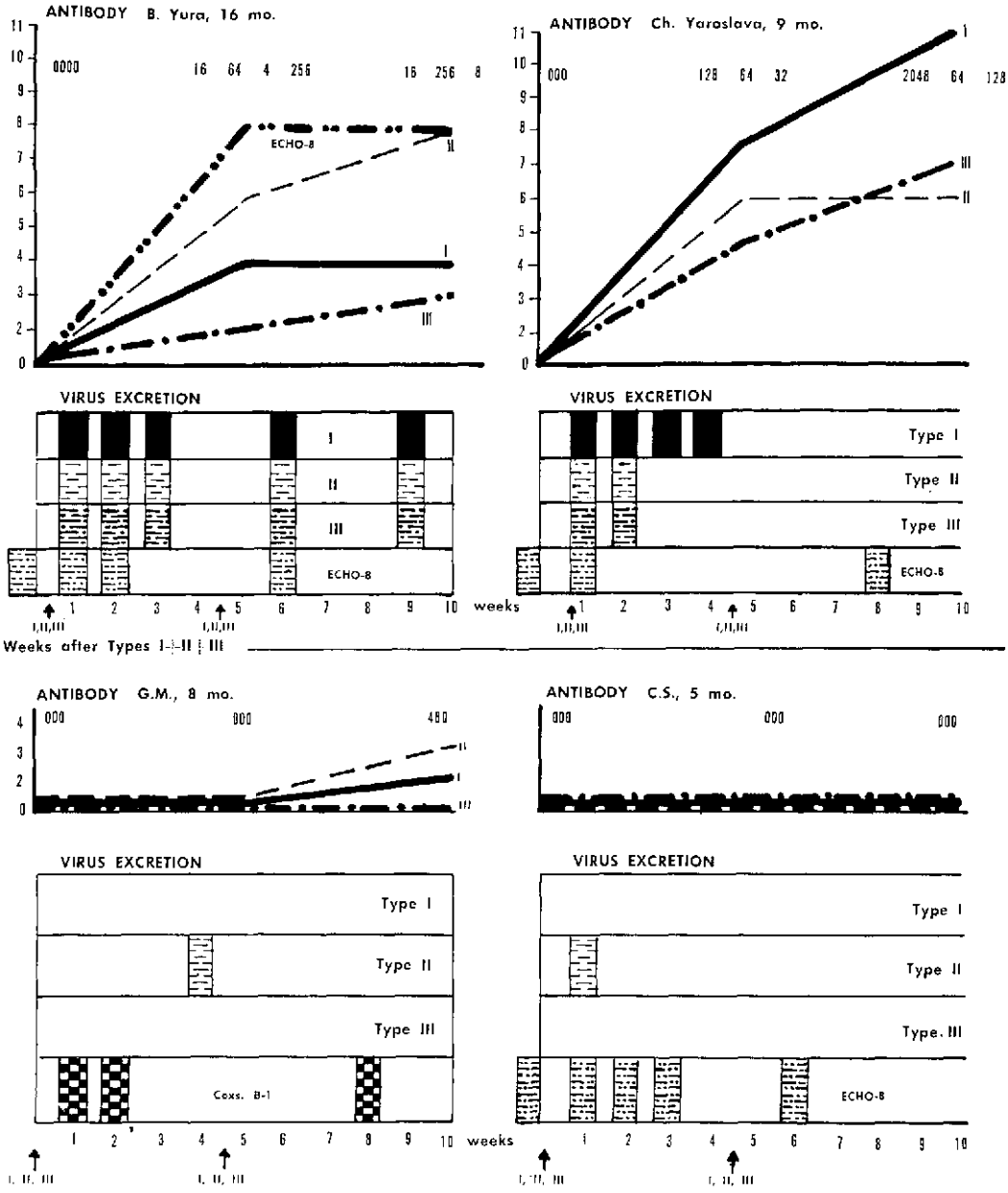


FIG. 13. Examples of interaction between vaccine strains and non-polio enteroviruses on two feedings with trivalent live vaccine.

DISCUSSION

CHAIRMAN GEAR: The last three papers are now open for discussion, that is, the papers by Dr. Prem, Dr. Vonka, and by Dr. Voroshilova.

DR. HODES: The Chairman stated that he would grant me a few minutes to speak about a new method for determining antibody. This method has been described to a number of my North American colleagues, but it has not been seen by others since the results of this are in press at the moment. We believe the method to be simple enough to be used for antibody testing on a mass basis. We believe also that it is more sensitive than any antibody method now in use.

The method which I shall describe very briefly is based on the fact that a specific antibody retards the spread of a radioactive virus up a strip of filter paper. We think the test may have practical importance because it requires only 0.1 ml. of serum. This can be obtained by finger puncture, which is the way we have done it. The test can be completed in a few hours. Moreover, the materials required, cost approximately five cents.

I do not think that we have time to go into it, but believe we can make this a very sensitive test, perhaps more so than any now available. In conducting the test hundreds of times, and in comparing it with standard neutralization tests, we have obtained a very high degree of correlation, something over 95 per cent.

The present method we use is described in Fig. 1. A strip of filter paper is used. Although the figure shows only the first 23 centimeters, we actually have it 29 centimeters long, ruled off in centimeter spaces. This piece of filter paper is then suspended from a cork, and put into a test tube. At the bottom of the test tube there is 0.5 cc. of radioactive poliovirus suspension.

The particular virus in this test was Type 2 with a titer of 15,000 TCD_{50} per 0.1 ml. and radioactivity of 15,000 counts per minute. One tenth of 1 ml. of the serum to be tested is spread across space 3. The bottom part of the filter paper

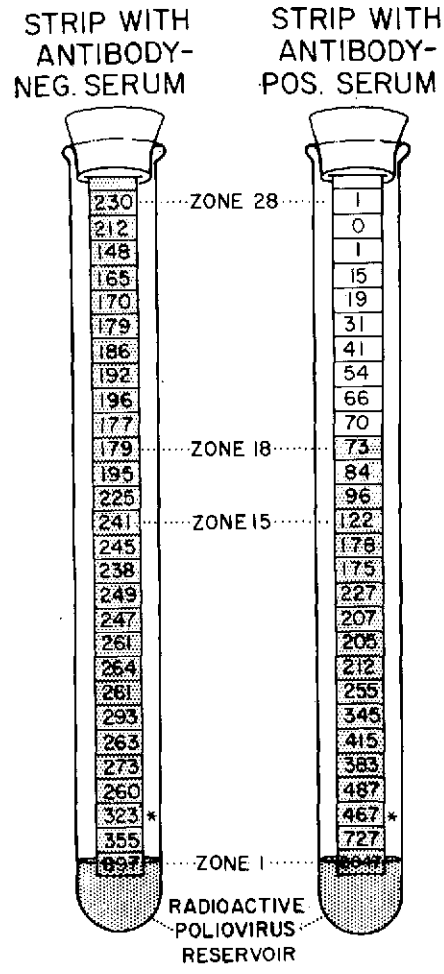


FIG. 1

(space 1) is dipped into the virus, the paper being allowed to become wet.

When the paper is wet all the way up to the top, it is taken out and put into an oven at 140° C.; this renders it non-infectious and makes the radioactive phosphorus stick to the paper. The paper strip is then fed into an automatic scanning device which plots the distribution of radioactivity on a graph.

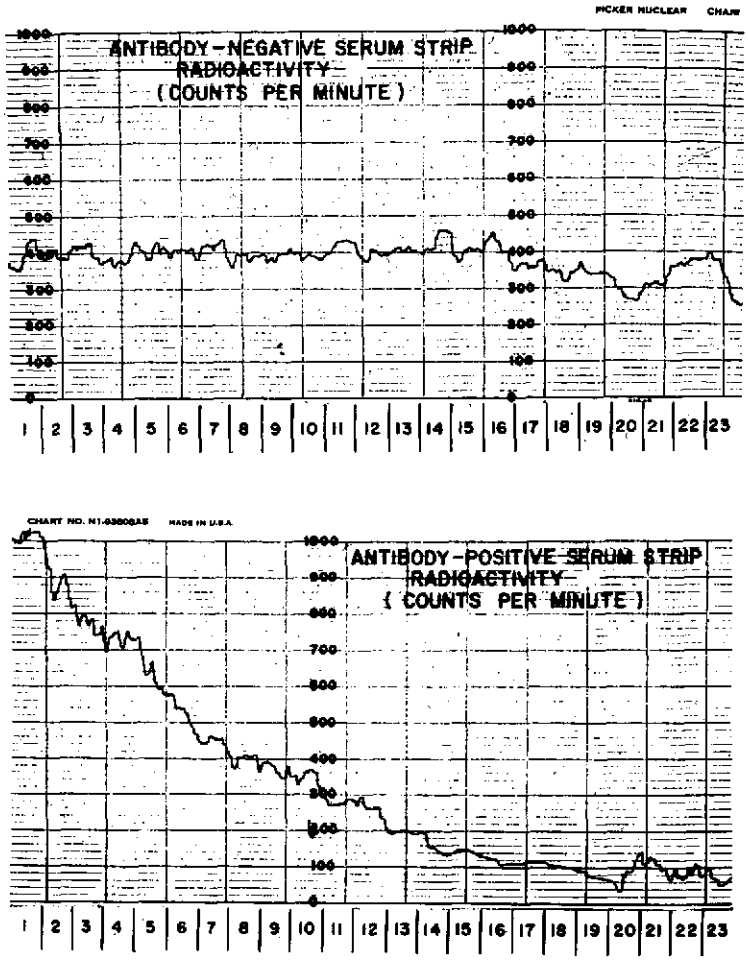


FIG. 2

In the case of the strip which has an antibody-negative serum on it, an almost straight line is plotted. The bottom space moves somewhere around 400 counts per minute and stays at this level all the way up the strip, reaching 300 counts per minute at the top of the strip.

In contrast to this, when 0.1 ml. of serum with a neutralizing antibody titer of 1:32 (as judged by cytopathogenic methods) was put on the second strip (space 3), the spread of radioactive virus is retarded (is kept down), and it piles up on the lower spaces. It starts out at a count about 1,000 per minute, then plots a curve that goes down to under 100. This whole procedure takes about eight minutes for the counting.

I wish to point out that we could merely have counted the bottom few spaces of the strip or the top few spaces, which would have taken only a minute or so, and the proper answer would have been obtained.

A mathematical expression can be found for the curve of radioactivity of the immune serum strip and a second one for the antibody-negative strip. We therefore believe this to be a reproducible physical phenomenon.

Figure 3 shows other examples of the effect of specific antibody on movement of radioactive Type 1 poliomyelitis up the paper strips.

DR. GARD: I should like to ask Dr. Vonka whether he has tested the stability of the two different activities he measured, the pH inhibition and the cytopathogenic inhibition in some other sera showing the greatest discrepancy; for instance, permanent stability of the two factors and

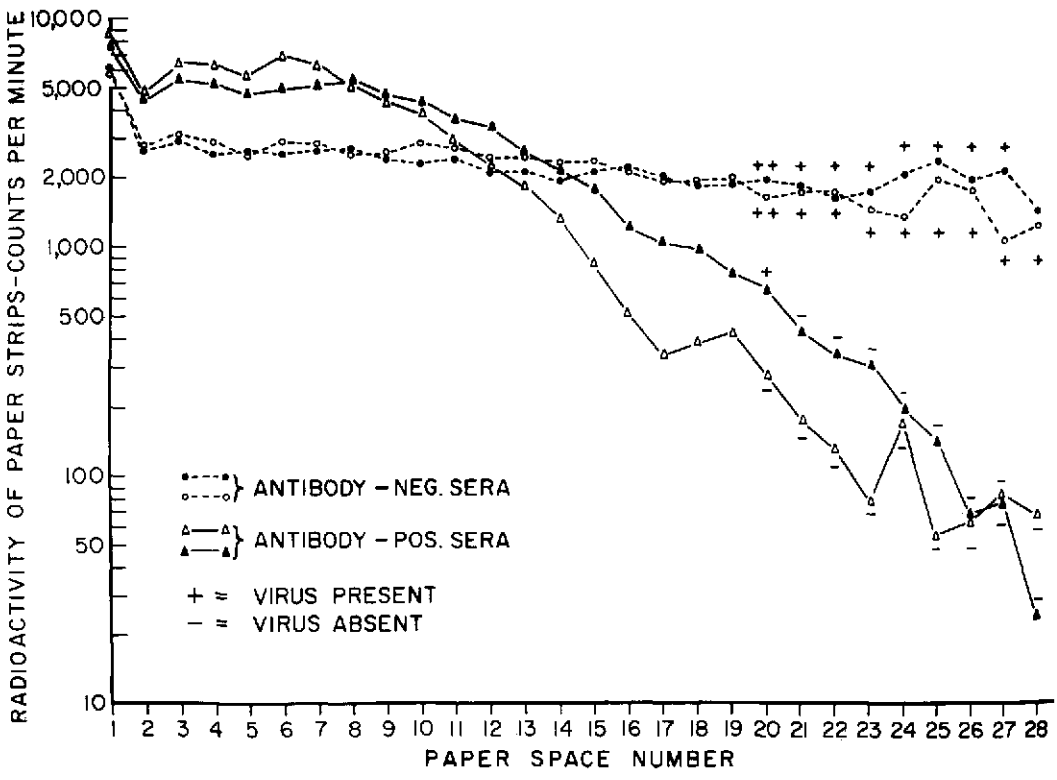


FIG. 3

the possibility of removing some activity with codeine or similar procedures.

DR. VONKA: We are planning such experiments. I agree perfectly with Dr. Gard that the first step in further investigation should be the analysis of all the possible non-specific factors which can play any role.

I should like to present some other results concerning the problem of antibody avidity, which may be of some interest. We investigated the RVA in virus-serum mixtures, containing sera, in which great discrepancies between the pH and CP tests were estimated, by means of virus titrations in tubes and by the plaque-counting method, as recommended by Dr. Dulbecco.

These results were obtained within the last few days and are shown in Fig. 1.

We have investigated two serum specimens taken in subject 18/HB as shown in the figure. The first one was obtained three weeks after virus was fed, the second nine weeks later. Mixtures were prepared containing the final dilution of serum 1:50 and 100,000 TCD_{50} of virus per 0.1 ml. They were incubated at 37° C. and at different intervals samples were drawn and diluted 1:1000, in order to have about 100 TCD_{50} of virus per 0.1 ml. inoculum.

The results achieved in the investigation of the first serum specimens show that the virus inactivation proceeded much more quickly when the plaque-counting method was used for RVA-estimation than in tube titrations. On the other hand, when the second serum specimen was tested, approximately the same results were achieved by both methods. I believe it rather difficult to explain this phenomenon. It is possible, however, that the conditions under agar overlay may prevent the virus antibody dissociation in the case of low avidity antibody, which readily occurs when tube tissue cultures are used. If this is true, the comparison of results achieved

in the investigation of RVA by the plaque counting method and by titrations in tissue culture tubes may be helpful in estimating the quality of antibodies.

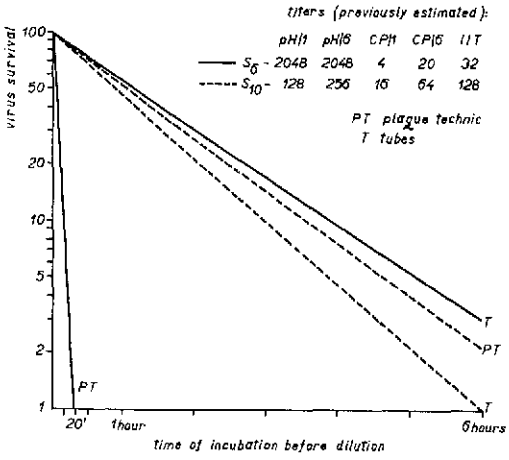


FIG. 1. The results of the residual virus activity estimated by plaque counting method and by virus titrations in tubes, in samples drawn from virus-serum mixtures containing 1:50 dilution of sera taken three weeks after virus was fed (S_6) and nine weeks later (S_{10}).

DR. BARON: I should like to ask Dr. Prem a question concerning the calculated abortion rate on the women fed vaccine virus before the 13th week of pregnancy.

Since in order to damage the fetus, the virus must pass through the blood from the intestinal tract to the fetus, were these abortion rates calculated on the basis of the pregnant women who had no prior poliovirus antibody so that the abortion rate would reflect vulnerable fetuses?

DR. PREM: No. This abortion rate was calculated from the entire group of women vaccinated up to the 13th week of pregnancy. It includes all, whether they had pre-feeding antibody titers or not. Two of the four women who aborted had unmeasurable antibodies prior to feeding. When measured after feeding, these two had significant rises. There were at least 7 women in the entire group who did not abort and who produced one normal, negative, pre-feeding titer, that changed to positive after feeding.

CHAIRMAN GEAR: The program has been arranged so as to continue with two papers on a similar subject. These will be presented by Dr. Goldblum on "Virological Findings and Antibody Response of Infants Fed Multiple Type Oral Poliomyelitis Vaccine", and by Dr. Pagano on "Routine Immunization with Attenuated Poliovirus of 850 Children Living in Philadelphia." In view of the time factor involved, the discussion on these papers will take place during the fifth session.

3. VIROLOGICAL FINDINGS AND ANTIBODY RESPONSE OF INFANTS FED MULTIPLE TYPE ORAL POLIOMYELITIS VACCINE. A PRELIMINARY REPORT*

SONNIA LEVINE AND NATAN GOLDBLUM

The Virus Laboratory
Ministry of Health, Tel Aviv-Yaffo, Israel

DR. GOLDBLUM *(presenting the paper)*: Paralytic poliomyelitis in Israel, as in certain other areas of the world, is of a strictly infantile character, and a high incidence of paralysis occurs in infants, four to 12 months of age.[1, 2] Antibody studies on infants of this age group have shown that they are usually devoid of maternal antibodies.[3] Thus, active immunization against poliomyelitis at this age is highly desirable. Attention has recently been called to the effect of single—and multiple—type oral poliomyelitis vaccines for immunization of infants.[4–7]

In view of our specific interest to investigate the advisability of early age immunization with an attenuated oral vaccine under local conditions, the present study was set up with the purpose of determining the following:

1. Effectiveness of a multiple type vaccine when fed to newborns.
2. Effectiveness of the vaccine when fed to infants, 1-4 months of age.
3. Immunological response to one or two feedings.
4. Intestinal resistance to repeated feedings.
5. Possible interference between poliomyelitis types fed.
6. Possible interference by naturally occurring enteroviruses with implantation of fed polioviruses.

Location of the field trial. The experiment is being carried out in Kyriat Shmone, a newly developed settlement in upper Galilee. This somewhat isolated town has a population of 14,000 composed of new immigrants representing many ethnic groups. A health center, under the auspices of the Ministry of Health, covers preventive health services. A maternity ward and child welfare clinic are attached to the center. An average of 50 infants are born monthly in the maternity ward. Seven infant-welfare subclinics serve the population. Physicians and qualified public health nurses are in charge of the child welfare work in both the health center and subclinics. This work was carried out with the full cooperation of these physicians and public health nurses.

Plan of the study. The study involves approximately 500 newborns and infants. Virological and immunological follow-up was carried out according to the following schedule:

Infants, 1-4 months old. There were approximately 200 infants of this age group in Kyriat Shmone when this work was initiated. During the month of December 1959, these infants were brought to the health center and fed the oral vaccine after pre-feeding blood and stool specimens were taken. Infants, one to two months old, at the initial feeding were re-fed in April, 1960. Post-feeding stool specimens were collected at weekly intervals for a period of four weeks from all infants fed. Second blood specimens were collected when the infants reached the age of six to seven months, which is three months after feeding.

Newborns. Approximately 300 newborns are included in this study. Prior to discharge from the maternity ward, the newborns, three to five days old, were fed the oral vaccine. Those born in December 1959 and January 1960 were re-fed in February and March, respectively, two months after the initial feeding. Post-feeding stool specimens were collected at weekly intervals for a period of four weeks. Cord blood was collected

* Aided by a grant from the Lederle Laboratories, Division of the American Cyanamid Company, Pearl River, New York.

at time of birth and a second blood specimen will be taken from the infants when they reach the age of six months.

Oral poliomyelitis vaccine. Individual doses of trivalent oral vaccine, "Orimune", lot No. 7-1238-804A, in vials containing 2 ml., kindly supplied by the Lederle Laboratories, Pearl River, New York, were used for feeding. Prior to feeding, the vaccine was titrated for whole virus content and found to contain $10^{6.2}$ TCD_{50} per dose. Since Type 2 poliovirus was found to be absent in numerous post-feeding stool specimens tested, a titration was carried out for the quantitative estimation of the poliovirus types. The trivalent vaccine was found to contain: Type 1-$10^{5.7}$ TCD_{50}, Type 2-$10^{4.2}$ TCD_{50}, and Type 3-$10^{6.2}$ TCD_{50}.

A new lot of poliomyelitis oral vaccine, No. 7-1238-812A1, was received in March 1960. Titration for whole virus content showed $10^{6.3}$ TCD_{50} per dose. Titration for estimation of the individual type concentrations showed: Type 1-$10^{6.2}$, Type 2-$10^{5.7}$, and Type 3-$10^{7.7}$. The new lot of vaccine is being used for refeeding of the infants and continued feeding of newborns.

Laboratory studies. Virus isolations from stool specimens: rectal swabs were collected in 2 cc. medium consisting of Hanks' solution and 0.5 per cent lactalbumin enzymatic hydrolysate. Specimens were kept at —20° C. until used. For preparation of the stool suspension, the swabs were thoroughly rinsed in the medium and centrifuged at 3000 rpm. for 20 minutes. The supernatant fluid was decanted and antibiotics added as follows: 200 units of penicillin, 200 micrograms of streptomycin, and 100 units of mycostatin per ml. of stool suspension. Monkey-kidney cell cultures (MKCC) were prepared from Rhesus monkeys in growth medium consisting of Hanks' solution, 0.5 per cent lactalbumin enzymatic hydrolysate, 2 per cent calf serum, and 0.22 per cent sodium bicarbonate with a final pH of 7.6. Stool suspensions, in 0.2 cc. amounts, were inoculated into three MKCC tubes and observed for CPE for a period of seven to 10 days.

Identification of strains. Pre-feeding stool specimens showing CPE were typed by the neutralization test using immune sera against poliovirus Types 1, 2, and 3, ECHO virus Types 1-14, Coxsackie B virus Types 1-5, and Coxsackie A9. The immune poliovirus horse sera were kindly supplied by Dr. V. J. Cabasso, Lederle Laboratories, New York. Post-feeding cultures showing CPE were carried through neutralization tests, using pooled pairs of poliovirus immune sera. Specimens containing mixtures of polioviruses with other previously identified enteroviruses were typed by adding the immune serum of the identified enterovirus to the pooled pairs of immune poliovirus sera.

Antibody tests. Neutralization tests were carried out on the pre- and post-feeding sera simultaneously. All sera were inactivated for 30 minutes at 56° C. prior to testing. The serum samples were then prepared in fourfold dilutions. Approximately 100 TCD_{50} per 0.25 ml. of poliovirus Types 1, 2, and 3, respectively, were added to an equal volume of the respective serum dilutions. The mixtures were incubated at 37° C. for two hours and then inoculated into MKCC tubes. Results were recorded after seven days of incubation at 37° C.

RESULTS

This preliminary report deals only with two aspects of the study: (1) virological and serological findings on 65 infants, three to four months old, who had been given a single dose of oral multiple poliomyelitis vaccine, and (2) effect of naturally occurring enteroviruses on the virological and serological response to the poliovirus types fed, in 14 infants, one to four months of age.

Table 1 is a summary of the virological findings in the post-feeding stool specimens taken from 65 infants, three to four months old, whose pre-feeding stools were free from enteroviruses. At first glance, the complete absence of Type 2 poliovirus is noted, while both Types 1 and 3 were repeatedly isolated from 71 per cent of the infants. Failure to isolate any of the poliovirus types fed occurred in only 6 per cent of the infants examined. Isolation of a single type only was found in 14 per cent for Type 3 and 9 per cent for Type 1 poliovirus. A consideration of the total number of infants who excreted poliovirus Type 1 or Type 3, singly or in combination, shows the percentage to be 80 for Type 1 and 85 for Type 3.

TABLE 1. VIROLOGICAL ISOLATIONS FROM INFANTS, 3-4 MONTHS OF AGE, AFTER FEEDING ORAL POLIOMYELITIS VACCINE
NO PRE-FEEDING ENTEROVIRUSES FOUND

POLIOVIRUS TYPES EXCRETED	Nº OF CHILDREN	PERCENT POSITIVE
T_1 AND T_3	46	71
T_1 ONLY	6	9
T_3 ONLY	9	14
NONE	4	6
TOTAL	65	100

• T = type.

TABLE 2. IMMUNOLOGICAL RESPONSE OF INFANTS, 3-4 MONTHS OF AGE, TO ORAL POLIOMYELITIS VACCINE
NO PRE-FEEDING ENTEROVIRUSES FOUND

POLIOVIRUS TYPE	Nº OF INFANTS WITH ANTIBODY RESPONSE	PERCENT
T_1 AND T_3*	42	68
T_1 ONLY	6	9
T_3 ONLY	11	18
NONE	3	5
TOTAL	62	100

• T = type.

The corresponding immunological response is summarized in Table 2. Again we find a high per cent of the infants responding to poliovirus Types 1 and 3, isolated from their stool specimens. Considering response to single types, it is seen that 77 per cent of the infants responded to Type 1, and 86 per cent to Type 3.

Details of the immunological response are illustrated in Fig. 1. Here are shown pre- and post-feeding antibody titers to Types 1 and 3 poliovirus. The infants show comparatively high titers in their post-feeding specimens, as compared to their pre-feeding specimens. The antibody titers for Type 3 are somewhat higher than those for Type 1 which may indicate that this Type 3 poliovirus is a better antigen.

Correlation between the virological findings and serological response are indicated in Table 3, which demonstrates almost complete correlation between both methods of followup of the poliomyelitis virus fed. Incomplete correlation is noted in five children only. Three infants who had excreted Type 3 poliovirus in their stool specimens, showed antibody response to both Type 3 and Type 1, while two other infants who had excreted Types 1 and 3 simultaneously, re-

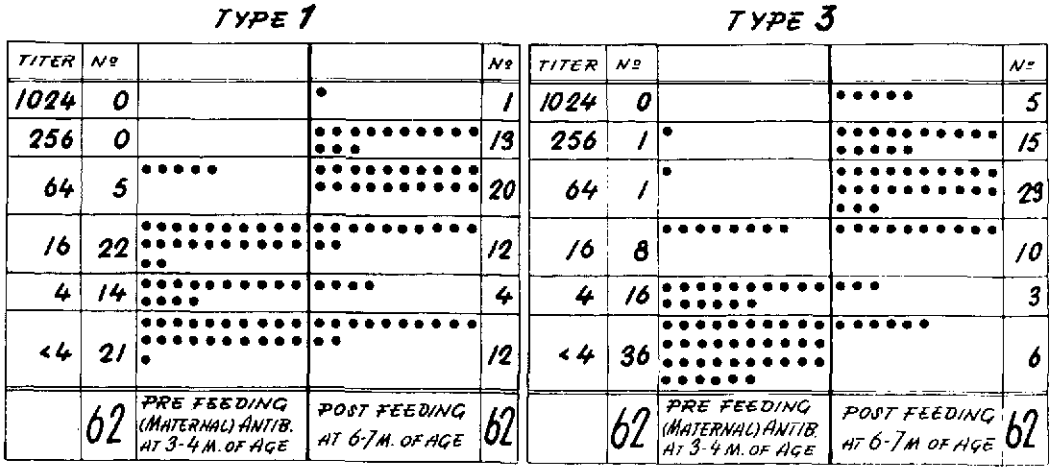


FIG. 1. Antibody titers in pre- and post-feeding blood specimens of infants 3-4 months old, after feeding oral poliomyelitis vaccine.

TABLE 3. CORRELATION OF VIROLOGICAL AND SEROLOGICAL FINDINGS AFTER FEEDING ORAL POLIOMYELITIS VACCINE

VIROLOGICAL FINDINGS: TYPES OF POLIOVIRUS	No. CHILDREN	SEROLOGICAL RESPONSE TO: T_1	T_3	T_1 AND T_3	NONE
T_1 ONLY	4	(4)			
T_3 ONLY	10		(7)	3	
T_1 AND T_3	35	1	1	(33)	
NONE	3				(3)

sponded serologically to a single Type 1 or 3, respectively.

Table 4 shows virological findings and antibody titers in the post-feeding specimens of 14 "triple negative" children, i.e. children without maternal antibody. Attention is called to child 76, the only infant who excreted poliovirus Type 2 and showed a corresponding rise in antibody titer. In the other infants, high antibody titers were obtained to polioviruses isolated from their stool specimens, again indicating adequate antibody responses to the types of poliovirus isolated from their stool specimens.

TABLE 4. POLIOVIRUS ISOLATIONS AND ANTIBODY RESPONSE IN "TRIPLE NEGATIVE" INFANTS, 3-4 MONTHS OLD, AFTER FEEDING ORAL POLIOMYELITIS VACCINE

NO PRE-FEEDING ENTEROVIRUSES FOUND

CHILD Nº	POLIOVIRUS TYPES ISOLATED	ANTIBODY TITER TO: T_1	T_2	T_3
13	T_1 AND T_3 *	64	0	64
56	"	64	0	256
63**	"	0	0	64
69	"	256	0	1024
74	"	16	0	64
76	T_1, T_2, T_3	64	64	256
102	T_1 AND T_3	64	0	256
103	"	16	0	16
108	"	16	0	16
114	"	256	0	64
115	"	256	0	64
143	"	16	0	16
105	T_3	4	0	64
118	T_3	0	0	64

* T = type.

** Type 1 was isolated in one stool specimen, Type 3 was isolated from all 4 stool specimens.

TABLE 5. POLIOVIRUS ISOLATIONS AND ANTIBODY RESPONSE IN INFANTS, 3-4 MONTHS OLD, WITH MATERNAL ANTIBODY TITER OF 1:16 TO 1:64 AFTER FEEDING ORAL POLIOMYELITIS VACCINE

CHILD Nº	POLIOVIRUS TYPE ISOLATED	ANTIBODY TITER TO: T_1	T_3
1	T_1 AND T_3	256	256
17	"	64	256
53	"	64	64
81	"	256	256
95	"	16	16
99	T_3	0	1024
46	T_3	4	64

In Table 5 virological and serological results are shown in seven infants whose pre-feeding sera showed the presence of antibodies in titers of 1:16 to 1:64 to both Types 1 and 3 poliovirus. The relatively high antibody titers to the homologous types of polioviruses excreted, demonstrate little or no effect of maternal antibodies on both virus excretion and the resulting antibody titers.

Of approximately 153 infants, one to four months old, 16 per cent showed the presence of an enterovirus in their pre-feeding stool specimens. Complete results of the virus isolations in pre- and post-feeding stool specimens, as well as antibody titers on pre- and post-feeding blood samples, have thus far been obtained on 14 of these infants.

Tables 6a and 6b summarize the virological and serological findings. In Table 6a are included seven infants from whose pre-feeding stool specimens enteroviruses were isolated and identified as Coxsackie B5 and A9, and ECHO 2 and 14.

Children Nos. 40 and 8 excreted Coxsackie B5 in both the pre- and post-feeding stools. This did not seem to interfere with the multiplication and excretion of the vaccine polioviruses, nor did it affect the antibody response which was high to both poliovirus Types 1 and 3 excreted. Infant No. 91 did not show the Coxsackie strain in the

TABLE 6A. VIROLOGICAL FINDINGS AND SEROLOGICAL RESPONSE TO ORAL POLIOMYELITIS VACCINE IN INFANTS WITH PRE-FEEDING ENTEROVIRUS IN THEIR STOOLS

PRE-FEEDING ENTEROVIRUS IDENTIFIED

CHILD Nº	PRE-FEEDING ENTEROVIRUS	POST-FEEDING STOOL ISOLATIONS AT WEEKLY INTERVALS				PRE-& POST FEEDING ANTIBODY TITER TO	
		1	2	3	4	T_1	T_3
40	Cox B5	T_1 T_3 Cox B5	T_1 T_3 Cox B5	T_1	T_3	0* 64	0 256
8	Cox B5	–	T_3 Cox B5	T_1	T_1	16 64	16 64
91	Cox B5	–	T_1	T_1	T_1	0 16	0 0
149	Cox B5	T_1 . T_3	T_1 . T_3	T_3	–	4 0	0 64
133	Cox A9	T_1 T_3	T	T_1	T_1	0 64	0 4
101	ECHO 2	–	–	T_1 T_3	T_1 . T_3	4 256	0 64
18	ECHO 14	–	T_1 . T_3	T_3	T_1	64 64	0 256

* Upper numbers—pre-feeding, lower numbers—post-feeding.

post-feeding stool specimens. Type 1 poliovirus only was excreted and a somewhat lower antibody response was found to the type excreted. Infant No. 149 also excreted Types 1 and 3 in the post-feeding stool specimens, but responded serologically only to Type 3 and failed to show antibodies to Type 1 poliovirus. Child No. 133 excreted Type 3 in one stool specimen but Type 1 in all four stool specimens. The serological response, as observed, was mainly to Type 1 and very low to Type 3 poliovirus. Infants Nos. 101 and 18 excreted both Types 1 and 3 and showed a comparatively high antibody titer to both.

Table 6b illustrates results obtained on seven

TABLE 6B. VIROLOGICAL FINDINGS AND SEROLOGICAL RESPONSE TO ORAL POLIOMYELITIS VACCINE IN INFANTS WITH PRE-FEEDING ENTEROVIRUS IN THEIR STOOLS

PRE-FEEDING ENTEROVIRUS UNIDENTIFIED

CHILD Nº	POST-FEEDING STOOL ISOLATIONS AT WEEKLY INTERVALS				PRE-& POST FEEDING ANTIBODY TITER TO	
	1	2	3	4	T_1	T_3
19	0	T_3	T_3	0	16 0	0 256
30	T_1 T_3	T_1 . T_3	T_1 . T_3	T_1	16 64	0 64
45	0	0	–	0	16 0	4 0
71	–	UNIDENTIFIED.		–	64 4	4 0
72	–	0	0	0	0 0	16 4
111	0	0	0	0	0 0	0 0
126	–	T_1	T_3 . T_1	T_1	0 64	16 4

T = Types of poliovirus.
– = Not done.

Upper number = Pre-feeding.
Lower number = Post-feeding.

additional infants from whose pre-feeding stool specimens an enterovirus was isolated but was not identifiable with the immune sera on hand. Here the picture is somewhat different; only child No. 30 showed consecutive isolations of Types 1 and 3 poliovirus with a corresponding serological response. Two infants, Nos. 19 and 126 responded to a single type only. Four children did not excrete any of the vaccine viruses nor did they show any antibody response in their post-feeding sera. An unidentified virus isolated in the pre-feeding specimen from infant No. 71 was again isolated from two post-feeding stool specimens.

COMMENT

Several points emerge from these findings. It has been shown that infants three to four months old, readily excrete the poliovirus strains fed for a period of at least four weeks. Only 6 per cent of the infants fed did not excrete any of the poliovirus types fed. These findings compare favorably with results obtained in older children.[8-11] Results of the virological findings, together with the corresponding homologous serological responses, indicate by the percentage of positives, as well as the high antibody titers, that infants three to four months of age may be effectively immunized with a multiple type oral poliomyelitis vaccine. It is regretted that no adequate information has been obtained with regard to the Type 2 poliovirus. Although the Lederle Type 2 strain has been previously reported to be poorly excreted,[5, 12] the almost total absence of this strain in the post-feeding stool specimens of infants fed the oral vaccine and the corresponding negative antibody response may be assumed to be due to the low concentration of this poliovirus type in the trivalent vaccine used for feeding.

A very high correlation between poliovirus types isolated and the corresponding antibody response has been demonstrated, indicating that the techniques used for virus isolations and identifications were satisfactory. In approximately 70 per cent of the infants studied, two types of the vaccine polioviruses multiplied adequately as judged by virus isolations and the immunological response. In a small per cent only a single type multiplied; this may be due to either interference between the poliovirus types[13] or to factors associated with individual responses.

Presence or absence of maternal antibodies did not seem to affect virus excretion or homotypic antibody response although there were too few children with high antibody levels to draw definite conclusions. The studies on the children excreting enteroviruses other than polioviruses demonstrate that the problem of interference is not as serious as previously reported. Of the 14 infants observed, 10 showed multiplication of at least one, and mostly of both poliovirus types fed, sometimes in combination with the pre-feeding enteroviruses. Lack of interference in the majority of the infants may be the result of the high dose of the multiple type oral vaccine administered, as previously suggested by Cox *et al.*[12] and by Sabin.[14]

REFERENCES

1. Yekutiel, P., Levinger, E. L., Muhsam, H. V., and Yekutiel, M. P.: Poliomyelitis outbreak in Israel, 1950-1951. *Bull. World Health Org.* **12:** 651-676, 1955.
2. Davies, M. A., Marberg, K., Goldblum, N., Levine, S., and Yekutiel, P.: Epidemiology of poliomyelitis in Israel, 1952-1959 with evaluation of Salk vaccination during a three-year period. *Bull. World Health Org.* In press.
3. Spigland, I. and Goldblum, N.: Immunization of infants with formalinized poliomyelitis vaccine. *Pediatrics.* In press.
4. Koprowski, H., Norton, T. W., Hummeler, K., Stokes, Jr., J., Hunt, A. D., Flack, A., and Jervis, G. A.: Immunization of infants with living attenuated poliomyelitis virus. *J. Am. M. Assn.* **162:** 1281-1288, 1956.
5. Martins da Silva, M., McKelvey, J. L., Prem, K. A., Bauer, H., Cooney, M. K., and Johnson, E. A.: Studies of orally administered attenuated live virus poliomyelitis vaccine in newborns and infants under six months. *Univ. Minn. M. Bull.* **29:** 133-150, 1957.
6. Plotkin, S. A., Koprowski, H., and Stokes, J.: Clinical trials in infants of orally administered attenuated poliomyelitis viruses. *Pediatrics* **23:** 1041-1062, 1959.
7. Prem, K. A., McKelvey, J. L., and Fergus, J.: Immunologic response of infants under six months of age to oral trivalent poliomyelitis vaccine. In: *Live Poliovirus Vaccines, First International Conference.* Scient. Pub. no.

44 of the Pan American Health Organization, pp. 254-259, 1959.

8. Levine, S., Goldblum, N., Moscovici, O., and Horetzski, H.: Field trial with the Lederle Type 3 attenuated oral poliomyelitis vaccine. To be published.
9. Barr, R. N., Bauer, H., Kleinman, H., Johnson, E. A., Martins da Silva, M., Kimball, A. C., and Cooney, M. K.: The use of orally administered live attenuated polioviruses as a vaccine in a community setting. A controlled study. *J. Am. M. Assn.* **170:** 893-905, 1959.
10. Verlinde, J. D. and Wilterdink, J. B.: A small-scale trial on vaccination and revaccination with live attenuated poliovirus in the Netherlands. In: *Live Poliovirus Vaccines, First International Conference.* Scient. Pub. no. 44 of the Pan American Health Organization, pp. 355-366, 1959.
11. Horstmann, D. M., Niederman, J. C., and Paul, J. R.: Attenuated Type 1 poliovirus vaccine; its capacity to infect and to spread from "vaccinees" within an institutional population. *J. Am. M. Assn.* **170:** 1-8, 1959.
12. Cox, H. R., Cabasso, V. J., Markham, F. S., Moses, M. J., Moyer, A. W., Roca-García, M., and Ruegsegger, J. M.: Immunologic response to trivalent oral poliomyelitis vaccine. In: *Live Poliovirus Vaccines, First International Conference.* Scient. Pub. no. 44 of the Pan American Health Organization, pp. 229-248, 1959.
13. Koprowski, H.: Immunization of man against poliomyelitis with attenuated preparations of living virus. *Ann. N. York Acad. Sc.* **61:** 1039-1049, 1955.
14. Sabin, A. B.: Present status of attenuated live virus poliomyelitis vaccine. *Bull. N. York Acad. M.* **33:** 17-39, 1957.

4. ROUTINE IMMUNIZATION WITH ATTENUATED POLIOVIRUS OF 850 CHILDREN LIVING IN PHILADELPHIA. PRELIMINARY REPORT*

JOSEPH S. PAGANO, M.D., STANLEY A. PLOTKIN, M.D., CARL C. JANOWSKY, M.D., AND HILARY KOPROWSKI, M.D.†

The Wistar Institute and the Philadelphia Department of Public Health, Philadelphia, Pennsylvania

DR. PAGANO *(presenting the paper):* The incidence of poliomyelitis increased in Philadelphia in 1958; half the cases occurred in children less than five years old who lived in a densely populated section of the City. A study was undertaken, as an outgrowth of this increase, to explore whether orally administered polio vaccine could be used as a practical public-health measure in city clinics. The antibody responses and reactions to vaccination with attenuated poliovirus of infants and children attending health clinics for routine immunizations were studied in the section of the City where most of the cases had occurred the previous year.

This is the first study in the United States of the use of living attenuated poliomyelitis vaccine in a substantial number of susceptible children living normally within a large city. Other investigations have been conducted in housing projects [1] and as household studies.[2, 3] This study was conducted within the population shown by several urban epidemics to be most in need of immunization and least likely to get it: children less than five years of age in the lower income groups.[4–6]

Children in the Study. Between January and July 1959, 850 children were given Type 1 vaccine; 805 of these children were also given Type 3 vaccine, and 335 were given Type 2, in sequence, at four-week intervals. The age distribution of the children is given in Table 1; 79 per cent were less than two years old.

Viruses Used for Immunization. The viruses and concentrations were: $10^{5.7}$ $TCID_{50}$ CHAT (Type 1), $10^{5.7}$ $TCID_{50}$ W-Fox (Type 3),[7] and $10^{6.2}$ $TCID_{50}$ P-712 (Type 2).[8] The vaccines were administered by health-center nurses who

TABLE 1. AGE DISTRIBUTION OF THE CHILDREN IN THE STUDY

Age	No.	Per cent Bled*
1-1/2 to < 2 mo.	34	47
2 to < 3 mo.	152	31
3 to < 4 mo.	109	28
4 to < 5 mo.	78	33
5 to < 6 mo.	72	31
6 to < 12 mo.	157	20
1 to < 2 yr.	71	31
2 to < 3 yr.	56	30
3 to < 4 yr.	53	21
4 to 5 yr.	62	21
Unknown	6	33
All Ages	850	28

* Before vaccination.

* This study was supported by a U.S. Public Health Service Grant, National Institute of Allergy and Infectious Disease.

† Dr. Pagano and Dr. Plotkin (Research Associates, The Wistar Institute; Epidemic Intelligence Service Officers, Communicable Disease Center, U.S. Public Health Service, Atlanta, Georgia); Dr. Janowsky (Director, Division of Epidemiology, Philadelphia Department of Public Health); and Dr. Koprowski (Director, The Wistar Institute). The participation of Drs. Pagano and Plotkin in this study does not imply endorsement by the Public Health Service; the opinions are those of the authors.

squirted the strain of virus, suspended in one cubic centimeter of phosphate-buffered saline solution, into an ounce of milk in the infant's bottle or into a cup of chocolate milk.

Serologic Sampling. Twenty-eight per cent of the children were bled at random before vaccination so that a significant sample (about 20 per cent) of paired sera would be obtained (Table 1). Neutralizing antibody titers were determined in paired pre- and post-vaccination sera from the same child by the metabolic inhibition test.[6]

Serologic Findings Before Vaccination. In the group one and one-half to three months old only 17 per cent of the sera were without any detectable antibodies (titers $<1:8$), as shown in Table 2. However, by the age of four to five months the percentage of sera without antibodies had risen to 60 per cent, a reflection of the rapid loss of transplacentally acquired antibodies. Antibodies had dropped to nondetectable levels in virtually all children one-half to one year old (Table 2). Thereafter, the percentage of sera without antibodies declined until it was 40 per cent in the three-to-five-year group, presumably as a consequence of natural infection with poliovirus.

Susceptibility of Population. During a period of about three years—from four months to three years of age—the children in the study were highly susceptible to poliomyelitis: 66 per cent of the children in this group were without polio antibodies of any type. In addition, most of the children less than four months, who had maternal polio antibodies before vaccination, could be expected to become susceptible within two months, as indicated by the sharp increase in children without antibodies in the four-to-five-month group (Table 2).

Health Inquiries After Vaccination. All children were seen approximately four weeks after receiving each dose of vaccine by a physician, nurse, or city health worker in the clinic or at home if the child failed to return. A summary of the visits and the distribution of illnesses by weekly intervals after vaccination is shown in Table 3. The majority of children were well during the course of immunization, but many illnesses were uncovered, mostly simple colds without fever. Gastrointestinal illnesses and fever without other symptoms were infrequently reported.

TABLE 2. DISTRIBUTION OF POLIOMYELITIS ANTIBODIES BY AGE

Age of Children	Serum Titrations	Antibody Status Before Vaccination			
		Triple-Negative	Type 1 Negative	Type 2 Negative	Type 3 Negative
	(No.)	(%)	(%)	(%)	(%)
1-1/2 to 3 mo.	52	17	27	54	54
4 to 5 mo.	20	60	75	85	80
1/2 to < 1 yr.	17	82	88	94	100
1 to < 2 yr.	16	62	81	100	81
2 to < 3 yr.	11	55	91	82	73
3 to < 5 yr.	10	40	60	80	80
All Ages	126	44	58	70	67

The miscellaneous illnesses were: earaches (eight), mumps (four), insomnia (two), wheezing, teething, pharyngitis, fretfulness, thrush, and undescribed (one each). Seven children were hospitalized: four for pneumonitis, two for bacterial meningitis proved by cerebrospinal fluid culture, and one for herniorrhaphy. There was one death: a three-month-old boy was found dead five weeks after receiving Type 1 and ten days after Type 3 vaccine; during this time the child had seemed well.*

dren indicated a random distribution. In addition, no excess of any one type of illness was observed in both study districts at a particular interval after administration of a strain of vaccine.

Community Surveillance. There were approximately 3800 persons, including 991 children five years old or younger, in the 830 households of vaccinated children.

Between the end of January and the end of July 1959, 97 cases of aseptic meningitis,

TABLE 3. CONDITION OF CHILDREN AND DISTRIBUTION OF ILLNESSES BY INTERVALS AFTER VACCINATION

Vaccine	Interval After Vaccination (days)	Kind and Number of Illnesses: Cold	Cold & Fever	Exanthema	Fever	Cough	Gastrointest.	Misc.	Total Illnesses	Well (No.)	Delinquent or Dropped (No.)	Not Vaccinated (No.)	Total Children (No.)
Type 1	1-7	21	6	6	8	4	5	2	52	547	16		850
	8-14	24	6	5		3	4	4	46				
	15-21	40	2	2§	1	1	3	4	53				
	22-28	39	16‡	11§	4	1	2	6	79				
	Other*	25	10	7	3	6	3	3	57				
Type 3	1-7	16	3	4	8		6		37	550	39	45	850
	8-14	18	8	3			4	1	34				
	15-21	24	10	2	1		4	3	44				
	22-28	27	8	5	2	4	7	2	55				
	Other*	25	9	6	1	1	2	2	46				
Type 2	1-7	1	3	1	2		2		9	251	16	515	850
	8-14	8	3	1	1			1	14				
	15-21	8	4	3		1			16				
	22-28	8	3		1	1	1		14				
	Other*	4	2	6		1	2		15				

* Illnesses occurring after 28 days and after an unknown interval.
‡ Two from one district, 14 from the other.
§ Three from one district, eight from the other.

The incidence of illnesses was similar during each of the four intervals after administration of each type of vaccine except for a slight predominance during the 22-28-day interval, possibly because the mothers tended to recall the illnesses that had occurred during the period just before the clinic visit.

Safety of Vaccine. This was not a safety test. However, no case of poliomyelitis, paralytic or nonparalytic, occurred in a vaccinated child. Analysis of the minor illnesses in vaccinated children indicated a random distribution. In addition, no excess of any one type of illness was observed in both study districts at a particular interval after administration of a strain of vaccine.

encephalitis, and miscellaneous paralytic disease that occurred in the entire City of Philadelphia were screened as possible cases of poliomyelitis. Laboratory studies,† performed in each case, gave no evidence of poliomyelitis. On the basis of these studies and the clinical findings, none of the 97 cases was considered to be poliomyelitis by the Department of Public Health.

The first isolations of poliovirus (Type 3) were made in July 1959 from two children in whom the onset of paralytic disease was June 11 and June 19. The third case occurred July 19. The three children, the only persons known to have contracted poliomyelitis during the study, lived in

* Complete postmortem study, including histologic examination of the brain, the medulla, and the spinal cord, and virologic study by The Pennsylvania State Virologic Laboratory of frozen specimens of these tissues in chick embryo, mice, and tissue culture failed to reveal the cause of death or a diagnosis. No lesions of poliomyelitis were found.

† Courtesy of Dr. Klaus Hummeler, Chief, Commonwealth of Pennsylvania Virus Diagnostic Laboratory, Children's Hospital of Philadelphia.

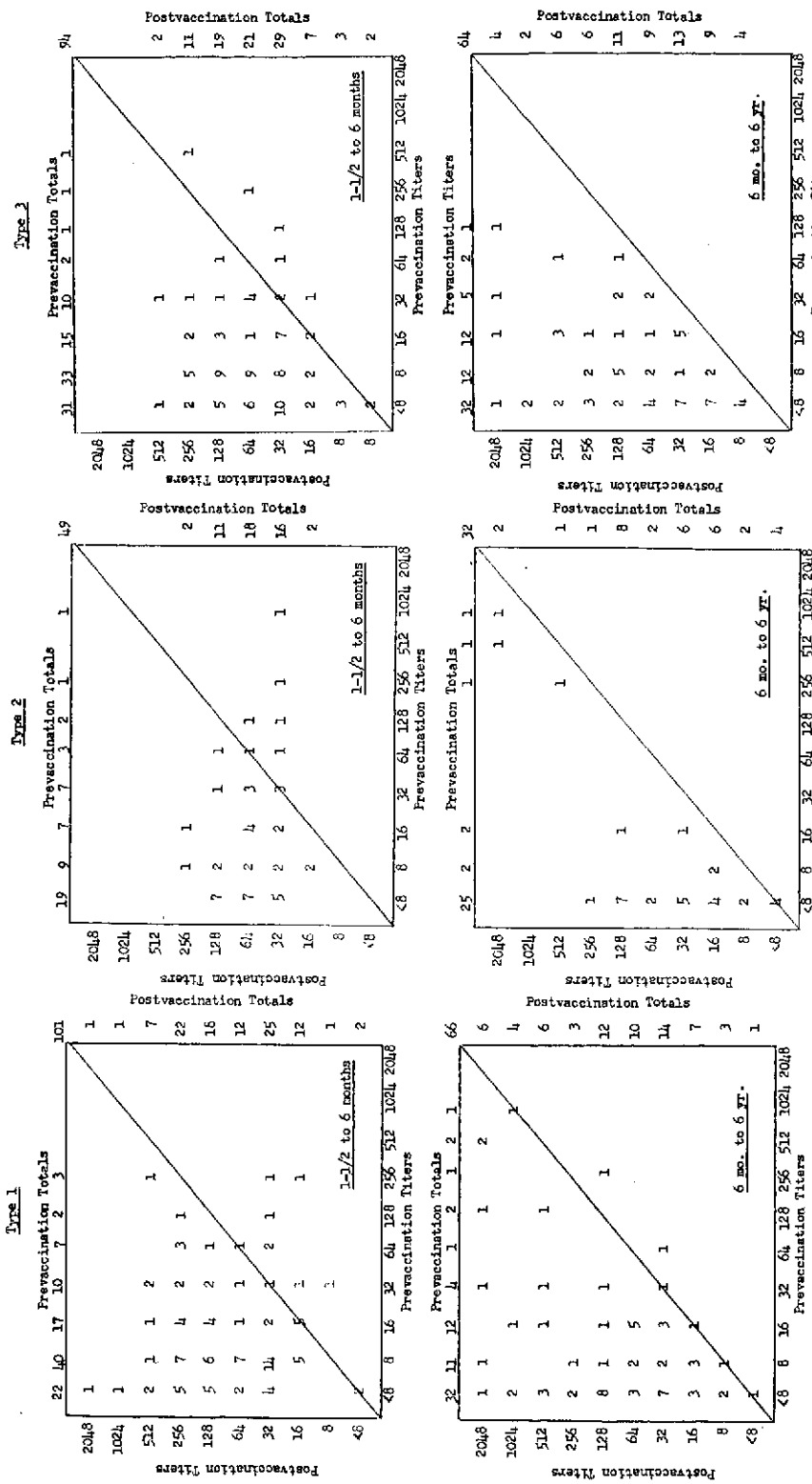


FIG. 1. Distribution of Poliomyelitis Antibody Titers in Children Before and After Vaccination by Age Group. The distribution of the prevaccination antibody titers is given by the pre-vaccination totals for each titer along the tops of the six boxes; the post-vaccination totals are given for each titer along the right sides of the boxes. Above each pre-vaccination titer is a vertical column of figures that indicates the distribution of post-vaccination titers. The numbers above the diagonal lines represent instances of an increase in titer after vaccination, those on the diagonal lines had the same titer before and after, and the numbers below the diagonal lines represent instances of a fall in titer after vaccination. In the 1½-to-6-month age group the pre-vaccination antibodies were assumed to be maternal in origin; in instances in which vaccination was unsuccessful these antibodies would be expected to show a decline in titer of more than two dilutions (see text).

TABLE 4. SUCCESS OF VACCINATION AND MEDIAN ANTIBODY TITERS BY AGE AND SUSCEPTIBILITY —Summary—

VACCINE	AGE GROUP AND PREVACCINATION ANTIBODIES	PAIRED SERA	MEDIAN TITERS*		OUTCOME OF VACCINATION	
			BEFORE VACC.	AFTER VACC.	SUCCESSFUL (4-FOLD RISE)†	UNSUCCESSFUL (LESS THAN 4-FOLD RISE)
		(No.)			(%)	(%)
Type 1	1½ to 6 mos.					
	Present‡	79	8	64	97	3
	Absent	22	<8	128	91	9
	(Total)	(101)	(8)	(64)	(96)	(4)
	6 mo. to 6 yr.					
	Present§	34	16	64	65	36
	Absent	32	<8	64	97**	3
	(Total)	(66)	(8)	(64)	(77)	(23)
Type 2	1½ to 6 mos.					
	Present‡	30	16	64	93	7
	Absent	19	<8	64	100	
	(Total)	(49)	(8)	(64)	(96)	(4)
	6 mo. to 6 yr.					
	Present§	7	16	128	29	71
	Absent	25	<8	32	84**	16
	(Total)	(32)	(8)	(32)	(66)	(32)
Type 3	1½ to 6 mos.					
	Present‡	63	8	64	100	
	Absent	31	<8	32	94**	6
	(Total)	(94)	(8)	(64)	(95)	(5)
	6 mo. to 6 yr.					
	Present§	32	16	128	69	31
	Absent	32	<8	32	100**	
	(Total)	(64)	(<8)	(64)	(78)	(22)

* Titers expressed as reciprocals of serum dilutions.
† In some infants <6 mos. old with prevaccination antibodies, fourfold rises were calculated by comparison with the expected titer based on the predicted decline of maternal antibodies (see text).
‡ Presumed to be of maternal origin.
** Includes 2, 2, 3, and 4 sera, respectively, with a postvaccination titer of 8.
§ Presumed acquired by natural infection.

the study districts but were unvaccinated and did not live in households of vaccinated children. Thereafter, 19 more cases of clinically verified poliomyelitis (16 paralytic) occurred during the remainder of 1959 in Philadelphia, eight in the study districts but none in vaccinated children or their households. Laboratory evidence of five Type 3 and four Type 1 infections was obtained.

An impression of the safety of the vaccine for the community was obtained from the fact that the vaccinated children were scattered through the heart of the city, in which there were about 50,000 children less than five years old. About half of these children were probably susceptible

to poliomyelitis.* The three cases of poliomyelitis that occurred in Philadelphia during the study were consistent with the pattern of the disease, which has been seen early in previous poliomyelitis seasons, both in Philadelphia and in neighboring New Jersey.

Response to Vaccination. Vaccinations were judged successful if paired sera showed either a fourfold rise in titer or a change in titer from <1:8 to 1:8 (the latter applied to 11 sera). In children less than six months old who had congenitally acquired antibodies, vaccination was considered successful if the observed titer after vaccination was at least four times greater than the expected titer at the end of three months (based on a predicted decline of maternal antibodies of approximately four twofold dilutions in three months).[10]

The patterns of antibody responses are analyzed in Table 4 and Fig. 1. Type 1 antibodies appeared in 91 per cent of the children less than six months old who had lacked them. Significant levels of Type 1 antibodies were also found in 97 per cent of infants less than six months old that had had antibodies at the time of vaccination. A rise in Type 1 antibody titer was observed in only 65 per cent of children more than six months old who had had antibodies; however, in the same age group, 97 per cent of children without antibodies had evidence of immunity after vaccination. The Type 2 and the Type 3 results in general showed similar rises (Table 4).

The median titers for the three types showed at least a fourfold rise in every group after vaccination (Table 4). In contrast, the median titer of 25 corresponding paired sera from children who did not receive Type 2 vaccine remained <1:8 after vaccination.

Table 5 shows the distribution of the three types of antibodies in the same children before and after vaccination. Before vaccination sera from 33 children were without antibodies; after vaccination 26 of these children had all three types of antibodies, and seven had two types. None of the 12 children with the three types of pre-vaccination antibodies, which were in many instances maternal in origin, lost them afterward.

Vaccination effected a substantial rise in antibody titer against all three types of poliovirus in every age group, including infants, regardless of the state of immunity (Table 4 and Fig. 1). (We surmised that the transmission of virus to unvaccinated children was not a significant factor in the analysis of this study, because the susceptibility of children entering the study did not vary greatly, and because 92 per cent of the children who did not receive Type 2 vaccine did not have a rise in Type 2 antibodies.)

After vaccination all three types of antibodies were detected in 85 per cent of the sera from children given all three vaccine strains compared with 19 per cent before vaccination (Table 5). Few children appeared to be immunized against only one type but left susceptible to other types of poliovirus.

SUMMARY AND CONCLUSION

Immunization with living attenuated poliovirus of infants and children living normally in a large city was essayed as a practical public-health measure in routine immunization clinics. CHAT, Type 1, vaccine was given to 850 children one-and-one-half months to six years old; 805 of these children were also given W-Fox, Type 3, and 335 were given P-712, Type 2, vaccine in sequence.

Serologic sampling disclosed that 44 per cent of all the children were without any poliomyelitis antibodies before vaccination; 66 per cent of those four months to three years old lacked antibodies of all three types before vaccination.

The illnesses uncovered during the study appeared unrelated to vaccination. None of the 22 cases of poliomyelitis in Philadelphia in 1959 occurred in vaccinated children or their households.

Of children less than six months old 91 per cent to 100 per cent had a significant antibody response to Type 1, Type 2, or Type 3 vaccines regardless of the presence of antibodies of maternal origin. Children more than six months old without antibodies before vaccination responded with a fourfold rise in titer in 84 per cent to 100 per cent of instances. The proportion of children with antibodies to all three types of poliovirus was increased from 15 per cent to 85 per cent after vaccination with the three strains of virus.

* Approximation based on figures in Table 2, assumed for the purposes of this estimate to be roughly representative of the population in the study districts.

TABLE 5. RESPONSE TO VACCINATION OF CHILDREN WITH VARIOUS PATTERNS OF SUSCEPTIBILITY

Antibodies Present Before Vaccination	Paired Sera*	Antibodies Present After Vaccination	
		Sera With 3 Types	Sera With 2 Types
Types	(No.)	(No.)	(No.)
0,0,0	33 →	26	7
1,0,0	6 →	4	2
0,2,0	3 →	3	
0,0,3	5 →	5	
1,2,0	2 →	2	
1,0,3	1 →	1	
0,2,3	0		
1,2,3	12 →	12	
Entire Group	62	53	9

* The completely titrated sera obtained from the 335 children given all 3 types of vaccine.

The vaccines appeared to be practical immunizing agents of high efficacy in conferring serologic immunity during infancy and childhood on a population at high risk.

ACKNOWLEDGMENTS

We acknowledge with pleasure the contributions of many persons in the Philadelphia Department of Public Health; the Statistics Section, Communicable Disease Center, U. S. Public Health Service; the Computing Center, University of Pennslyvania; the Pennslyvania State Virus Diagnostic Laboratories, Children's Hospital of Philadelphia; and the Wistar Institute.

REFERENCES

1. Barr, R. N., Bauer, H., Kleinman, H., Johnson, E. A., Martins da Silva, M., and Kimball, A. C., and Cooney, M. K.: Use of orally administered live attenuated polioviruses as a vaccine in a community setting—A controlled study. *J. Am. M. Assn.* **170:** 893-905 (June 20) 1959.
2. Gelfand, H. M., Potash, L., LeBlanc, D. R., and Fox, J. P.: Intrafamilial and interfamilial spread of living vaccine strains of polioviruses. *J. Am. M. Assn.* **170:** 2039-2048 (Aug. 22), 1959.
3. Plotkin, S. A., Koprowski, H., Richardson, S. N., and Stokes, Jr., J.: Vaccination of families against poliomyelitis by feeding and by contact spread of living attenuated virus. *Acta Paediat.*, 1960. In press.
4. U. S. Public Health Service, Poliomyelitis Surveillance Unit, Communicable Disease Center: Poliomyelitis in United States, 1959. Report no. 191, Atlanta, Ga., Nov. 30, 1959.
5. Nathanson, N., Thrupp, L. D., Hall, W. J., Langmuir, A. D., Cornell, R. G., Forester, H. E., Church, R. E., Hall, J. B., Hildebrand, M., Shaughnessy, H. J., and Morrissey, R.: Epidemic poliomyelitis during 1956 in Chicago and Cook County, Illinois. *Am. J. Hyg.* **70:** 107-168, (Sept.) 1959.
6. Molner, J. G., Brody, J. A., and Agate, G. H.: Detroit poliomyelitis epidemic—1958, Preliminary report. *J. Am. M. Assn.* **169:** 1838-1842 (Apr. 18) 1959.
7. Koprowski, H.: Discussion of Dr. Sabin's paper "Properties of attenuated polioviruses and their behavior in human beings." *New York Academy of Sciences*, Monograph no. 5: 128-133, 1957.
8. Sabin, A. B.: Properties and behavior of orally administered attenuated poliovirus vaccine. *J. Am. M. Assn.* **164:** 1216-1223 (July 13) 1957.
9. Lipton, M. M. and Steigman, A. J.: Simplified colorimetric test for poliomyelitis virus and antibody. *Proc. Soc. Exp. Biol.* **88:** 114-118 (Jan.) 1955.
10. Plotkin, S. A., Koprowski, H., and Stokes, Jr., J.: Clinical trials in infants of orally administered attenuated poliomyelitis viruses. *Pediatrics* **23:** 1041-1062 (June) 1959.

FIFTH SESSION

WEDNESDAY, 8 JUNE 1960, 9:00 A.M.

Chairman

DR. HERMAN E. HILLEBOE
Commissioner of Health
New York State Department of Health
Albany, New York

TOPIC III. EFFICACY. (A) LABORATORY EVIDENCE *(continuation)*

(1) ANTIBODY RESPONSE

(b) Influence of Age

Presentation of Papers by:

Dr. Joseph S. Pagano

Dr. Stanley A. Plotkin

Dr. Frederick C. Robbins

Dr. Henry M. Gelfand

Dr. Saul Krugman

(DISCUSSION)

(c) Influence of Dosage and Regimen

Presentation of Papers by:

Dr. Herald R. Cox

Dr. Herman Kleinman

TOPIC III. EFFICACY. (A) LABORATORY EVIDENCE *(continuation)*

5. EXPERIMENTAL INFECTION WITH CHAT ATTENUATED POLIOVIRUS IN PREMATURE INFANTS*

JOSEPH S. PAGANO, M.D., STANLEY A. PLOTKIN, M.D., DONALD CORNELY, M.D., AND HILARY KOPROWSKI, M.D.†

The Wistar Institute and the Philadelphia General Hospital
Philadelphia, Pennsylvania

DR. PAGANO *(presenting the paper):* Several investigators have established that infants less than six months old can be successfully vaccinated with living attenuated poliovirus administered orally.[1-4] It has also been observed that vaccination failures occur with some frequency in infants less than 60 days old, in contrast to the usual success of vaccination in infants more than 60 days old.[3] Investigation of this age-variation of the response to attenuated poliovirus was extended to vaccination of premature infants, in order to elucidate whether the resistance to vaccination that was occasionally observed in full-term infants was somehow related to biologic immaturity.[3, 5]

Opportunities were incidentally afforded to study experimentally several aspects of virus infection including: the immunologic response of premature infants to a viral antigen, the influence of passively acquired antibodies on vaccination with attenuated virus, and an estimation of the half-life of antibodies of maternal origin in premature infants.

In addition, the CHAT poliovirus was readministered orally to some of the infants, in particular to those that had not responded previously with the formation of detectable antibodies, despite obvious intestinal infection with the virus. The refeeding of these infants was done to discover whether immunologic tolerance had been induced, that is, whether in such infants there would be failure to produce antibodies after re-exposure to the same antigen later in life.

Subjects, Attenuated Virus Used. Forty-nine premature infants ranging in weight from 990 to 2100 grams were given $10^{5.7}$ $TCID_{50}$ CHAT Type 1 poliovirus [6] by gavage on the third day of life; 14 infants not given virus were retained as controls.

Specimens. Specimens of feces collected twice weekly for the duration of the hospital stay (four to 10 weeks) were tested for cytopathogenic effect; viruses isolated from the feces were identified by serum-neutralization tests.[7]

Neutralizing antibody titers were determined in paired sera from the same infant by a modification‡ of the immune-inactivation test.[8] Blood specimens were obtained from the umbilical cord

* This study was supported by a U.S. Public Health Service grant from the National Institute of Allergy and Infectious Disease.

† Dr. Pagano (Research Associate, The Wistar Institute); Dr. Plotkin (Epidemic Intelligence Service Officer, Communicable Disease Center, U.S. Public Health Service, Atlanta, Georgia); Dr. Cornely (Director of Newborn Nurseries, Philadelphia General Hospital); and Dr. Koprowski (Director, The Wistar Institute). The participation of Drs. Pagano and Plotkin in this study does not imply endorsement by the Public Health Service; the opinions are those of the authors.

‡ With HeLa-cell tissue culture and 10^6 PFU Mahoney virus.

and by heel puncture at approximately monthly intervals up to four to six months after birth.

Readministration of Attenuated Virus. Fourteen infants were given a second feeding of $10^{5.7}$ $TCID_{50}$ CHAT three to five months after the first administration; four stool specimens were collected at home during a two-week period after readministration of the virus.

General Observations. As a preliminary trial, five infants were given attenuated poliovirus and were found to excrete virus without symptoms and without evidence of transmission of the virus to other infants in the nursery. Additional infants were then given CHAT virus.

Ninety per cent of the infants were infected intestinally, as evidenced by repeated isolations of Type 1 poliovirus from fecal specimens beginning within a day of feeding (Table 1). Only 10 per cent of the infants were not infected.*

TABLE 1. INTESTINAL INFECTION AND ANTIBODY RESPONSE WITH CHAT VIRUS

Intestinal Infection	Infants	Antibody Response	
		Positive	Negative
Infected	90% (44)*	56% (14)	44% (11)
Not Infected	10% (5)	0	100% (5)
All Infants	100% (49)	47% (14)	53% (16)

* Number of infants in parentheses.

However, despite a well-established infection of this sort an antibody response was frequently not observed (Table 1). Among infants who excreted poliovirus only 56 per cent had a significant antibody response; the significance of this lack of response despite infection will be taken up later.

Birth Weight and Excretion of Virus. There was no notable relation (Table 2) between birth weight and the duration of excretion of virus. Excretion regularly began soon after administration of the vaccine and continued for an average of three or four weeks regardless of the size of the child.

The frequency of infection (Table 1) and the lack of relation to birth weight indicated that the capacity of human beings to serve as hosts for poliovirus is well developed up to three months before term birth. Evidently the intestinal lymphoid or epithelial tissue in which poliovirus may multiply is "mature" enough to support virus growth in premature infants. Consequently the speculation that the failure of full-term infants to become infected with attenuated virus is somehow related to biologic immaturity [3] was not supported. We inferred that some transitory phenomenon lasting about two months occurs sometime *after* birth to induce resistance to infection in a proportion of vaccinated full-term infants, an inference supported by subsequent studies.[6] The converse interpretation is that the susceptibility of newborn infants to attenuated virus is transitorily lost in some infants.

Transplacentally Acquired Antibodies and Intestinal Infection. Almost all the premature infants had transplacentally acquired poliomyelitis antibodies before vaccination. The findings in these infants (Tables 3 and 4) allow us to be increasingly certain that both resistance to intestinal infection with attenuated poliovirus and failure of antibody response are unrelated to the presence of transplacentally acquired antibodies in the infant's circulation. The percentages of infants that were infected, as well as the mean duration of excretion, were neither diminished by high pre-vaccination titers nor increased by low cord-blood titers (Table 3).

Transplacentally Acquired Antibodies and Immune Response. The antibody response that followed administration of attenuated virus did not appear clearly related to the presence or the titer of such congenital antibodies, again, within the limits of the titers of maternal antibodies that were encountered, as indicated in Table 4.

There is, however, a suggestion of a trend that higher titers of maternal antibodies may interfere with the development of active antibodies.

However, as we test the equivocal group, shifting them into the positive or negative groups, we have been able to evaluate additional data. There is no clear-cut trend. If there is an effect, it is difficult to discern within these levels of antibodies.

* Coxsackie B-5 virus was isolated from one of these infants.

TABLE 2. BIRTH-WEIGHT, INTESTINAL INFECTION, AND DURATION OF EXCRETION OF VIRUS

BIRTH-WEIGHT	INFECTED INTESTINALLY	DURATION OF EXCRETION (WEEKS)	
		MEAN	RANGE
(Grams)			
990 to 1200	100% (8)*	4 (4)	2 to 6
1201 to 1800	91% (19)†	3 1/2 (6)	3 to 6
1801 to 2100	90% (17)†	3 (7)	1–1/2 to 9

* Number of infants in parentheses.
† Two infants in this weight group were not infected.

TABLE 3. TRANSPLACENTALLY ACQUIRED ANTIBODIES, INTESTINAL INFECTION, AND DURATION OF EXCRETION OF VIRUS

CORD-BLOOD TITER*	INFECTED INTESTINALLY	DURATION OF EXCRETION (WEEKS)	
		MEAN	RANGE
512–256	90% (9)†	3 (5)	1–1/2 to 7
128–64	83% (10)	4–1/2 (5)	3 to 9
32–16	91% (10)	3–1/2 (3)	1–1/2 to 7
8–<8	100% (15)	3 (6)	2 to 6

* Type 1 antibody titers before vaccination; reciprocals of serum dilutions.
† Number of infants in parentheses. Four additional infants with cord-blood titers of 32, 128(2), and 256 were not infected.

TABLE 4. TRANSPLACENTALLY ACQUIRED ANTIBODIES AND ANTIBODY RESPONSE

CORD-BLOOD TITER*	NUMBER OF INFANTS	ANTIBODY RESPONSE AFTER VACCINATION		
		POSITIVE†	NEGATIVE	EQUIVOCAL
		(No.)	(No.)	(No.)
512–256	11	1	2	8
128–64	12	3	4	5
32–16	10	4	4	2
8–<8	20	8	6	6

*Type 1 antibody titers before vaccination; reciprocals of serum dilutions.
† Fourfold rise or, in a few cases, persistent twofold rise.

TABLE 5. ANTIBODY TITERS BEFORE AND AT INTERVALS AFTER VACCINATION AT BIRTH AND IN UNVACCINATED INFANTS

ANTIBODY RESPONSE	BEFORE VACCINATION	AFTER VACCINATION			
	Age →				
	0 mo.	*1 mo.*	*2 mo.*	*3 mo.*	*4–6 mo.*
*Positive**					
Median	8 (16)†	32 (12)	32 (9)	32 (7)	8 (7)
Mean**	12	42	30	35	14
*Negative**					
Median	32 (16)	8 (13)	8 (7)	<8 (8)	<8 (12)
Mean	27	7	6	2	2
Unvaccinated					
Median	128 (14)	16 (7)	8 (7)	8 (9)	<8 (10)
Mean	93	14	9	9	5
*Equivocal**					
Median	128 (23)	32 (20)			
Mean	59	44			

* All infants infected.
** Titers of $<8 = 0$ for calculation of geometric means.
† Number of infants in parentheses.

The larger number of equivocal antibody responses seen in the children with high pre-vaccination titers is probably a reflection of the fact that the high titers of acquired antibodies often prevented detection of masked antibody responses.

Magnitude of Antibody Response and Decline. Table 5 shows that among infants with a positive antibody response the rise in median titer, although significant, was not great; that a positive response was usually evident (in infants with a low cord-blood titer) within a month of vaccination; and that the titer in these infants may diminish after four to six months.

Infants with a negative antibody response despite definite and prolonged infection (a group constituting 44 per cent of infants, when those with an equivocal antibody response are excluded, as shown in Table 1) are of particular interest. Table 5 shows the rapid decline of pre-vaccination antibodies in this group, a decline apparently uninfluenced by vaccination, which is comparable to the decline of transplacentally acquired antibodies in the unvaccinated infants.

These rates are illustrated graphically in Fig. 1 in the form of geometric mean antibody titers at monthly intervals after birth and vaccination. (Preliminary analysis of these data indicated

that the half life of maternal poliomyelitis antibodies in premature infants appears to be about 23 days).

Readministration of Virus. When the same dose of the same virus was given a second time three to five months after birth to infants that had previously been infected and had had a positive antibody response (Table 6), in other words, successfully vaccinated, the response was as expected. Such infants were resistant to reinfection: only two of six infants were detectably reinfected, and these two infants excreted virus for less than five days, compared with an average excretion of over four weeks in the six infants after the first feeding. Three of the six infants had a rise in antibody titer.

In contrast the highly interesting group of five infants, who although infected had not had

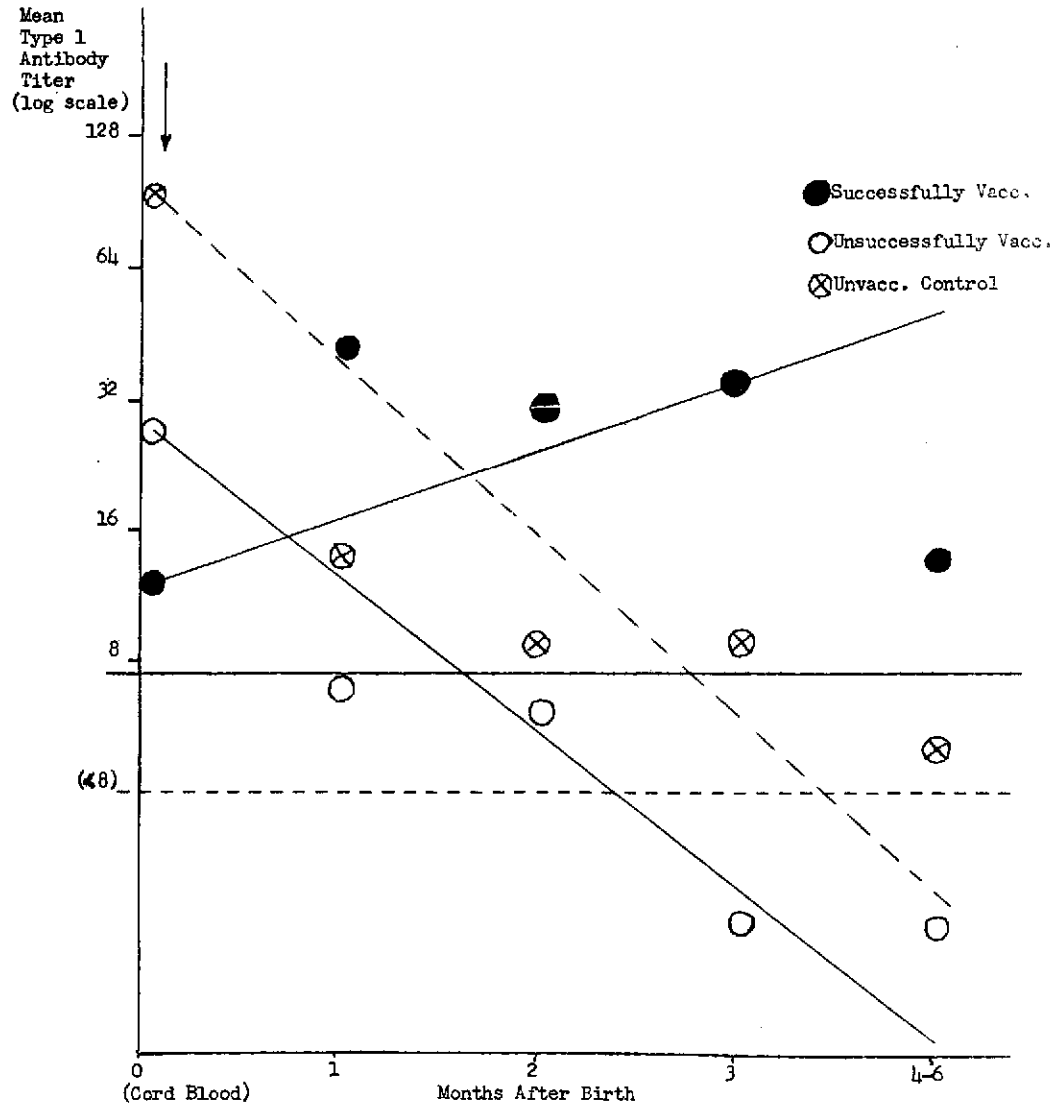


FIG. 1. Antibody levels at intervals after birth in successfully and unsuccessfully vaccinated premature infants and in unvaccinated control infants.

TABLE 6. READMINISTRATION OF CHAT VIRUS THREE TO FIVE MONTHS AFTER BIRTH

FIRST ADMINISTRATION		SECOND ADMINISTRATION	
OUTCOME	INFANTS	REINFECTED	POSITIVE ANTIBODY RESPONSE
	(No.)		
Infected, POSITIVE Antibody Response	6	2/6	3/6
Infected, NO Antibody Response	5	5/5	5/5*
NOT Infected, NO Antibody Response	2	0/2	0/2

* Response in one infant uncertain.

an antibody response after the first administration of virus, were readily reinfected in all five cases (Table 6). Excretion of virus after the second feeding was detected as long as the stools were examined (up to two weeks), despite a previous average excretion after the first feeding of over four weeks. That is to say, instead of having been rendered resistant to reinfection these five infants appeared to be as susceptible to infection as previously unexposed infants. However, the infants were not immunologically tolerant, as shown (Table 6) by the antibody response that followed reinfection in at least four of these five infants.

SUMMARY AND CONCLUSION

We found that neonatal premature infants were highly susceptible to infection with poliovirus, but that detectable antibody production was stimulated in only about half the instances of infection. The presence of passively acquired antibodies in the circulation of the infants seemed to have no effect on either intestinal infection or active antibody response to living attenuated poliovirus. Intestinal infection with the same virus was readily reestablished in infants who had previously been infected but were without antibodies, in contrast to the resistance to reinfection of successfully vaccinated premature infants. However, immunologic tolerance to poliovirus was not demonstrated, for all the infants who were infected were shown to be capable of an immune response to the attenuated poliovirus.

ACKNOWLEDGMENTS

The assistance of Walter Lauterer, M.D., Jane Sitnek, R.N., and Toby Goldschneider, and the cooperation of Paul Gyorgi, M.D., in carrying out this study, is gratefully acknowledged.

REFERENCES

1. Koprowski, H., Norton, T. W., Hummeler, K., Stokes, Jr., J., Hunt, A. D., Flack, A., and Jervis, G. A.: Immunization of infants with living attenuated poliomyelitis virus. *J. Am. M. Assn.* **162:** 1281-1288 (Dec. 1) 1956.
2. Martins da Silva, M., McKelvey, J. L., Prem, K. A., Johnson, E. A., and Cox, H. R.: Immunization with orally administered attenuated live-virus poliomyelitis vaccine. *Am. M. Assoc. J. Dis. Child.* **96:** 624-625 (Nov.) 1958.
3. Plotkin, S. A., Koprowski, H., and Stokes, Jr., J.: Clinical trials in infants of orally administered attenuated poliomyelitis viruses. *Pediatrics* **23:** 1041-1062 (June) 1959.

4. Pagano, J. S., Plotkin, S. A., Janowsky, C. C., and Koprowski, H.: Routine immunization with attenuated poliovirus of 850 children living in Philadelphia—Preliminary report (See this volume pp. 277-283).

5. ———, ———, and Koprowski, H.: Variation of response in early life to vaccination with living attenuated poliovirus and lack of immunologic tolerance. *Lancet.* To be published, 1960.

6. Koprowski, H.: Discussion of Dr. Sabin's paper "Properties of attenuated polioviruses and their behavior in human beings." New York Academy of Sciences, Monograph No. 5, pp. 128-133, 1957.

7. ———, and others: Clinical investigations on attenuated strains of poliomyelitis virus. *J. Am. M. Assn.* **160:** 954-966 (March 17) 1956.

8. Gard, S.: Immuno-inactivation of poliovirus. *Arch. ges. Virusforsch.* **7:** 449-460, 1957.

6. VACCINATION OF FULL-TERM INFANTS WITH ATTENUATED POLIOVIRUSES*

STANLEY A. PLOTKIN, M.D., JOSEPH S. PAGANO, M.D., AND HILARY KOPROWSKI, M.D.†

The Wistar Institute of Anatomy and Biology
Philadelphia, Pennsylvania

DR. PLOTKIN *(presenting the paper)*: For vaccination with living attenuated polioviruses perhaps the most important age group is infants less than six months old. If not vaccinated, these infants become highly susceptible to poliomyelitis after they lose the passive protection of transplacentally acquired antibodies. Furthermore, from a public health point of view, vaccination in hospitals shortly after birth would be maximally effective in reaching groups refractory to vaccination campaigns.

Previous studies from this laboratory have shown that it is possible to vaccinate infants successfully in the presence of maternal antibody,[1] even on the day of birth.[2] However, when performed at ages under two months, vaccination was found to be less effective than when done in older infants.[2] This relative resistance has been further explored [3] and is the chief subject of the present communication.

The subjects of these studies were infants living in the nursery of an institution, under close supervision. Because of their young age, contacts between infants included in these trials rarely occurred. Specimens consisted of stools and pharyngeal swabs collected twice weekly and blood specimens obtained at approximately monthly intervals. Isolation of virus from the stools and tests for neutralizing antibodies against poliomyelitis were performed in the manner just described by Dr. Pagano.

The viruses used were CHAT (Type 1),[4] P-712 (Type 2),[5] and W-Fox (Type 3);[4] all feedings were done by allowing the infant to ingest, by means of a dropper, the virus diluted in milk.

A successful antibody response, as in the case of the premature infants, was judged by the presence of an antibody titer after vaccination, which was at least four times greater than the titer which would be predicted from the uninfluenced normal decline of transplacental antibodies. The normal rate of decline was determined from paired serum specimens obtained before vaccination. The arithmetic and geometric means determined from these data are given in Table 1, from which it was computed that the average half-life of transplacental antibodies is approximately three weeks. Variability in the half-life figure was moderate, the standard deviation being eight days.

These data may be compared with the half-life values of 23 days which we have observed in premature infants; 21 days reported by Perkins and colleagues in England; 25 days in a paper which Dr. Gelfand very kindly allowed us to read, to be published shortly; 21 half-day life of gamma globulin reported by Dixon *et al.*; and the 37-day figure reported by Dr. Martins da Silva and associates.

Age at Vaccination. The success of vaccination in infants of different ages is summarized in Tables 2 and 3. Intestinal infection with living virus was established in more than 90 per cent of infants fed during the first week of life and also those fed beyond the age of 70 days. Paradoxically, between eight and 70 days of age, intestinal infection occurred at the significantly lower rate of approximately 70 per cent (Table 3).

* These studies were supported by grants from the National Institute of Allergy and Infectious Disease.

† Dr. Plotkin and Dr. Pagano (Epidemic Intelligence Service Officers, Communicable Disease Center, U.S. Public Health Service, Atlanta, Georgia, on assignment at The Wistar Institute); and Dr. Koprowski (Director, The Wistar Institute). The participation of Drs. Plotkin and Pagano in this study does not imply endorsement by the Public Health Service; the opinions are those of the authors.

TABLE 1. GEOMETRIC MEAN HALF-LIVES OF POLIOVIRUS ANTIBODY

Virus Type	Number of Determinations	Geometric Mean Half-Life (Days)
1	9	17
2	17	18
3	9	18
All	35	18
		Arithmetic Mean = 19 Standard Deviation = ± 0

TABLE 2. AGE AT VACCINATION IN RELATION TO INTESTINAL INFECTION WITH ATTENUATED POLIOVIRUSES

Age (Days)	Positive Intestinal Infections	
	Ratio	Per Cent
Birth-7	37/39	95
8-35	55/75	73
36-70	81/109	74
71+	70/76	92

$X^2 = 17.4$, $n = 3$, $p < .01$.

TABLE 3. VARIATION WITH AGE OF ANTIBODY RESPONSE TO ATTENUATED POLIOVIRUS

Age of Infants (Days)	Infections Followed by Positive Antibody Responses	
	Ratio	Per Cent
Birth-7	10/19	53
8-35	21/30	70
36-70	32/40	80
71+	26/29	90

$X^2 = 9.75$, $n = 3$, $p < .05$.

In infants in whom an intestinal infection was established, the frequency with which a detectable antibody response was observed varied directly with the age of vaccination, rising from 53 per cent within the first week of life to 90 per cent after 70 days of age (Table 3). This variation was also statistically significant.

TABLE 4. INTESTINAL INFECTION IN RELATION TO LEVEL OF TRANSPLACENTALLY ACQUIRED ANTIBODIES IN INFANTS LESS THAN 70 DAYS OLD

Homotypic Antibody Titer at Vacc.	Infections Established	
	Ratio	Per Cent
512-256	9/9	100
128-64	14/21	67
32-16	22/25	88
8- <8	40/48	83

TABLE 5. ANTIBODY RESPONSE TO INFECTION IN RELATION TO LEVEL OF TRANSPLACENTALLY ACQUIRED ANTIBODIES AT VACCINATION OF INFANTS LESS THAN 70 DAYS OLD

Homotypic Antibody Titer at Vacc.	Positive Antibody Response	
	Ratio	Per Cent
512-256	2/7*	29
128-64	8/10	80
32-16	17/19	89
8 - <8	23/35	66

* $X^2 = 4.5$ for twofold comparison, $p < .05$.

Transplacental Antibodies. In Tables 4 and 5, the possible influence of transplacental antibodies on live virus vaccination is examined. The 512 category includes a few values of 10/24 also. Only vaccinations done at less than 70 days of age are considered in these tables because high levels of maternal antibody were not frequently encountered beyond that age. No demonstrable effect of high titers of transplacental antibodies on intestinal infection was noted (Table 4). The infection rate in the presence of titers of 1:256 or more was not less than the infection rate at lower antibody levels. Analysis of the effect of passively acquired maternal antibody on the active response to vaccination is more difficult (Table 5). Pre-vaccination titers of 1:256 or greater, apparently tended to depress the rate of response, but only seven feedings are included in that group. Furthermore, the frequency of positive antibody responses did not show a progressive increase with decreasing pre-vaccination titers below 1:256.

Post-vaccination Antibody Response. The post-vaccination antibody response is examined

TABLE 6. ANTIBODY LEVELS AT INTERVALS AFTER VACCINATION OF INFANTS IN WHOM INTESTINAL INFECTION DEVELOPED

Age When Vaccinated (days)	Mean Pre-vaccination Antibody Titer*	Mean Postvaccination Antibody Titers* (Days After Vaccination)		
		21 - 45	46 - 75	76 - 135
0 - 7	30 (16)**	21 (10)	9 (10)	14 (14)
8 - 35	24 (17)	15 (11)	15 (16)	34 (17)
36 - 70	10 (18)	17 (27)	54 (17)	128 (4)
71 - 140	7 (10)	151 (21)	83 (5)	51 (7)
Entire Group	16 (61)	29 (69)	26 (48)	31 (42)

* Geometric means; reciprocals of serum dilution. Titer of <8 taken as 4.
** Number of determinations in parentheses.

qualitatively as well as quantitatively in Table 6, in which are analzyed the geometric mean antibody titers according to the age of the child at vaccination. In children older than 70 days at vaccination, a prompt response was observed, resulting in an average antibody level 20 times greater than before. The response of infants 36 to 70 days old also reached moderately high levels, but two months rather than one were required to attain such levels. Still younger infants in the eight-to-35-day-old group required two to three months to show a low level antibody response. Finally, newborn infants vaccinated during the first seven days of life reacted poorly to the antigenic stimulus of attenuated poliovirus, showing only a slight rise in titer at three months after vaccination. These data are presented graphically in Figure 1. It is interesting to note that the time of development of immunologic competence, as judged by the earliest appearance of a definite antibody response, occurred between eight and 12 weeks of age.

Immunologic Tolerance. The identical concern mentioned by Dr. Pagano regarding the possible induction of immunologic tolerance in premature infants led to the closer study of full-term infants in whom detectable antibody response failed to develop, despite intestinal infection with living virus. As shown in Table 7, second infections of six such infants, three by wild and three by attenuated virus, led to the development of antibodies.

Dosage. We have not systematically investigated problems of dosage, but a number of feedings have been done with less than $10^{5.0}$ $TCID_{50}$, the majority being between four and five logs. A few feedings were done with more than six log doses. In Table 8 we see that within the limits of approximately four to six logs, and under these controlled conditions, the virus dose did not exert a marked effect on either intestinal infections or the antibody response of subjects in whom multiplication was established.

However, the incidence of pharyngeal infection did appear to be directly related to dosage, as shown in Table 9. Despite the small numbers in the high dosage group, the difference in the rate of pharyngeal infection is highly significant. It was noted also that none of six pharyngeal virus excretors so far tested had detectable prevaccination antibody titers. The probability of this association occurring by chance alone is less than 1 in 1,000.

Sequence of Feeding. Various sequences of feeding monovalent vaccines were employed with three to four weeks as the usual interval between the feedings. With the CHAT, P-712 and W-Fox viruses, no clear evidence of interference was

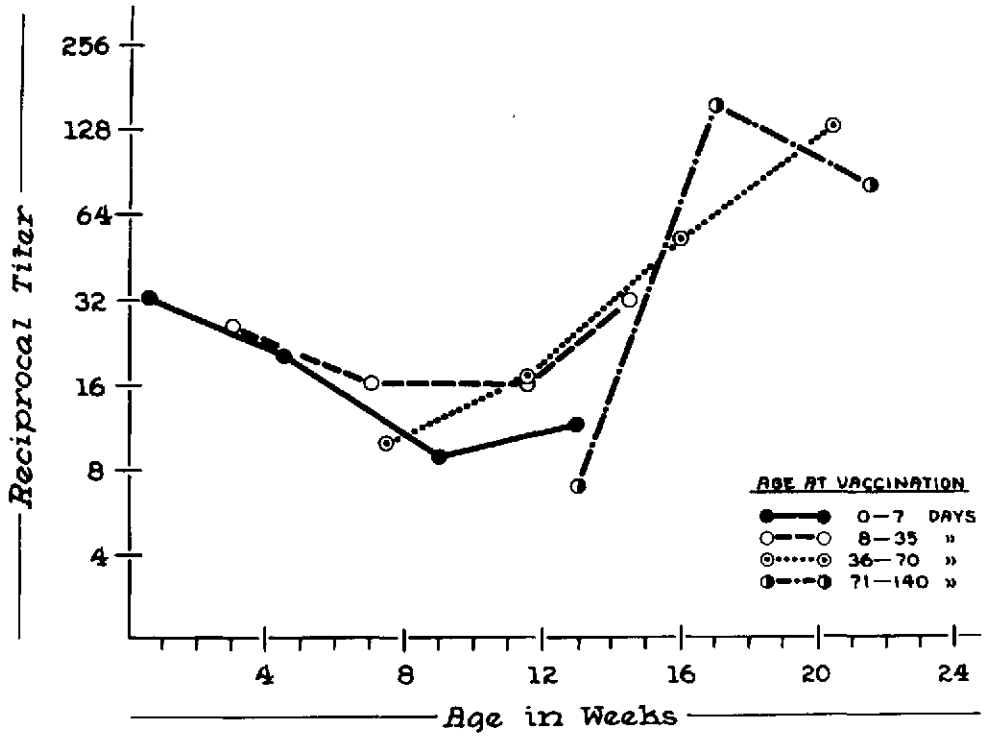


FIG. 1. Antibody titers at approximately 1, 2, and 3 months after the vaccination of infants are shown. The infants are divided into those vaccinated at 0-7, 8-35, 36-70, and more than 71 days of age. The first value for each group represents the mean titer at the time of vaccination.

TABLE 7. TESTS OF HYPOTHESIS OF IMMUNOLOGIC TOLERANCE IN FULL-TERM INFANTS

Infant No.	First Infection (Attenuated Virus)			Second Infection			
	Age	Fecal Virus	Post-Vacc. Antibody	Virus	Age	Fecal Virus	Post-Vacc. Antibody
103	4 d.	+	<8	Atten.	17 m.	+	16
119	3 d.	+	<8	Atten.	3.5 m.	+	512
139	2 d.	+	<8	Atten.	7 m.	+	512
56	2.5 m.	+	<8	Wild	29 m.	?	128
99	3 d.	+	<8	Atten. Wild	24 d. 18 m.	0 ?	<8 >1024
100	3 d.	+	<8	Atten. Wild	24 d. 18 m.	0 ?	<8 >1024

TABLE 8. VIRUS DOSAGE AS A FACTOR IN THE OUTCOME OF VACCINATION

Dosage (log_{10} $TCID_{50}$)	Intestinal Infection		Antibody Response*	
	Ratio	Per Cent	Ratio	Per Cent
<5.0	37/48	77	19/26	73
>5.0	117/147	80	64/86	74

* In infected subjects.

TABLE 9. INFLUENCE OF VIRUS DOSAGE ON THE INCIDENCE OF PHARYNGEAL EXCRETION

Dosage (log_{10} $TCID_{50}$)	Pharyngeal Virus Detected	
	Ratio	Per Cent
<6.0	4/94	4
6.0-6.9	2/9	22
>7.0	3/5	60

$X^2 = 22.6$, $n = 2$, $p < .01$.

found for any of the sequences of feeding, as shown in Table 10, although there was a suggestion that Type 2 virus was less effective when given after Types 1 or 3. It was noted, however, that in feedings given after 70 days of age, no such difficulty was observed.

Simultaneous Administration of the Three Types. Simultaneous administration of all three virus types has been studied. Our lack of success is illustrated in Table 11. The Type 3 W-Fox virus appears to dominate the other two strains, perhaps because of higher infectivity. I might note in passing, in relation to a suggestion made earlier by Dr. Horstmann, that it has been possible in some infants to overcome interference by Type 3 against Type 1, by feeding larger doses of Type 1, maintaining the Type 3 dose of five logs.

Persistence of Immunity. Persistence of humoral immunity has been studied in 12 vaccinated infants who have been followed for periods up to 41 months. Table 12 gives the current antibody titers of six babies given Types 1 or 2 viruses, 23 to 41 months before. Moderate to high antibody levels are still present in each case. Heterotypic antibodies, in contrast, are largely absent.

TABLE 10. INTESTINAL INFECTION BY ATTENUATED POLIOVIRUSES FED IN VARIOUS SEQUENCES

Position in Sequence	Type 1	Type 2	Type 3
First	92/111(83)*	16/17(94)	31/38(82)
After Type 1	-	9/13(69)	37/47(79)
After Type 2	5/6 (83)	-	7/8 (88)
After Type 3	7/9 (78)	27/37(73)	-

* Per cent.

TABLE 11. SIMULTANEOUS VACCINATION WITH THREE TYPES

Subject No.	Age	Log $TCID_{50}$ Doses of Virus			Antibody Responses		
		Type			Type		
		I	II	III	I	II	III
110	7 d.	5	5	5	0	0	+
111	9 d.	5	5	5	+	+	+
WB101	10 y.	5	5	5	0	0	+
B43	38 y.	5	5	5	0	0	+

TABLE 12. PERSISTENCE OF ANTIBODIES AFTER LIVE-VIRUS VACCINATION OF INFANTS (I)

Infant No.	Age at Feeding (days)	Months Since Vacc.	Reciprocal of Titer Against Poliovirus					
			1-3 Months Post-Vaccination			Current Determinations		
			I	II	III	I	II	III
8	42	41	1024	(16)	(<8)	64	(<4)	(<4)
9	108	39	256	(48)	(4)	64	(<4)	(<4)
15	117	40	1024	1024	(4)	128	128	(<8)
16	117	36	256	(16)	(4)	1024	(32)	(16)
41	75	27	256	(4)	(6)	1024	(16)	(8)
48	15	23	64	(<4)	(<4)	>1024	(<16)	(<16)

( ) Titers in parentheses are against types not fed.

Six infants vaccinated sequentially with all three types are shown in Table 13 to have retained polio antibodies for 13 to 17 months after vaccination with Types 2 and 3. Type 1 antibody persisted in five cases, but became undetectable in the sixth.

Summary and Discussion. With the strains and dosages described, the full-term infant is highly susceptible at birth to intestinal infection with living attenuated poliovirus. At about one week of age, however, he develops a significant degree of relative resistance to infection, which persists until about 70 days of age. Beyond 70 days of age, the infant again appears to be highly susceptible, which is confirmed by other studies using these same viruses.[6-8] Transplacental antibody plays no part in this phase of resistance.

The cause of this phenomenon is unknown. Possible explanations include the following three hypotheses:

(1) A physiological change in the gastro-intestinal tract increases the resistance of receptor cells to poliovirus during the age period of eight to 70 days.

(2) The infant does not develop full susceptibility to poliovirus until about 70 days of age;

TABLE 13. PERSISTENCE OF ANTIBODIES AFTER LIVE-VIRUS VACCINATION OF INFANTS (II)

Infant No.	Age at Feeding (days)	Months Since Vacc.	Reciprocal of Titer Against Poliovirus					
			1-3 Months Post-Vaccination			Current Determinations		
			I	II	III	I	II	III
64	67-106	17	512	128	64	16	16	64
65	3-80	17	256	512	128	32	32	256
68	53-93	17	64	32	32	<8	32	32
82	1-78	16	32	128	512	16	64	64
84	10-86	14	512	64	64	128	32	64
89	7-49	13	32	64	138	128	128	128

that is, he is born with a relative resistance. However, an endocrine factor transmitted from the mother via the placenta, enhances susceptibility for about a week after birth.

(3) The active colonization of the intestinal tract by enteric bacteria, which occurs during the first two months of life, leads to destruction of the poliovirus in the intestine by bacterial enterotoxins.[9]

The antibody response to live virus vaccination varies directly with the age at the time of vaccination, the poorest response occurring in newborns and the best in infants older than two months. These observations are consistent with what is known concerning the development of human immunologic capability: for example, the fact that gamma globulin is first produced by the infant at about six weeks of age.[10]

Thus there appear to be two main reasons for considering the period under 70 days af age less than optimal for live attenuated poliovirus vaccination: difficulty in causing infection, and the immaturity of the antibody forming mechanisms. In addition, a small amount of data suggested that transplacental antibodies of 1:256 or more at the time of vaccination may depress the active antibody response to live virus.

On the other hand, public health considerations may dictate that vaccination of newborns be performed in an effort to immunize at least some part of the population ordinarily refractory to vaccination campaigns. Evidence has been presented that vaccination of newborns does not carry the risk of induction of immunologic tolerance, and that when an antibody response does occur, it may persist for extended periods despite the young age at vaccination.

REFERENCES

1. Koprowski, H., Norton, T. W., Hummeler. K., Stokes, Jr., J., Hunt, A. D., Flack, A.. and Jervis, G. A.: Immunization of infants with living attenuated poliomyelitis virus. *J. Am. M. Assn.* **162:** 1281-1288, 1956.
2. Plotkin, S. A., Koprowski, H., and Stokes, Jr., J.: Clinical trials in infants of orally administered attenuated poliomyelitis viruses. *Pediatrics* **23:** 1041-1062, 1959.
3. Pagano, J. S., Plotkin, S. A., and Koprowski, H.: Variation of Response in early life to vaccination with living attenuated poliovirus and lack of immunologic tolerance. *Lancet.* In press.
4. Koprowski, H.: Discussion of Dr. Sabin's paper "Properties of attenuated polioviruses and their behavior in human beings." New York Academy of Sciences, Monograph No. 5, pp. 128-133, 1957.
5. Sabin, A. B.: Properties and behavior of orally administered attenuated poliovirus vaccine. *J. Am. M. Assn.* **164:** 1216-1223, 1957.

6. Plotkin, S. A., Koprowski, H., Richardson, S. N., and Stokes, Jr., J.: Transmission of attenuated polioviruses during a vaccination trial in American middle economic group families. *Acta Paediat.* In press.
7. Buser, F. and Schär, M.: Schutzimpfung gegen poliomyelitis mit lebenden avirulenten viren. *Schweiz. med. Wschr.* **88:** 1282-1284. 1958.
8. Pagano, J. S., Plotkin, S. A., Janowsky, C. C., Richardson, S. N., and Koprowski, H.: Routine immunization with oral attenuated poliovirus. *J. Am. M. Assn.* In press.
9. Likar, M., Bartley, E. O., and Wilson, D. C.: Observations on the Interaction of poliovirus in host cells *in vitro.* III. The effect of some bacterial metabolites and endotoxins. *Brit. J. Exp. Path.* **40:** 391-397, 1959.
10. Good, R.: Report of a Conference on Resistance to Infection and Immunity in Early Infancy. March 1958, Columbus, Ohio, Ross Laboratories.

7. THE RESPONSE OF NEWBORN INFANTS TO VACCINATION WITH LIVING TYPE 1 POLIOVIRUS (SABIN-LSc, 2ab) PRELIMINARY REPORT*

MARTHA LIPSON LEPOW, M.D., ROBERT J. WARREN, M.D.,† NIGEL GRAY, M.B.B.S., AND FREDERICK C. ROBBINS, M.D.

Department of Pediatrics and Contagious Diseases
Cleveland Metropolitan General Hospital
and
Department of Pediatrics
Western Reserve University School of Medicine
Cleveland, Ohio

DR. ROBBINS *(presenting the paper)*: The purpose of this study is to investigate the response of the newborn infant to vaccination with living attenuated polioviruses. Newborn infants are to be compared to those three months of age. The influence of certain other factors upon the success of vaccination is also being considered. These include the antibody titer in the infant's blood at the time of feeding and the acidity of the stomach. Data are also being obtained relative to the persistence of antibody, the immune response as determined by the susceptibility of the bowel on refeeding and by giving a booster injection of killed vaccine, the spread of virus within the family, and the genetic attributes of the virus excreted by the infants as compared to those of the virus fed.

Unfortunately, the investigation is still in its early stages and relatively few data are available at the time of writing. Therefore, this can be no more than a brief preliminary report and will concern primarily the pattern of virus excretion and antibody response of newborns, as contrasted with a group of three-month-old infants. Data concerning the influence of serum antibody upon virus excretion and a few observations on familial spread of the virus will also be included. Because of the preliminary nature of this report we have not attempted to refer to the work of others.

* Aided by a grant from The National Foundation.
† Epidemic Intelligence Officer, Public Health Service.

MATERIALS AND METHODS

Virus. The virus employed has been the Sabin Type 1 (LSc, 2ab) strain and was obtained from Dr. Sabin in sealed ampules of 1 ml. each. When titrated in this laboratory in monkey-kidney cell cultures, an ID_{50} of $10^{-7.5}$ per ml. was obtained. This agrees well with the titers observed on repeated titration in Dr. Sabin's laboratory.

Each newborn infant received 1 ml. of virus, or $10^{7.5}$ $TCID_{50}$ administered by dropper into the mouth. In order to conserve materials, the older infants have received 1 ml. of a 1:32 dilution of the same virus, given in the same manner.

Antibody Determinations. Serum neutralizing antibody has been determined by the metabolic inhibition test of Salk, Youngner, and Ward. Sera are diluted in twofold steps starting at a dilution of 1:4, 100 ID_{50} of virus are added to each tube, and monkey-kidney cells are employed as the indicator system.

COLLECTION OF SPECIMENS

Blood. Blood samples are secured from pregnant mothers in the prenatal clinic one month to six weeks before their expected date of delivery and cord blood is collected in the delivery room. A further sample is obtained from each infant at the age of three months and additional ones will be taken as indicated.

Those infants who are three months old at the time of feeding are bled before the vaccine is ad-

ministered, one month afterwards, and thereafter, as circumstances dictate.

Feces. In the case of newborns, three fecal specimens are collected from each infant during their stay in the hospital, which is on the average of four days. After their discharge home two specimens are obtained per week for a minimum of six weeks. In selected instances, fecal specimens are also collected from other members of the family. In addition to the specimens from the infant, a specimen is obtained from the mother at the time of delivery and other infants in the nurseries are sampled once a week.

Specimens are secured from the older infants twice weekly for six weeks after feeding.

VIRUS ISOLATION

Virus isolations are performed in monolayer cultures of monkey-kidney cells by methods that have been in use in this laboratory for a number of years.

THE STUDY POPULATION

Newborns. Mothers are interviewed in the prenatal clinics at the Cleveland Metropolitan General Hospital and at University Hospitals of Cleveland. The program is explained to them and if they agree to participate, they are asked to sign a permit for their baby to be fed the vaccine. The infant is fed virus within the first 24 hours of life. In some instances, this is done before he has received any nourishment; in others, shortly following a meal. The majority of the babies are Negroes and the families come from the lower socio-economic stratum of society.

Three-Month-Old Infants. The three-month-old infants have been recruited from two sources. One group has consisted of those attending the Well Baby Clinics conducted by the Department of Health of the City of Cleveland. They come from the same population group as the newborns.

The other group consists of occupants of a Catholic home for children. Most of them are awaiting adoption. A higher proportion of these children are white and the details of their backgrounds are not available to us.

All of the infants accepted into the study, except for those in the institution, are followed by the staff conducting the study who assume responsibility for supervision of their well baby care.

RESULTS

Viral excretion. To date, 150 newborn infants and 58 infants three months of age have been vaccinated. The data are still much too fragmentary to permit any extensive presentation of results. It is apparent, however, that approximately 80 per cent to 85 per cent of the newborn infants excrete virus in the feces for a significant period of time. A small number is found to have virus in the feces only during the first few days after feeding. It is doubtful that this represents true infection of the bowel and more likely is the result of simple transit of the fed virus through the gastrointestinal tract. Unfortunately, no quantitative estimations of the amount of virus in the feces are available as yet. In contrast to the newborns, all of the 26 infants three months of age on whom there are adequate observations have excreted virus for one week or more.

The length of time that the infants have continued to excrete virus is summarized in Table 1. It will be seen that approximately 70 per cent of the newborn infants tested in the first week after feeding was positive. Only about 50 per cent is found to have virus in their feces during the second week, and there is a steady decrease in the numbers excreting virus during the subsequent weeks. However, a few are still positive in the seventh week. In examining the individual protocols, it is striking that many of the newborn infants have negative specimens interspersed with positive ones. The feces may be free of virus for a week or more, after which time virus reappears. It is possible that this represents reinfection from family contacts that were themselves infected from the vaccinated infant. Our data do not provide an answer to this question. No such irregularities in isolation of virus from the consecutive specimens from three-month-old infants was noted.

It is interesting to compare the results during the second week in the older infants with those in the newborns. Although the numbers are small, 100 per cent of the 15 older infants were excreting virus as compared to only 55 per cent of the newborns. This would suggest further that the gastrointestinal tract of the older infant is

TABLE 1. DURATION OF EXCRETION OF VIRUS BY NEWBORN AND THREE-MONTH-OLD INFANTS FOLLOWING FEEDING OF TYPE 1 POLIOVIRUS (SABIN LSc, 2AB) VACCINE

WEEKS AFTER VACCINATION	NEWBORNS			THREE-MONTH-OLD INFANTS		
	NO. TESTED	NO. EXCRETING VIRUS	% EXCRETING VIRUS	NO. TESTED	NO. EXCRETING VIRUS	% EXCRETING VIRUS
1	120	85	71	21	20	95
2	110	60	55	15	15	100
3	93	37	40	6	6	100
4	75	22	29			
5	54 (62)*	16	30 (25)†			
6	30 (62)*	7	23 (10)†			
7	12 (62)*	5	42 (8)†			

* Number in parentheses: Those babies tested + those who were no longer being followed because of consistently negative specimens.

† Number in ( ) = adjusted % calculated on the basis of babies tested plus those whose stools had been consistently negative.

more readily infected than that of the newborn.

Relationship of maternal antibody to infection of the infant. In attempting to determine the influence of maternal antibody upon the likelihood of the infant to become infected we have been handicapped by the fact that all but a rare mother has a significant level of neutralizing Type 1 poliomyelitis antibody in her serum. Thus, few data are available concerning the response in the absence of antibody and it has been necessary to consider the data according to the titer of maternal antibody. Such an analysis is presented in Table 2. From these data it is evident that of the 19 babies who failed to become infected, 13 were born to mothers who possessed titers of neutralizing antibody in their sera of 256 or greater. Looked at in another way, 69 women had titers of 128 or less and only six (9 per cent) of their infants were not demonstrated to be infected, whereas 19 of 45 (28 per cent) from mothers with higher levels of antibody, did not excrete virus. Thus, if the maternal titer of homotypic antibody is high enough, 256 or higher, there is apparently some reduction in susceptibility to poliovirus infection. Of course, it is possible that sufficient antibody is present in the secretions of the lower bowel of the infant to neutralize the virus even though multiplication is occurring at a higher level. This seems unlikely, but it can only be evaluated when the immune response of these infants has been assessed. It is of interest, however, to note that a number of infants whose mothers possessed serum titers of 512 or higher became infected and excreted virus in excess of a week.

Antibody response of vaccinated infants. Antibody determinations are available on 24 newborn infants and 37 infants three months of age. In the newborns, the antibody titer of the cord-blood is compared with that of the infants' serum at three months of age, whereas for the older infants the serum titer before immunization is compared with that one month later. In Table 3 it will be seen that three of 20 newborn infants who excreted virus demonstrated an eightfold or greater rise in antibody titer, 15 showed no change, and only in two had the titer fallen to a significant degree. On the other hand, none of the four babies who did not excrete virus, demonstrated a rise in titer and three showed a significant fall.

TABLE 2. RELATIONSHIP OF HOMOTYPIC NEUTRALIZING ANTIBODY TITER IN THE MOTHER'S SERUM TO THE EXCRETION OF TYPE 1 POLIOVIRUS (SABIN LSc, 2AB) BY HER NEWBORN INFANT FOLLOWING VACCINATION

TITER OF TYPE 1 NEUTRALIZING POLIO ANTIBODY IN MOTHER'S SERUM	NO. INFANTS WITH NO VIRUS EXCRETION	DURATION OF VIRUS EXCRETION IN INFANTS' FECES		
		1 WEEK ONLY	>1 WEEK	TOTAL
<4		2	4	6
4			1	1
8			11	11
16		2	3	5
32	3	1	9	13
64	2	1	10	13
128	1	3	16	20
SUBTOTAL: <4—128	6	9	54	69
256	2	1	12	15
512	8	2	10	20
1024	2	2	4	8
2048			1	1
4096 or>	1		0	1
SUBTOTAL: 256—4096	13	5	27	45
TOTAL	19	14	81	114

Although we have not attempted to analyze our data accurately on the basis of the half-life of the maternal antibody, most of those babies recorded as showing no change in titer would be interpreted as having responded if this criterion were applied. It is too early to draw many conclusions from these data. However, provided those infants who have not shown the expected drop in antibody can be assumed to have responded satisfactorily, it would appear that the

TABLE 3. ANTIBODY RESPONSE OF NEWBORN AND THREE-MONTH-OLD INFANTS FOLLOWING VACCINATION WITH TYPE 1 POLIOVIRUS VACCINE (SABIN LSc, 2AB)

FECAL EXCRETION OF VIRUS	ANTIBODY RESPONSE					
	NEWBORNS*			THREE-MONTH-OLD INFANTS†		
	RISE	NO CHANGE	FALL	RISE	NO CHANGE	FALL
PRESENT	3	15	2	21	16	0
ABSENT	0	1	3	0	0	0
TOTAL	3	16	5	21	16	0

* Neutralizing antibody titer in cord blood compared with titer in infant's blood approximately three months after vaccination.

† Neutralizing antibody titer before vaccination compared with that one month after.

majority of the babies who excreted virus probably did develop antibodies. Only after a longer period of observation will it be possible to determine the frequency of antibody responses.

The observations on the three-month-old infants are presented in the same table. It will be seen that good antibody responses were noted in 21 of the 37, whereas 16 showed no change. In no instance did the titer fall to a significant degree, but it should be noted that the period of observation is only one month. The influence of the serum antibody level at the time of immunization of these infants upon the frequency with which a rise in titer was demonstrated, is analyzed in Table 4. It will be seen that 19 of 22 infants with a pre-immunization titer of eight or less demonstrated a rise, as compared to only two of 15 of those whose initial titer was 16 or greater. The highest pre-immunization titer noted in this group was 256.

The data might be interpreted to mean that the higher levels of passive antibody prevented the infant from forming antibody. However, it would seem to us more likely that the amount of antibody formed by the infant was in many instances too little to be reflected as a significant rise in the face of the large amount already present. Quantitatively expressed, if the pre-immunization titer was 64, the baby would have to manufacture 192 units of antibody in order for the total to add up to a titer of 256, which would be a fourfold rise in titer.

pH of gastric contents. Just before giving the vaccine, the gastric content of 39 newborns was aspirated and its pH determined. The majority were found to have a pH of between 1.0 and 3.0, although the range was from 1.0 to 5.8. The variation from baby to baby was too little to permit any correlation of the stomach acidity and the rate of infection. However, no difference has been noted when the virus was given before, as compared to shortly after a milk feeding.

Familial spread of vaccine viruses. The only data so far collected on spread of vaccine viruses are those from two families. In one instance, one of a pair of twins was fed virus, the other was not. The vaccinated infant excreted virus for approximately 12 days. On the 12th day the other twin became infected and excreted for about two weeks. Virus then re-appeared on the 33rd day in the feces of the baby who had been fed originally. Other members of the family, including two siblings three and four years of age and the mother and father, did not become infected.

In the second family a four-year-old sibling became infected three weeks after the vaccinated infant was brought into the home. The three-year-old sibling and the parents did not excrete virus during an observation period of eight weeks.

SUMMARY

From the limited data so far available it would appear that when fed in the first day of life, the Sabin Type 1 poliovirus vaccine establishes an infection in approximately 80 per cent to 85 per cent of the babies. Infants three months of age are more uniformly susceptible.

The level of maternal antibody would appear to have some influence upon the susceptibility of the infant to infection. Most of those babies who never were found to have virus in their feces had maternal antibody levels of 256 or higher. However, this was not an "all or none" phenomenon since a number with maternal antibody

TABLE 4. CORRELATION OF THE PRE-IMMUNIZATION NEUTRALIZING ANTIBODY TITER WITH THE DEMONSTRATION OF A RISE IN ANTIBODY FOLLOWING IMMUNIZATION IN THREE-MONTH-OLD INFANTS

PRE-IMMUNIZATION ANTIBODY TITER	ANTIBODY RESPONSE		TOTAL
	RISE	NO CHANGE	
8 OR <	19	3	22
16 TO 256	2	13	15
TOTAL	21	16	37

titers of 512 or higher excreted virus for one week or more.

Relatively few of the newborn infants showed a significant rise in neutralizing antibody three months after feeding (three of 20), as compared to approximately 21 of 16 three-month-old infants who responded with an eightfold or better rise in antibody titer. However, it is postulated that the high levels of passive antibody in the serum of most of the infants studied, masked the antibody produced by the infant, and only later observations will give the true picture in regard to serologic response of these infants.

Family spread from newborn infants did occur to young siblings in two families, but was limited in extent.

8. PRELIMINARY REPORT ON THE SUSCEPTIBILITY OF NEWBORN INFANTS TO INFECTION WITH POLIOVIRUS STRAINS IN AN ATTENUATED VIRUS VACCINE

HENRY M. GELFAND, DOROTHY R. LEBLANC, ALFONSO H. HOLGUIN, AND JOHN P. FOX*

DR. GELFAND *(presenting the paper)*: A number of studies have been reported during the past several years wherein the efficacy of oral vaccination of infants with attenuated poliovirus vaccines was investigated.[1-5] If practicable, the practice of routine infant vaccination would provide the easiest solution to the problem of maintaining the immunity of a community against poliomyelitis after safety and efficacy of a live virus vaccine has been assured and it has been received by the majority of the general population.

In previous studies, use was made of virus strains developed by Dr. Hilary Koprowski and by the Lederle Laboratories, administered in monotypic or trivalent form, and under various conditions of infant age, dose, and method of evaluation. Nevertheless, it was generally demonstrated that infection may be induced in very young infants, even in the presence of high titers of maternally-derived neutralizing antibody. It had been suggested, however, that there may be relative resistance to infection in the intestinal tracts of very young infants, a resistance which might be overcome by the administration of larger doses than are required by older individuals.

The present investigation was undertaken to extend these observations by a study of infant susceptibility to infection with the "set" of attenuated poliovirus strains developed by Dr. Albert B. Sabin. By means of the administration of graduated doses of each of the three types separately, we hoped to perform infectivity titrations in newborn infants in order to determine the smallest effective doses which would result in virus excretion and subsequent antibody response in a satisfactory proportion of babies of an age which would be suitable for routine use. In addition, the course of infection following trivalent vaccination was to be examined in detail, and certain other variables of age and inoculation regime were to be investigated.

MATERIALS AND METHODS

Study group. The original plan called for the recruitment of 230 mothers and their two- to three-day-old infants at the time of discharge from the maternity ward of a hospital in New Orleans, and 45 mothers and their 30-day-old babies at the time of the first post-partum visit. Only lower-economic-group Negro mothers and babies who were in good health following an uncomplicated pregnancy and delivery were selected. The program was fully explained and written agreement to participate was obtained from the mothers by the nurse-epidemiologist (D.R.L.).

The entire group of babies was divided into the subgroups shown in Table 1. Groups 1, 2, and 3 act as the basic titration of each of the vaccine types separately, and group 5 serves to compare older infants with the younger with reference to Type 1 vaccine virus. Group 4 was established to determine whether multiple feeding of the same virus type on three successive days and in maximum concentration would increase the likelihood of infection. Group 6 consists of babies who received trivalent vaccine either once or on three successive days. Group 7 received no vaccine, and serves as an illness and "wild virus" infection control. Since groups 1-5 were fed only one virus type, they also serve as built-in "wild virus" infection controls for the types not being fed.

Only babies of group 6 were selected on the basis of birth order. Since these infants received

* Dr. Gelfand and Dr. Holguin (Communicable Disease Center, U.S. Public Health Service, Department of Health, Education, and Welfare, Atlanta, Georgia); Miss LeBlanc (Division of Epidemiology, Tulane University School of Medicine, New Orleans, Louisiana); and Dr. Fox (Division of Epidemiology, Public Health Research Institute, New York, N. Y.).

TABLE 1. DESCRIPTION OF VACCINE FEEDING SUBGROUPS

Subgroup number	Vaccine administered			Age of baby (days)	Number of babies	
	Virus type	Dilution	No. doses		Limited study	Detailed study
1 a	1	undiluted	1	2 - 3	10	10
b	1	1:10	1	2 - 3	15	0
c	1	1:100	1	2 - 3	15	0
2 a	2	undiluted	1	2 - 3	10	10
b	2	1:10	1	2 - 3	15	0
c	2	1:100	1	2 - 3	15	0
3 a	3	undiluted	1	2 - 3	10	10
b	3	1:10	1	2 - 3	15	0
c	3	1:100	1	2 - 3	15	0
4	1	undiluted	3	2 - 3	20	0
5 a	1	undiluted	1	30	5	10
b	1	1:10	1	30	15	0
c	1	1:100	1	30	15	0
6 a	1,2,3	undiluted	1	2 - 3	10	10
b	1,2,3	undiluted	3	2 - 3	10	10
7	none	-	-	2 - 3	20	0
Total	-	-	-	-	215	60

trivalent vaccine, it was desirable to minimize as much as possible the opportunity for sibling contact infection early after vaccine administration with resulting intrafamilial circulation of virus and subsequent reexposure of infants to a virus type which had failed to cause primary infection because of intertypic interference. Therefore group 6 babies were all the first-born in the family.

Vaccine feeding was done in cycles rather than by subgroup, two babies in each subgroup (a total of 32) constituting a cycle. There were two advantages to this method. First, it avoided the possibility that all babies of a single subgroup might be fed at a time of a hospital epidemic of an illness unrelated to vaccination, and false association of the illness with a specific vaccine type or schedule. Secondly, it permitted us to make continuous comparison of results of vaccination while new recruitment was still going on, and therefore to modify the protocol if indicated.

Vaccine administration. The vaccine strains were kindly provided by Dr. Albert B. Sabin from single large pools of each type designated LSc, 2ab (Type 1), P 712, Ch, 2ab (Type 2), and Leon 12 a_1b (Type 3). As reported by Sabin,[6] these pools contained approximately $10^{8.0}$, $10^{7.8}$, and $10^{7.4}$ TCD_{50} per ml. of Types 1, 2, and 3, respectively, as titered in Cynomolgus monkey-kidney cell tissue-culture tubes which had had the serum contained in the growth medium leached out of the cells, and held in a roller drum following inoculation. Using Rhesus cell cultures held in stationary position after inoculation, Dr. Dorothy Clemmer in our Tulane University Laboratory obtained titers of $10^{7.6}$, $10^{6.8}$, and $10^{6.4}$ TCD_{50} per ml. for Types 1, 2, and 3, respectively, in the vaccine lots actually used in this trial. Prior to the field use of any vaccine, the stock vials of each type were thawed, emptied, and pooled, diluted as required in Hanks' BSS, and put up in 1 ml. quantities (except for trivalent use) in individual screw top vials which were then stored at —20° C. until just before use. A trivalent vaccine dose consisted of 3 ml. containing 1 ml. of each of the virus types, and it was prepared and stored in that amount.

The vaccine was administered to the babies without reference to the last meal. A smooth-tipped "medicine dropper" of 1 ml. capacity was filled with recently thawed vaccine, gently inserted into the infant's mouth, and emptied by a combination of slow squeezing of the rubber bulb and the sucking action of the baby. The vaccine was not squirted into the mouth, and therefore no more than a drop or two was ever lost.

Specimen collection and virus isolation. A venous blood specimen was collected from the mother at the time of recruitment. Since previous work in our laboratory [7] and elsewhere has indicated that the neutralizing polio-antibody titers in an infant's umbilical cord serum do not differ significantly from his mother's, the maternal specimen will be used as the baseline for measuring serologic change. A blood specimen will be collected from each infant at three to four months of age and again at six months. These sets will be tested by titration in parallel. No serologic study has yet been undertaken.

A fecal specimen was collected from almost every infant at the time of vaccine feeding. Occasionally this was impossible, and one was procured by the mother several hours after vaccination, placed in an ointment jar, and refrigerated until the nurse's visit. A fecal sample was collected from every child six days after vaccination. In addition, certain babies were designated for "detailed study" (see Table 1), and a series of specimens collected. From such infants in groups 1, 2, 3, and 5 (monovalent vaccine feeding), they were collected on days: 0, 2, 4, 6, 8,

10, 14, 21, and 28. From infants in group 6 (trivalent vaccine feeding), they were collected on days: 0, 1, 2, 3, 4, 5, 6, 8, 10, 14, 21, 28, 35, 42, 49, 56, 63, 70.

Fecal samples were prepared into 20 per cent extracts by shaking with Hanks' BSS, followed by slow speed centrifugation in the cold for about two hours to remove gross particulate matter. Acid extracts were brought to approximately pH 7.4 by the addition of a drop or two of dilute NaOH solution, and then stored with antibiotics in screw-topped vials at —20° C. until tested. For virus isolation, each of four monkey-kidney cell culture tubes was inoculated with 0.25 ml. extract. The tubes were examined every second day for eight days. If negative at that time, supernates of the four tubes were pooled, and 0.2 ml. of the pool inoculated into each of two new MKC tubes. If the passage tubes showed no evidence of a cytopathic effect after eight days, the specimen was considered negative for virus.

"Positive" supernates were tested by mixing an aliquot of a standard 1:100 dilution with potent Type 1, Type 2, Type 3, polyvalent (three types), and normal control antisera. These mixtures were incubated for one hour at room temperature, and then inoculated into two tubes each of MKC culture. If the results of this test indicated that more than one poliovirus type was present, a second similar test was performed using antisera with the following mixtures of antibody types: 1 and 2, 1 and 3, 2 and 3, all three types, and normal control. Combinations of any 2 or 3 types could thereby be identified. It should be noted that if small quantities of a second virus type were present in a fecal extract, it might be missed because of the overgrowth of the predominant type. Because of this possibility (among infants fed polyvalent vaccine), mixtures will be made at a later date of fecal extracts and antisera against the virus type(s) already isolated, and a new attempt at virus isolation will be made. Such retesting has not yet been done, and the following results may be deficient to that extent.

Clinical observations. At the time of a home visit to pick up the sixth-day fecal specimen, the nurse examined the infant briefly and discussed with the mother his health during the interval since vaccination. A formal health record was made at one and two weeks after vaccine feeding on all babies designated for detailed study and those in the illness control group.

RESULTS

Until the time of this writing (15 May, 1960), 138 babies have received vaccine under one or another of the indicated schedules, and another 11 have been observed as group 7 controls. There have been no untoward reactions attributable to the vaccine, i.e., no diarrhea not readily explainable on another basis, no febrile or allergic responses, no symptoms referable to the central nervous system. Two serious illnesses have occurred in vaccinated infants, one case of pulmonary atelectasis, edema, and pneumonitis, perhaps related to congenital cardiac defects, which terminated fatally, and one case of hemolytic jaundice probably caused by severe bacterial infection.

Infection has thus far been investigated only by the attempt at virus isolation, and for those infants in the "limited study" category only one specimen (six days after vaccine administration) was available. It is therefore possible that some infections have been missed, and will be picked up only upon serologic examination later. No virus cytopathogenic in monkey-kidney cell tissue culture has been detected in a fecal specimen collected prior to feeding, but two enteroviruses, as yet unidentified but not poliovirus, have been found in the excreta of children 28 days after vaccine administration, who were 30 and 56 days of age, respectively. Before it was realized that a fecal specimen collected several hours after oral vaccination was unsuitable, several were obtained at two, four, or six hours after administration when none was available at the time of feeding. In almost every such instance, homologous virus was isolated from the feces, undoubtedly representing the very rapid intestinal passage of vaccine.

As results were accumulated in the laboratory, changes in the study protocol seemed justified, and new recruitment goals were established, as indicated in Table 2. (See Table 1 for description of subgroups.) Because the end-point of infectivity had not been reached for any of the vaccine types, new subgroups 1d, 2d, and 3d were

added recently to comprise babies receiving vaccine in 1:1000 dilution. None of these has yet received the vaccine. This table also shows the number of babies vaccinated to date in each subgroup and the numbers demonstrated to have become infected or to have failed to become infected as indicated by the presence or absence of homologous virus in the sixth-day or later specimen. Considering first the monovalent feedings in very young infants (groups 1-3), and realizing

TABLE 2. PRELIMINARY RESULTS OF ORAL VACCINATION BASED ON FECAL EXCRETION OF VIRUS OF HOMOLOGOUS TYPE

Subgroup number(X)	Number of babies		Presence of homologous virus in 6-day specimen		
	Modified (new) goal	Fed vaccine to date	yes	no	incomplete study
1 a	10	10	9	0	1
b	10	12	9	0	3
c	10	11	4	4	3
d	10	0			
2 a	6	6	6	0	0
b	10	10	7	1	2
c	10	10	7	0	3
d	10	0			
3 a	10	10	9	1	0
b	15	10	5	3	2
c	15	10	6	2	2
d	10	0			
4	0	2	1	1	0
5 a	10	10	8	1	1
b	10	6	6	0	0
c	10	6	6	0	0
6 a	20	15	9	1	5
b	10	10	10	0	0
7	15	11*	0	8	3
Total	201	149	102	22	25

(X) See Table 1 for description of subgroups.
* Recruited but not vaccinated.

the small numbers available in each subgroup, the Type 2 component of the vaccine seems to be the most highly infectious, followed by Types 1 and 3 in that order. A single failure was found with Type 2 in 1:10 dilution, but none with the 1:100. The 50 per cent end-point of infectivity with Type 1 vaccine may be at about the 1:100 dilution since only four of eight babies became infected. The results following Type 3 administration are most scattered, some failures being recorded throughout the range of dilutions used.

Since a single dose of undiluted Type 1 vaccine virus appeared to result in infection, it was decided to discontinue the recruitment of babies for group 4, those who were to be fed this material on three successive days. Only two infants were fed on this regime, and it was very surprising to note that one failed to excrete virus on the sixth day after the first dose. Serologic study later may reveal that infection had occurred nonetheless.

The babies approximately 30 days old (group 5) who have so far been fed have become infected with the sole exception of one child who received undiluted vaccine. The latter unquestionably received the full dose without wastage, and almost certainly failed to be infected since fecal specimens collected every second day were consistently virus-free. Whether this group as a whole demonstrates the greater susceptibility of month-old babies over newborns is difficult to decide from the small numbers involved.

The duration of fecal excretion, and the consistency with which virus may be isolated from serial specimen collections is illustrated in Table 3. Note that the choice of the sixth day was a fortunate one for the collection of a single specimen from the majority of children, since from only one of these babies who were at some time shown to have become infected was a negative specimen obtained on the sixth day. However, note also that no single specimen can guarantee to demonstrate the infection of every infected

TABLE 3. DURATION AND CONSISTENCY OF FECAL EXCRETION OF HOMOLOGOUS VIRUS FOLLOWING ORAL ADMINISTRATION OF UNDILUTED VACCINE TO FIRST 24 BABIES OBSERVED IN LONGITUDINAL FASHION

Subgroup number (X)	Vaccine virus type	Presence or absence of virus in specimen collected on indicated day after vaccination								
		0	2	4	6	8	10	14	21	28
1 a	1	– NC*	+ +	+ +	+ +	– –	– –	– –	– –	– –
		– –	+ +	NC +	+ +	+ +	– –	– –	– –	– –
		– –	+ +	+ +	+ +	+ +	– –	+ –	+ –	– –
2 a	2	– – – – –	+ + NC + +	– – NC + +	+ + + + +	+ – + NC –	– + + + +	+ – – + +	+ – + – +	+ – – + +
3 a	3	NC – NC – – –	+ + + + – +	+ + + + + +	+ + + + + +	NC + + + + +	+ + + NC + +	– + + + + +	– + – + – +	– – – + – –
5 a	1	– NC – + – –	+ + + + + +	+ + + + + +	+ + + + + +	+ + + + + +	+ + + + + –	+ + + + + –	– – + + + –	– – – + + –

(X) See Table 1 for description of subgroups.
* Not collected.

child; negative "skips" in the series of positives were encountered in six of these 24 children. The duration of fecal excretion in these babies appears to be somewhat shorter than is usually observed following the administration of the Sabin vaccine strains, and there is, moreover, a suggestion that duration may be somewhat longer among 30-day-old babies (subgroup 5a) than among two-three-day olds (subgroup 1a), all of whom were fed the same dose of Type 1. By contrast, our previous observations of orally vaccinated older children and adults [8] showed the substantially longer mean and median durations summarized in Table 4.

TABLE 4. DURATION OF FECAL EXCRETION OF "SABIN VACCINE" VIRUSES FOLLOWING INFECTION OF OLDER CHILDREN AND ADULTS*

Virus type	Number of individuals	Duration of virus excretion	
		Median	Mean
1	24	11 - 15 days	21 days
2	17	16 - 20 "	21 "
3	20	31 - 40 "	39 "

* From reference (9).

All except one of the babies fed trivalent vaccine so far have become infected with at least one virus type. The sole exception again is not explainable on the basis of faulty vaccine administration or the inadequacy of fecal observation; virus was not isolated from any of the fecal specimens collected on nine occasions during two weeks after feeding. Among those who did become infected, the pattern of virus excretion has been rather consistent, but with minor variations. Table 5 presents the isolation results from the first six babies studied. Note that fecal specimens are dated from the first dose received, and that, therefore, those receiving three doses had a longer opportunity to excrete passively the vaccine material itself. Type 2 appears to become predominant in every individual. Nevertheless, Types 1 or 3 may appear from time to time and even, as in baby No. 61, may be excreted for a prolonged period of time.

DISCUSSION AND CONCLUSIONS

The major purpose of this investigation was to titrate in two-to-three-day-old infants each of the virus types included in the "Sabin vaccine," in order to determine the 50 per cent and 90 per cent end-points of infection in children of this

TABLE 5. SERIAL RECORD OF POLIOVIRUSES ISOLATED FROM FIRST SIX INFANTS WHO RECEIVED UNDILUTED TRIVALENT VACCINE

Day after first vaccine dose	Poliovirus type(s) isolated on indicated day					
	Babies given 1 dose			Babies given 3 doses		
	Baby # 28	Baby # 60	Baby # 61	Baby # 30	Baby # 31	Baby # 62
0	0	0	0	0	0	0
1	1,3	0	1,2	1,2,3	0	2,3
2	0	1,3	1,2,3	1,2,3	1,2	2,3
3	2	1	0	1,2,3	1,2	2
4	2	1	2	1,2,3	2	2
5	2,3	-	2	1,2	2	2
6	2,3	1	2	2	2,3	2
8	2	1,2	2	2	2	2
10	2	2	2	2	2	-
14	2	2	2	2	2	0
21	1	0	3	2	2	0
28	1	2	3	2	0	0
35	0	1	3	0	0	0
42	0	0	3	2	0	0
49	0	0	3	0	0	0
56	0	2	0	0	0	0
63	0	0	0	0	0	0
70	0	0	0	0	0	0

age. The D_{2-3} ID_{50} will be useful as the more accurate basis for comparison with other vaccine candidates and with other age groups. The D_{2-3} ID_{90} is probably the measure needed in actual practice since a vaccine can hardly be acceptable as an immunizing tool which is not effective in at least 90 per cent of those receiving it. The 30-day-old babies were included to permit comparison (using Type 1) with somewhat older children, and further comparison is valid with our previous experience with still older children [8] since the material, techniques, and investigative personnel involved were essentially the same. Our aim, therefore, was to complete Table 6.

TABLE 6. INFECTIVITY ENDPOINTS OF "SABIN VACCINE" VIRUS STRAINS IN CHILDREN OF DIFFERENT AGES

Virus type	Age	Infectivity endpoints	
		$I\ D_{50}$	$I\ D_{90}$
1	2 - 3 days 30 " > 1 year*	$< 10^{6.0}$	$< 10^{6.0}$
2	2 - 3 days > 1 year*	$< 10^{5.3}$	$< 10^{5.3}$
3	2 - 3 days > 1 year*	$< 10^{5.4}$	$< 10^{5.4}$

* From reference (9).

Since virologic study is still incomplete, and serologic study has not yet been started, it is too early to make final judgments. However, it appears now that Sabin's Type 2 vaccine is the most highly infectious of the three types in young infants, and a 1:100 dilution of this component may be capable of producing infection in almost every recipient. The Type 1 vaccine may require a greater concentration, and Type 3 may have to be administered in undiluted form to result dependably in infection. This result with Type 3 is surprising, since, on the basis of intrafamilial spread, our previous work had led us to conclude that this was the most infectious of the three component types.[8]

It should be noted that the administration of vaccine on which this work was based was done with extreme care, by one person only, and using a technique which permitted the baby to suck the contents of the medicine dropper used. Under the less optimal conditions of routine use, more failures of "take" would be anticipated.

The duration of virus excretion in the feces after infection appeared to be shorter among young infants than had been observed with the same materials in older children. It will be interesting to observe in the future whether or not antibody titers are also lower. If so the duration of effective post-vaccination immunity may be in question, and will require specific investigation. It is hoped that it might be possible to challenge some of the babies in the present study with a second dose of homotypic vaccine after several years have elapsed, and to make observations on intestinal resistance to reinfection. The possibility that the duration of excretion may be related to the level of passive antibody present in the babies' serum will have to await serologic study.

The ultimate result of vaccination with trivalent material will also have to wait upon serologic evaluation. The predominance of Type 2 virus infection, as found in virologic study of fecal extracts, might not be reflected in antibody response since there is already a suggestion that Types 1 and 3 may be continuously present in lower titer. The predominance of Type 2 may be related to the greater infectivity of this type in the concentration used, and it may be controllable by using a greater dilution of this component in a mixture.

The unexpected failure of infection in an occasional baby fed undiluted vaccine despite the demonstrated ability of a 100-fold dilution of the same type to cause infection is difficult to explain. It is possible that an occasional individual is refractory to infection, at least at the given time, for purely personal physiologic reasons, related to gastric acidity or some other mechanism. Similar occurrences among babies fed diluted vaccine would pass unnoticed because of the anticipated failures due to the smaller virus inoculum.

Finally, although expected, the complete absence of untoward reactions attributable to the vaccine in infected children should be noted.

REFERENCES

1. Koprowski, H., Norton, T. W., Hummeler, K., Stokes, Jr., J., Hunt, Jr., A. D., Flack, A., and Jervis, G. A.: Immunization of infants with living attenuated poliomyelitis virus. *J. Am. M. Assn.* **162:** 1281-1288, 1956.
2. Martins da Silva, M., McKelvey, J. L., Bauer, H., Prem, K. A., Cooney, M. K., and Johnson, E. A.: Studies of orally administered attenuated live virus poliomyelitis vaccine in newborns and infants under six months. *U. Minn. M. Bull.* **29:** 133-150, 1957.
3. Plotkin, S. A., Koprowski, H., and Stokes, Jr., J.: Clinical trials in infants of orally administered attenuated poliomyelitis viruses. *Pediatrics* **23:** 1041-1062, 1959.
4. Koprowski, H., Plotkin, S., Pagano, J., Norton, T. W., and Stokes, Jr., J.: Behavior of attenuated strains of poliomyelitis virus in relation to age, familial spread, and duration of immunity. In: *Live Poliovirus Vaccines, First International Conference.* Scient. Pub. no. 44 of the Pan American Health Organization, pp. 159-171, 1959.
5. Prem, K. A., McKelvey, J. L., and Fergus, J.: Immunologic response of infants under six months of age to oral trivalent poliomyelitis vaccine. In: *Living Poliovirus Vaccines, First International Conference.* Scient. Pub. no. 44 of the Pan American Health Organization, pp. 254-259, 1959.
6. Sabin, A. B.: Present position of immunization against poliomyelitis with live virus vaccines. *Brit. M. J.* **1:** 663-680, 1959.
7. Gelfand, H. M., Fox, J. P., LeBlanc, D. R., and Elveback, L: Studies on the development of natural immunity to poliomyelitis in Louisiana. V. Passive transfer of polioantibody from mother to fetus, and natural decline and disappearance of antibody in the infant. *J. Immun.* Accepted for publication, 1960.
8. ———, Potash, L., LeBlanc, D .R., and Fox, J. P.: Revised preliminary report on the Louisiana observations of the natural spread within families of living vaccine strains of polioviruses. In: *Live Poliovirus Vaccines, First International Conference.* Scient. Pub. no. 44 of the Pan American Health Organization, pp. 203-217, 1959.

9. IMMUNIZATION OF NEWBORN INFANTS WITH LIVE ATTENUATED POLIOVIRUS VACCINE*

SAUL KRUGMAN, M.D., JOEL WARREN, PH.D., MARVIN S. EIGER, M.D., PETER H. BERMAN, M.D., RICHARD H. MICHAELS, M.D., AND ALBERT B. SABIN, M.D.

Departments of Pediatrics and Obstetrics, New York University School of Medicine, Department of Biologic Research, Chas. Pfizer and Co., and the Children's Hospital Research Foundation, University of Cincinnati College of Medicine

DR. KRUGMAN *(presenting the paper):* Live attenuated poliovirus vaccine was administered to 400 newborn infants during a three-month period (October 1959-January 1960). This study was designed to obtain information which might provide answers to the following questions: (1) Would the ingestion of larger doses permit enough of the vaccine viruses regularly to pass the "acid barrier" of the stomach of newborn infants and result in regular multiplication in the intestinal tract? (Poliovirus may be destroyed below a pH of 2.5 and the gastric contents of newborns often have a pH of about 1.5); (2) Would it be possible to by-pass the potential handicap of high gastric acidity by swabbing the vaccine directly on the posterior pharyngeal wall?; (3) Would all three types multiply following administration of a mixture of large doses of Types 1, 2, and 3 poliovirus either by mouth or by throat as determined by virus excretion and antibody formation?

MATERIALS AND METHODS

Study Group. The study group included 400 infants born in Bellevue Hospital between October 1959 and January 1960. The mothers of most of the infants were of Puerto Rican extraction and came from a low socio-economic group, a relatively immune population. Consequently, it was likely that most of the babies would possess passively acquired maternal poliovirus antibodies.

Administration of Vaccine. The following strains of Sabin's poliovirus vaccine[1] were employed: (1) Type 1 (LSc, 2ab) containing $10^{7.0}$ TCD_{50} ml.; (2) Type 2 (P 712, Ch, 2ab) containing $10^{7.2}$ TCD_{50}/ml.; and (3) Type 3 (Leon, $12a_1b$) containing $10^{7.3}$ TCD_{50}/ml. The vaccine was given orally in the various dosage schedules listed in Table 1. It was administered by instilling a measured amount on the back of the tongue or by swabbing the tonsillar fauces and posterior pharyngeal wall with an absorbent cotton swab saturated with undiluted vaccine.

The first 330 infants were assigned in consecutive rotation to the 11 different groups listed in Table 1. The last 70 infants were placed in groups 5 and 10 and each 11th infant was an unvaccinated control. The first dose of vaccine was given within 11 hours of birth. The second dose was administered between 12 and 35 hours and the third dose between 36 and 59 hours.

Collection of Specimens. Stool specimens were obtained from most infants on the day of discharge from the hospital, usually the fourth or fifth day of life. In exceptional instances, specimens were obtained as early as the second day and as late as the 23rd day.

Cord-blood was obtained at the time of delivery. Subsequently, at about three months of age, a second blood specimen was obtained in the Well Baby Clinic. The serum specimens were kept in the deep freeze until paired samples were available for antibody determination.

Virus Isolation Studies. Ten per cent stool extracts, prepared as previously described,[2] were tested in Rhesus kidney-tissue cultures in the Cincinnati laboratory. When an initial test on a total of 0.6 ml. of 10 per cent extract in three tissue culture tubes was negative, the test was

* Aided by grants from Chas. Pfizer and Co., and The National Foundation.

TABLE 1. SCHEDULE FOR LIVE POLIOVIRUS VACCINE ADMINISTRATION TO NEWBORN INFANTS

VACCINE FED*	GROUP	METHOD	DOSE & DILUTION OF VACCINE	DAY GIVEN**
TYPE 1 ONLY	1	MOUTH	1 cc undiluted	0
	2	"	" "	0,1,2
	3	"	1 cc 1:10	0
	4	"	" "	0,1,2
	5	THROAT	Undiluted	0,1,2
TYPES I+II+III	6	MOUTH	1 cc undiluted	0
	7	"	" "	0,1,2
	8	"	1 cc 1:10	0
	9	"	" "	0,1,2
	10	THROAT	Undiluted	0,1,2
NONE	11	....	....	.....

* Sabin strains
Type 1 (LSc, 2 ab)
Type 2 (P 712, Ch, 2 ab)
Type 3 (Leon, 12 a_1b)

** 0 = birth to 11 hours
1 = 12 to 35 hours
2 = 36 to 59 hours

repeated in an additional five tubes with an additional 1 ml. of stool extract. The isolates were identified by appropriate neutralization tests. No cytopathic agents, other than polioviruses, were recovered.

Neutralizing Antibody Determinations. Tests for antibody were carried out simultaneously on the stored specimens of cord blood and on the three-month specimens by the pH, metabolic inhibition test at the Pfizer Research Laboratories. The sera were tested in two-fold dilutions in duplicate cups, and the titer was the dilution of serum which neutralized the virus in *both* cups.

RESULTS

Clinical Observations. All 400 infants were asymptomatic during the period of observation in the nursery. No untoward reactions were reported or observed in 270 infants examined at the one month and three-month follow-up visits.

Virus Isolations on Single Stool Specimens. The incidence of poliovirus isolations from single stool specimens is shown in Table 2. Virus was detected in about 80 to 90 per cent of the cases. A single 1 cc. dose seemed to be as effective as three daily 1 cc. doses of undiluted vaccine. Similar results were also obtained with three daily applications of undiluted vaccine by throat swab. None of the stools of 31 control patients yielded virus.

Extent of Poliovirus Multiplication. Table 3 shows the extent of poliovirus multiplication after a single feeding of either Type 1 or of trivalent vaccine. Stool specimens were obtained from 20 infants two to 14 days after vaccine administration. Ten infants received 1 cc. of Type 1 undiluted vaccine; the remainder received 3 cc. of the undiluted trivalent mixture.

Feeding of Type 1 poliovirus vaccine was followed by evidence of multiplication of virus on the fourth, fifth, and eighth post-vaccination day. The amount of virus recovered from the stool ranged from $10^{3.2}$ to $10^{4.7}$ TCD_{50} per gram. Triple-negative, five to 10-year-old children, who had received 0.01 ml. of the same vaccine, excreted $10^{4.7}$ to $10^{6.2}$ TCD_{50} per gram of stool at a comparable time.[3]

TABLE 2. INCIDENCE OF VIRUS ISOLATIONS ON SINGLE STOOL SPECIMENS OBTAINED ABOUT 4-5 DAYS AFTER ORAL POLIOVIRUS VACCINE

VACCINE FED	DOSE AND DILUTION	DAY GIVEN	RESULTS	
			ISOLATION ONLY	ISOLATION +ANTIBODY
TYPE I ONLY	1 cc Undiluted	0	8/10	9/10
	" "	0, 1, 2	7/10	7/10
	1 cc 1:10	0	5/10	≥ 6/10
	" "	0, 1, 2	7/10	8/10
	Throat Swab Undiluted	0, 1, 2	12/21	≥ 13/21
TYPES 1+2+3	3 cc Undiluted	0	9/10	9/10
	" "	0, 1, 2	9/10	9/10
	3 cc 1:10	0	13/20	≥ 13/20
	" "	0, 1, 2	6/16	≥ 9/16
	Throat Swab Undiluted	0, 1, 2	15/22	≥ 15/22
NONE	....	....	0/35	0/35

Administration of the trivalent vaccine was followed by excretion predominantly of Type 2 virus. Evidence of multiplication of virus was apparent on the second to the seventh day and on the 14th day following vaccination. The amount of virus recovered from the stool ranged from $10^{3.2}$ to $10^{6.0}$ TCD_{50} per gram.

Evidence of Antibody Response 3 Months After Administration of Poliovirus Vaccine to Newborn Infants. The decline of 54 poliovirus antibody titers from birth to three months in 19 unvaccinated control infants is illustrated in Table 4. The decline in titer ranged between four and 128-fold. Consequently, any infant with only a fourfold or greater decline in titer at three months was assumed to have had no antibody response. Evidence for active antibody response included a two-fold decline, no change or an increase in titer, although it was realized that lower levels of actively produced antibody would thus be masked by the higher levels of the residual maternal antibody.

The results shown in Table 5 are based on the above criteria. The administration of Type 1 poliovirus vaccine was followed by evidence of antibody response in about 30 per cent of a group of 109 infants at three months. The results were not significantly affected by the dose or route of administration.

A mixture of Types 1+2+3 vaccine induced evidence of an antibody response at three months in about 50 per cent of 134 infants on whom studies have been completed. The response was confined chiefly to Type 2 antibody.

DISCUSSION

The demonstration of virus multiplication in the intestinal tracts of 80 to 90 per cent of newborn infants fed live poliovirus vaccine based on examination of only a single stool specimen

TABLE 3. EXTENT OF POLIOVIRUS MULTIPLICATION AT INDICATED TIME AFTER SINGLE FEEDING OF UNDILUTED TYPE 1 OR TRIVALENT VACCINE

DAY STOOL OBTAINED	LOG 10 TCD_{50}/Gm. VIRUS IN STOOLS OF INFANTS	
	FED TYPE I ONLY	FED TYPES I + II + III
2		3.2 (II)
3	< 0.8	3.2 (II)
4	3.2 4.2 4.7	4.7 (II)
5	3.2 3.7 4.2 4.7	< 0.8 2.2 (II) 3.2 (I+II) 5.2 (I+II)
6	< 0.8	6.0 (II)
7		4.2 (II)
8	4.2	
14		5.2 (II)

(II) = Type 2 Poliovirus.

TABLE 4. DECLINE OF 54 PASSIVE POLIOVIRUS NEUTRALIZING ANTIBODY TITERS IN 19 UNVACCINATED INFANTS FROM BIRTH TO THREE MONTHS

ANTIBODY LEVEL IN CORD BLOOD	NO. OF TITERS*	ANTIBODY TITERS OBSERVED AT THREE MONTHS	FOLD-DECLINE IN TITER
1024	1	256	4
512	4	128, 128, 64, 64	4–8
256	9	64, 32, 16, 16, 16, 16, 16, 16, <4	4–128
128	8	32, 16, 16, 8, 8, 8, 8, 8	4–16
64	7	16, 16, 8, 8, 8, 4, 4	4–16
32	7	8, 4, 4, <4, <4, <4	4–16
16	18	All <4	4

* Types 1, 2, and 3 polioviruses.

TABLE 5. EVIDENCE OF ANTIBODY RESPONSE AT 3 MONTHS AFTER ADMINISTRATION OF LIVE POLIOVIRUS VACCINE TO NEWBORN INFANTS

VACCINE FED	GROUP	EVIDENCE OF ANTIBODY RESPONSE					
		NUMBER			PER CENT		
Type I Only	1	5/22			23		
	2	6/16			37		
	3	7/21			33		
	4	6/19			32		
	5	10/31			32		
		I	II	III	I	II	III
Types I + II + III	6	1/20	7/20	2/20	5	35	10
	7	2/24	14/24	2/24	8	58	8
	8	1/24	12/24	2/24	4	50	8
	9	2/20	8/20	3/20	10	40	15
	10	2/27	8/27	5/27	7	30	18
None	11	0/19	0/19	0/19	0	0	0

Note: See Table 1 for key to groups.

TABLE 6. ORAL POLIOVIRUS VACCINATION OF NEWBORN INFANTS

Lack of effect of level of maternal antibody on poliovirus multiplication in the intestinal tract

ANTIBODY TITER* OF CORD BLOOD	NO. OF INFANTS	LOG 10 TCD_{50}/GM. OF POLIOVIRUS EXCRETED
$\leq$ 16	7	3.7–6.2
32	8	2.7–5.2
64	22	1.9–5.7
128	13	1.4–5.7
256	11	2.0–4.7
512	5	3.2–6.0
1024	1	3.2

* Reciprocal.

(usually at four to five days) was a significant finding. Similar observations have been made by Gelfand [4] in infants fed the same lots of vaccine at two to three days of age. It would appear that the factor of high gastric acidity in the newborn infant does not present a serious handicap in the oral immunization of newborn infants, when the larger doses of vaccine are used.

Gelfand,[4] working with aliquots of the same lots of vaccine, has also observed a uniform predominance of excretion of Type 2 virus when trivalent vaccine is administered. The interference with Types 1 and 3 is further substantiated by the evidence of a predominant rise in Type 2 antibody.

The presence of high levels of maternally transmitted antibody had no effect on the extent of multiplication of virus in the intestinal tract. As indicated in Table 6 the amount of virus excreted in the stool was essentially the same in infants with low or high titers of maternally transmitted antibody.

Evidence of an active antibody response was more apt to occur in infants excreting virus than in those with negative tests for virus in the single stool specimens examined as shown in Table 7. An antibody response was present in 42 per cent of 55 infants with positive isolations as compared with 20 per cent of 30 infants with negative isolations. It is equally important to note that in spite of active multiplication of virus in the intestinal tract, 58 per cent of the infants had no evidence of an active antibody response on the basis of the criteria that had to be adopted. Although there was no correlation between the titer of antibody in the cord blood and the extent of virus multiplication in the intestinal tract (Table 6), the reverse was true for the relationship between antibody titer at birth with the evidence for active antibody response at three months. Figure 1 illustrates that antibody response was inversely related to the titer of passively transferred maternal antibody. The lower the titer the higher the percentage of responses. With an antibody titer of 1:64 or less in the cord blood the evidence for antibody response ranged between 55 and 77 per cent. With a titer of 1:128 to equal or greater than 1:1024, the percentages dropped from 33 to 9 per cent.

The absence of evidence of an antibody response in an infant with a high titer of antibody in the cord blood does not necessarily mean lack of active antibody production. It is evident that when the amount of antibody produced by a newborn infant is less than half the residual maternally transmitted antibody, its presence would be masked. It is apparent, therefore, that a more adequate evaluation of the active antibody response is not possible until at least six months or longer have passed.

CONCLUSIONS

These studies indicate that a single dose of 1 ml. of undiluted vaccine given on the day of birth results in the establishment of intestinal infection in about 80 to 90 per cent of the infants. It is possible that 0.1 ml. of undiluted vaccine may be equally effective, but a definitive conclusion on this question will not be possible until the final data are available. Feeding of the trivalent vaccine in maximal dosage led to regular multiplication only of the Type 2 virus and only rarely of Types 1 and 3. At three months after feeding, some children had definite evidence

TABLE 7. CORRELATION BETWEEN POLIOVIRUS ISOLATION AND ANTIBODY RESPONSE IN INFANTS ON WHOM BOTH TYPES OF DATA ARE AVAILABLE

VIRUS ISOLATIONS	NO. OF INFANTS	EVIDENCE OF ANTIBODY RESPONSE	
		PRESENT IN	ABSENT IN
POSITIVE	55	23 (42%)	32 (58%)
NEGATIVE	30	6 (20%)	24 (80%)

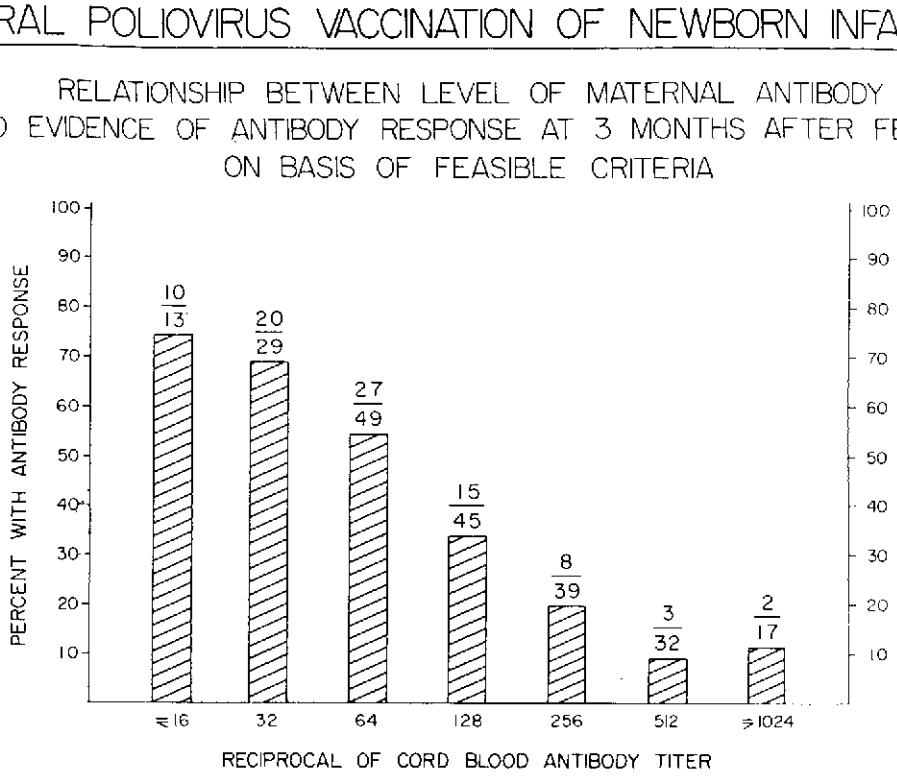


FIG. 1. Relationship between level of maternal antibody and antibody response in 224 newborn infants three months after oral poliovirus vaccination.

of active antibody formation, while in others it was either absent or still masked by high levels of residual placentally transmitted antibody. At the present time it seems wise to await the results of further antibody tests at six months of age and of studies on intestinal resistance and antibody response to reinfection, before reaching any decision about the use of live poliovirus vaccine during the first days after birth. One thing, however, is already clear: that monovalent rather than trivalent vaccine will have to be used, and the Type 1 should therefore be the first to be administered.

ACKNOWLEDGMENTS

We are indebted to Dr. Gordon W. Douglas, Professor of Obstetrics, New York University School of Medicine, Dr. Harold Jacobziner, Assistant Commissioner of Health, and Dr. Morris Greenberg, Director, Bureau of Preventable Diseases, New York City Department of Health, for their help and cooperation.

REFERENCES

1. Sabin, A. B.: Status of field trials with an orally administered live attenuated poliovirus vaccine. *J. Am. M. Assn.* **171:** 863-868 (Oct. 17) 1959.
2. ————, Krumbiegel, E. R., and Wigand, R.: ECHO Type 9 virus disease. *Am. M. Assn. J. Dis. Child.* **96:** 197-219 (August) 1958.
3. ————: Unpublished data.
4. Gelfand, H.: Personal communication (Unpublished studies).

DISCUSSION

CHAIRMAN HILLEBOE: Before we begin the discussion of the five papers presented, Dr. Payne has asked for the privilege of giving a brief report from Switzerland.

DR. PAYNE: I have a report from Dr. F. Buser and Dr. M. Schär of Bern, in cooperation with Dr. R. Martin Du Pan and Dr. M. Paccaud of Geneva, and Dr. P. Müller and Dr. R. Knoepfli of Basle, entitled "Vaccination of Children, Infants, and Newborns against Poliomyelitis with Live Avirulent Poliovirus."

The summary of their communication is as follows:

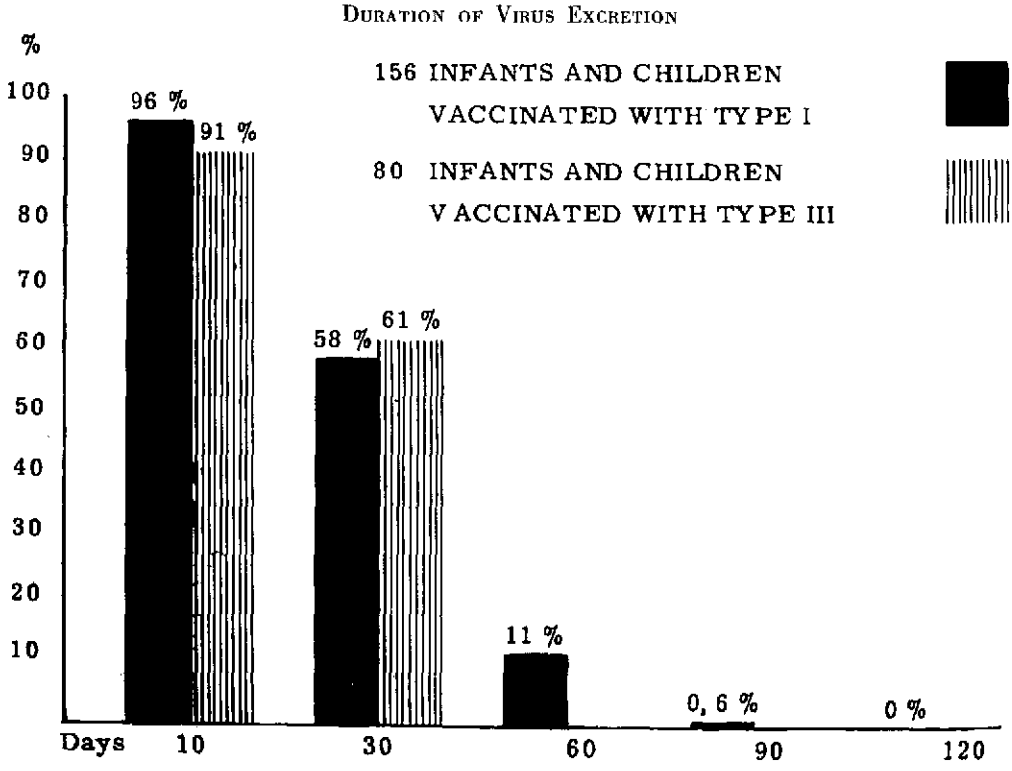
Four hundred individuals, including 145 triple-negative infants, children, and 100 newborns, have been vaccinated against poliomyelitis with the attenuated poliovirus Type 1, the CHAT strain; 90 of these individuals were also vaccinated with the Type 3 Fox strain.

Infection was established in 99 per cent for Type 1 and 95 per cent for Type 3 of all individuals without homologous antibody, whether or not they had been vaccinated with the Salk vaccine previously, as well as of all passively immune infants over two months of age. Naturally immune children and adults became less frequently infected, 66 per cent and 30 per cent, respectively.

Antibody titers over 1:200 were reached in almost 80 per cent of vaccinated persons, and only 2 per cent of the Type 1 and 5 per cent of the Type 3 vaccinees remained without antibody.

These high antibody levels remained practically unchanged during an observation period of two years.

After Type 3 vaccination, the heterologous response to Type 2 antibody, which did not persist, was observed in more than 50 per cent of the vaccinated individuals. The vaccine virus was transmitted from a vaccinated child to non-



vaccinated siblings in 16 out of 50 families, with two or more children. Nineteen out of a total of 75 contact children became infected.

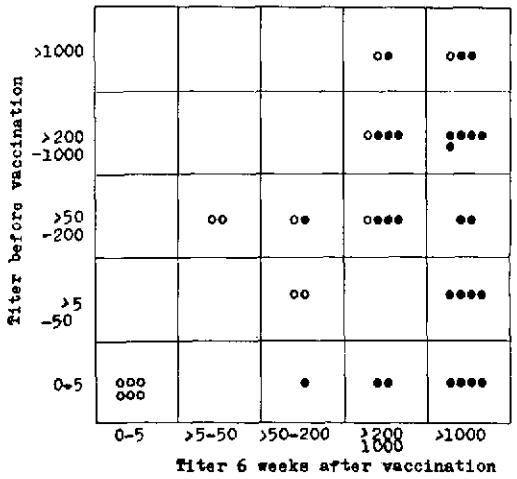
The virus excreted by the contact children did not show any increased virulence on intrathalamic injection in monkeys.

Contrary to the excellent results obtained by vaccination in infants over three months of age, intestinal infection developed in only about 60 to 70 per cent of newborns and infants younger than two months of age; however, those newborns and young infants who became infected with the vaccine virus developed as high antibody titers as older infants, regardless of the level of passively transmitted maternal antibody.

The following figures and tables give additional data on the results obtained by the Swiss group:

F. Buser, Bern and R. Martin Du Pan, Geneva

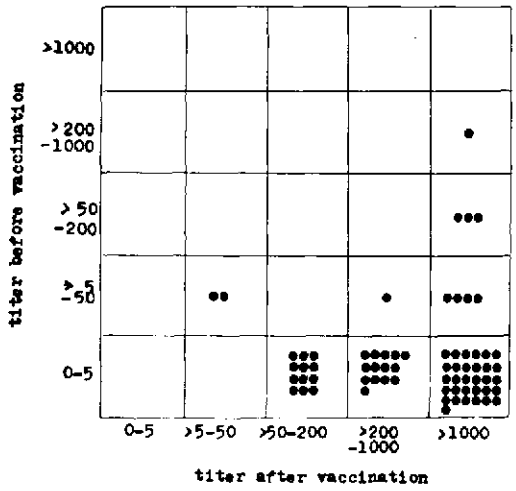
Level of antibodies against Type 1 poliovirus in 43 newborns before and after vaccination with live avirulent polio vaccine Type 1 (CHAT strain).



• Virus excretors
o no virus excreted

F. Buser and M. Schär, Bern

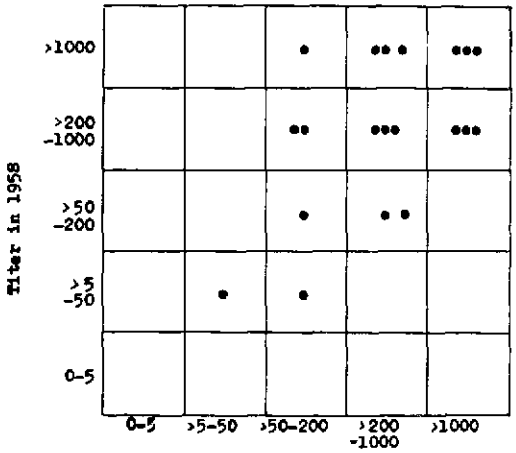
Antibody titer against Type 1 poliomyelitis virus in 63 2-12 months old infants before and after vaccination with avirulent Type 1 poliovirus (CHAT strain).



F. Buser and M. Schär, Bern

Persistence of antibodies against Type 1 poliovirus.

Retitration 2 years after the vaccination with avirulent Type 1 vaccine (CHAT strain) in 20 triple negative infants.



INFLUENCES OF ANTIBODY LEVEL ON VIRUS EXCRETION

	PREEXISTING TYPE I ANTIBODY TITER	NUMBER OF PERSONS	VIRUS EXCRETORS	
			NUMBER	PERCENTAGE
INFANTS & CHILDREN	0 < 5	156	156	100
	5 < 50	24	20	86
	50 and above	49	31	64
ADULTS	0 < 5	14	9	64
	5 < 50	8	4	50
	50 and above	17	4	23

CHAIRMAN HILLEBOE: Thank you, Dr. Payne. The papers you have heard are now open for discussion.

DR. LEPINE: I should like to ask Dr. Plotkin a question. Could he state whether the children who have shown active resistance to vaccination between the age of eight to 70 days were breast-fed children or bottle-fed children? In other words, had they received human milk or artificial milk?

The reason for my question is that a few years ago, when we studied the establishment of carrier state in chimpanzees after oral feeding of live poliovirus, we found that, whereas the injection of gamma globulin would have no effect on the establishment of a carrier state, if gamma globulins were simultaneously fed by mouth at the time of infection and continued for some days, the establishment of a carrier state could be prevented in some cases, and thus the antibody response resulting from infection greatly reduced.

DR. PLOTKIN: We have also been interested in this point, Dr. Lépine.

Unfortunately, we have been unable really to study this. In reply to your question, very few of the infants in this study were breast-fed, owing to the circumstances of the institution in which the study was conducted. Of course, in those cases where there was breast feeding, we did follow those infants; in those few we did not observe any effect of the breast milk on the feeding.

We did titrate some breast milk from the mothers; to be sure, there was polio antibody and although I do not recall the exact figures, they were not at a remarkably high level.

DR. COX: I was very much interested in Dr. Robbins' comments about feeding Dr. Sabin's Type 1 virus to infants, in whose stools virus was found for certain periods, followed by periods when virus was not found, and then followed by reappearance of virus again.

We also have been doing a study of this type. We have fed a number of children, but have the results on only a few at the present time.

However, one interesting observation has been made. We fed trivalent vaccine, containing 6.1 logs per strain per dose, to a child aged five and one half months, who happened to be the child of the senior technician in charge of our serology group. We found all three types to appear in the stool for three consecutive weeks. Then Type 2 disappeared for two consecutive weeks and we found only Types 1 and 3 to be present. Then, much to our amazement, Type 3 disappeared, and for two consecutive weeks we found the appear-

ance of Types 1 and 2. This is contrary to what we had expected because, as you all know, our Type 2 strain is what you might call the weak sister of the three types. Here is a case where Type 3 disappeared and Type 2 reappeared. Of further interest is the fact that this is the only child in the family. We know that the mother is solidly immune to all three types of polio, because we have re-fed her at six-month intervals for the past three years. However, we have no information concerning the serological status of the father.

At any rate, much to our surprise, the Type 2 strain disappeared and then reappeared and this, in our opinion, was contrary to all our expectations in view of what we know about our Type 2 strain.

DR. SABIN: Since the same material was used by Dr. Gelfand, Dr. Krugman, and Dr. Robbins, I should like to say that many recent tests have shown the titer to be $10^{7.3}$ plaque-forming units or $10^{7.6}$ TCD_{50} per ml. Therefore, the dose used by Dr. Robbins was about 10^6 TCD_{50} for three-month-old children and $10^{7.6}$ TCD_{50} for the newborn.

I should now like to comment on the question of breast feeding raised by Dr. Lépine. I have also been concerned about that because, as Dr. Lépine may recall, a number of years ago I carried out extensive studies on feeding human milk, which had been titrated and shown to contain antibodies for polio, to monkeys, which were then fed virulent poliovirus by mouth. The human milk was given, not only before the virus, but its feeding was continued for, I believe, seven or 14 days thereafter, several times a day; that had no effect upon the incidence of infection of paralytic polio in the monkeys.

I also have fed mixtures of gamma globulin and virulent virus, in which the antibody was in excess, and found that there was some dissociation in the intestinal tract of the monkeys, because some of them developed antibody.

For a time I thought that this showed that antibody-containing human milk had no effect on infection of the alimentary tract by poliovirus, until I realized that the pathogenesis after feeding in monkeys and chimpanzees is different from what it is in human beings. In monkeys and chimpanzees most of the infection occurs in the posterior pharyngeal wall, while in human beings the lower intestinal tract is also very susceptible.

So if monkeys swallow human milk and then are given large doses of the virus by mouth, they could have their posterior pharyngeal wall infected, and the milk in the stomach or in the intestinal tract would have very little effect.

Therefore, I do think that at the present time we should look into the question of what effect antibody-containing human milk can have even when taken in much larger quantities than I could ever administer to monkeys—because a child of a few weeks of age will take about a quart of milk or so. For this reason, I think it would be worthwhile if Dr. Krugman and Dr. Robbins might, as far as possible, obtain a history of breast feeding, both during the first few days and later, on those mothers who have been involved in this study. Because in many of the economically underdeveloped areas, subtropical, and tropical regions, certainly in the Far East, breast feeding goes on for a long time. I think that we should get more information on this.

There is one other point. Amazement has been expressed about the appearance and disappearance of individual types of virus in persons to whom a trivalent mixture of polioviruses was fed.

I think if one will refer to the Proceedings of the First Conference held here last year, one will see a number of studies on individuals to whom I fed trivalent or bivalent mixtures. These were young adults in a federal reformatory without contact with the outside, and there the same phenomenon was evident.

Now, whether the virus can remain for two or three weeks in the intestinal tract without being detected in the stools I do not know, but there is no question that when multiple types are fed at the same time this is a phenomenon that has been repeatedly observed and confirmed.

It is of importance because I think that this limited multiplication of any one type in the intestinal tract after trivalent mixtures are fed, even when all three types may appear, is reflected in a poor resistance of the intestinal tract to reinfection, months and years later.

DR. GOLDBLUM: I should like to ask both Dr. Plotkin and Dr. Krugman concerning the newborns and infants who were found to excrete virus and failed to produce antibodies.

Would they kindly give us the details on the degree and duration of virus excretion. I am bringing this up since this is in disagreement with what we have found in older infants. In our experience, there was excellent correlation between virus excretion and antibody response.

DR. PLOTKIN: In answer to that point, let me say that we have included only infants in whom excretion was established beyond a period of several days after feeding. As for the data regarding duration of excretion, we have not noticed a difference in the duration of excretion of the newborn infants and the infants fed later in life.

However, our data are somewhat influenced by the fact that most of the infants are fed the other virus types sequentially, so that there is an artificial cutoff of excretion.

It may be that if we allowed excretion to go on indefinitely, excretion beyond the period of three or four weeks after the feeding would occur in one group and not in the other, but insofar as I can answer your question, Dr. Goldblum, we have no reason to believe that the newborns excrete less than do the older infants.

DR. PERKINS: There is striking similarity between the problems that people are facing in obtaining antibody response after feeding of live virus vaccines and those that we had when attempting to immunize infants and the newborns with Salk vaccine. Both maternal antibody interference and the lower response of the newborn infants are presenting problems.

Latterly, we attempted to overcome the interfering effect of the maternal antibody in the newborn by giving not two but three doses of vaccine, each at four-week intervals, as a primary immunizing stimulus. While it appeared at first that the response was no better than there had been to two doses of vaccine, all these infants have now been recalled at 12 months of age, given a booster dose (this was the fourth dose of vaccine), and bled 10 to 14 days later. We now see that we have some excellent booster responses in these infants, so that it is quite clear that all those infants were sensitized in their primary immunization course to Type 2 and Type 3, and more than 90 per cent of them to Type 1.

The lessons that we have learned from this could also be applied to the live virus problem and these are twofold: the first is that the increase in antigenic stimulus has had a marked effect upon these infants, resulting in our being able to overcome maternal antibody interference; and the second is that one should delay one's assessment of the immunity state after the first feeding, until a booster or refeed is given, for it is at this time that one is better able to assess what has happened in the original feeding experiments.

DR. GEAR: The question of serologic tolerance has been raised, and to complete the picture, I wonder if one of our speakers could tell us the antibody response of infants whose mothers were fed virus before the infants were born.

We have seen a number of cases of poliomyelitis in mothers and in their newborn infants at the same time, nearly all of whom died. We have also seen several cases of mothers who contracted poliomyelitis during pregnancy and who subsequently gave birth to normal babies.

Two such babies have been followed up for one year. The purpose was to find out whether they would respond to formalinized vaccine by producing antibodies to the type causing the mothers' infection.

We found that the babies had antibodies against Type 1 virus at birth, at six months, and again at one year. Presumably, the later period antibodies resulted from active infection, possibly occurring before birth.

The question is whether there is any evidence of in utero infection in the vaccine-fed mothers, and if so, are their babies immunologically tolerant?

DR. PLOTKIN: Dr. Gear, I cannot tell you whether in utero infection has occurred. We have vaccinated infants of several women who were given live virus vaccine during pregnancy, and we did not detect a difference in their response. They apparently were still susceptible to infection and capable of producing antibodies. Whether an in utero infection might have occurred we cannot say.

I should like to take this opportunity to make several points concerning the evaluation of the effect of transplacental antibody. I think that some attention should be given to the means of administration of the virus, for the reason that

transplacental antibodies, or antibodies of any type, are present in the pharynx to a much greater degree than in the intestinal tract.

In our own premature study, the virus was given by gavage directly into the stomach. With full-term infants, the virus was given in a volume of milk, so that most of it passed down the esophagus and past the pharynx.

If I interpret Dr. Robbins' statements correctly, 1 cc. of the virus was delivered by dropper into the pharynx; in Dr. Krugman's study, a similar procedure was followed, I take it.

Could it be, then, that some of the influence of transplacental antibody shown in the results may reflect the implantation of the virus in the pharynx, whereas the relative lack of effect on infection that we have found, may reflect the administration of the virus as directly as possible into the intestinal tract?

Second, I have been interested—from a biological point of view—in the half-life of transplacental antibody, apart from the necessity of knowing this value for the evaluation of antibody responses of infants, and I took the liberty of calculating Dr. Krugman's data which he so kindly has given us. By my rapid computation, the geometric mean half-life of those data here would be something less than four weeks. Therefore, I think, as I am sure he realizes, that he has been perhaps too conservative in his criterion of antibody response of infants. If he had used a lower figure for the half-life, then more of the infants would have shown some apparent response.

Of course the six-month blood determination is certainly the most important and the most crucial in determining the response of these infants. Before that, it is sometimes hard to try to calculate what should have happened, but, at six months it is clear whether the infants have responded or not.

Dr. Cox: There is no doubt that Dr. Gear's question is very important. The data that we have are very meager, since they actually consist of only one case.

It so happens that my own daughter was fed our polio vaccine within the first two months of pregnancy. At the time I did not known that she was pregnant. However, she was fed trivalent vaccine even though she was negative to Types 1 and 3.

All three of our grandsons were fed trivalent vaccine in the third month of life. All three strains were found to be excreted in their stools. This past spring, they were re-fed, and only the youngest, now 16 months old, excreted virus, and only Type 2.

Our oldest grandson, who is now five years old, has been fed vaccine on four consecutive years, and the past two years he has failed to show excretion of virus.

These limited data do not mean much perhaps, but they may give us a general idea as to what to expect.

While I have the floor, I should like to tell my friend Dr. Sabin, that I really was not surprised to see the coming and going of virus strains being excreted, but I was surprised to see that Type 2 was being excreted so long, because in this respect our Type 2 is rather poor compared to Types 1 and 3.

I do not like to belabor the point, but I should like to point out that in many ways what we have done with live poliovirus vaccine has followed the almost perfect model of Newcastle disease in poultry. I am not sure that I mentioned this work this past year, but we had a very serious problem in trying to immunize newborn chicks against Newcastle disease. Like everything else in virology, we have two basic conditions to consider: quality of the strain and quantity of material.

We found out quite early that there was no particular problem in immunizing newborn chicks, derived from non-immune dams, with our particular strain of Newcastle, which is the Blacksburg strain, provided we used an amount of virus in excess of 4.5 logs. If we went below 4.5 logs, then we obtained very little protection.

We also found that we could immunize baby chicks derived from non-immune dams with 4.5 logs of virus or better, by any route.

On the other hand, when we came to the problem of immunizing newborn chicks derived from immune dams, we found that no matter how much virus we inoculated by the intramuscular or subcutaneous routes, we could get no real protection as determined by subsequent challenge.

However, regardless of whether baby chicks are derived from non-immune or immune dams,

if we give the Blacksburg Newcastle vaccine (4.5 logs or better), either intranasally or in the eye, we find that they are rendered immune. I think this observation is an extremely important one and of value in polio work.

Actually, the work which was done on Newcastle disease back in 1944 and 1945 by my colleague, Dr. Floyd S. Markham, highly influenced us to go in the direction of living virus vaccine in connection with the polio problem.

DR. LÉPINE: I should like to confirm what Dr. Perkins said regarding the response of infants to antigenic stimuli, especially in the case of inactivated vaccine.

A paper published recently by Dr. R. Grumbach *et al.*, containing a study made with my laboratory's participation and presented at the October meeting of the C.I.E. in Paris, shows the response of infants immunized with inactivated polio vaccine combined with other antigens, namely, diphtheria, tetanus, and pertussis on two groups of children. In one group, there were infants from two to six months of age; in the second, children over six.

After immunization, the response in infants, as judged by the antibody titers obtained, seemed to lag behind that in the older ones, and it therefore seemed at first that the results were not good. However, one year later, when they were given their booster dose, the infants' response jumped to exactly the same antibody level as that of the older children. So, even if the primary response in infants is not immediately so good as in the older children, they nevertheless keep the memory of the antigen and are later able to do as well as the older children.

DR. KRUGMAN: I believe that the comments by Dr. Lépine and Dr. Perkins tend to confirm our impression that placentally transmitted antibody masks evidence of active antibody formation. I am reminded of a study which was carried out in our department in 1952 by Doctors Osborn and Dancis. Their studies were concerned with the effect of passive transplacental antibody on the active immunization of newborn infants with diphtheria toxoid. An injection of toxoid was followed by a rising titer of diphtheria antitoxin in infants with low levels of passive antibody and a declining titer in those with high levels.

At six months, infants with the high level of transplacental passive antibody and lack of evidence of response were given another inoculation of diphtheria toxoid, which promptly induced a "booster" response. Accordingly, it appeared as if the initial high levels of passive antibody had "masked" the presence of active antibody. This phenomenon may explain the low percentage of antibody response in our high titer group.

DR. DULBECCO: I should like to ask a question of a more general nature, namely, what is the feeling about the possible complication caused by simian virus, some of which are known and some possibly unknown, in the feeding of newborn children?

In fact, the experimental work with animals has shown that at this age animals are particularly susceptible to carcinogenic viruses, of which some are active also in oral infections.

CHAIRMAN HILLEBOE: Does anyone wish to comment on this?

DR. KOPROWSKI: I should like first to comment about immunologic tolerance. Although I recognize this phenomenon as an important biological problem, we have no evidence that it plays any role in virus infection, except possibly the lymphocytic choriomeningitis infection of mice. Even in that case, the available evidence is still not completely satisfactory because the crucial experiments have not been performed.

I must say that we have completed a two-year study of attempts to induce tolerance in mice to the three viruses: rabies and two Arbor B viruses. If these observations are ever written up, they will be submitted to the "Journal of Negative Results."

If we want to study tolerance in virus diseases, our efforts should be directed to infections which do not induce formation of demonstrable antibodies, such as African swine fever, equine infectious anemia, or serum hepatitis. Our efforts are largely wasted in the case of diseases like poliomyelitis, where antigenic stimulus almost invariably causes antibody formation.

In one case of possible fetal infection of man, that of German measles virus, tolerance does not play any role. I have asked Sir Macfarlane Burnet on several occasions about children who were

born of mothers who had a German measles infection during early pregnancy, that is, children born with congenital defects. The German measles infection of these children runs a normal course.

Many of the protagonists of live virus vaccination would like to know how to discover a potential tumor-inducing agent in the monkey-kidney tissue. But this problem should be almost of equal importance to adherents of inactivated accines. Since inactivation rate of such a still imaginary virus is unknown, it could exist in the formalin-inactivated vaccine and cause tumors after parenteral injection, a route much more dangerous for an animal than administration through the alimentary canal.

Although newborn animals are prone to be more susceptible to tumor-inducing properties of such viruses as polyoma or milk tumor agent, there are many more factors than age involved in this problem. The genetic make-up, hormonal imbalance, and influence of neighboring cells are only a few of the factors to be mentioned. Conversely, newborn animals are more resistant than adults to non-tumor viruses such as polio or lymphocytic choriomeningitis. Where to place simian viruses in this respect is impossible to say.

DR. BODIAN: One of the things that have impressed me at this session is the increase, since the last Conference, in information about the subject of immunization of infants. Many questions which arose last year have at least been approached, and have partial, if not complete, solutions.

Among the ones that I think deserve considerable attention are, first, the differences in infectivity among virus strains fed to newborns and infants, and the question of whether this may lead to a solution by means of a single-strain feeding or, rather, by adjustment of dose.

The studies that we have heard about have included dosage ranges which vary from less than 5 logs to 7.5 logs. The results in response have varied to some extent correspondingly.

Second, another area about which we knew practically nothing last year and know a great deal about now is the effect of maternal passive antibody on the excretion of virus in these infants and on the antibody response. The balance of evidence, I think, suggests that there is an influence of maternal antibody, not only on virus excretion but on the antibody response. As I mentioned yesterday, this fits very well in terms of the critical level of passive antibody that we have obtained in young chimpanzees.*

DR. SABIN: While there are certain similarities in response of very young infants to dead vaccine and to feeding of live virus vaccines, there are also certain differences. When a preformed antigen is administered, it can combine with antibodies present in the individual.

I should like to recall some observations made by my good friend Dr. Cox when we worked together at the Rockefeller Institute 25 years ago. He showed that if you added too much antiserum to formalinized eastern equine encephalitis virus, it lost much of its effectiveness as an immunizing antigen.

It is also a fact that younger infants, during the first weeks of life, produce lower antibody levels. What has been demonstrated after feeding live virus vaccine is not that a higher level of placentally transmitted antibody interferes with antibody development in infants but, rather, that when the original levels are high they mask the demonstration of the lower levels of actively produced antibody—at least at three months of age. That was beautifully brought out in the chart shown by Dr. Krugman.

There is only one other comment I should like to make with reference to what Dulbecco said. We know of the so-called "carcinogenic," polyoma virus, but in order to demonstrate its effects, massive doses have to be administered by injection to newborn mice, while under natural conditions and after administration by the oral route, there is no evidence that this virus is "carcinogenic." If one thinks of the mouse mammary carcinoma "milk agent," one should remember that its activity is limited only to certain breeds of mice.

CHAIRMAN HILLEBOE: We shall now have the presentation of two papers. The first is by Dr. Cox on "Recent Experience with the Lederle Trivalent Oral Poliomyelitis Vaccine"; the second is by Dr. Kleinman on "Further Experiences with Oral Poliomyelitis Vaccines in Minnesota."

* *Bulletin of the Johns Hopkins Hospital*, 1960, In Press.

10. RECENT EXPERIENCE WITH THE LEDERLE TRIVALENT ORAL POLIOMYELITIS VACCINE

HERALD R. COX, SC.D., VICTOR J. CABASSO, SC.D., JUAN EMBIL, JR., M.D., FLOYD S. MARKHAM, PH.D., MAX J. MOSES, M.D., ARDEN W. MOYER, PH.D., MANUEL ROCA-GARCÍA, M.D., AND J. M. RUEGSEGGER, M.D.

Viral and Rickettsial Research Section, Industrial and Community Relations Section, and Medical Research Section, Lederle Laboratories, American Cyanamid Company, Pearl River, New York, and Municipal Children's Hospital, Havana, Cuba

DR. COX *(presenting the paper)*: Since apparently I am the only one given credit on the program, I should also like to give due credit to my associates, who are responsible, along with me, for the data we are going to report today: Dr. Victor Cabasso, Dr. Juan Embil from Cuba, Dr. Floyd Markham, Dr. Max Moses, Dr. Arden Moyer, Dr. Manuel Roca-García, and Dr. James Ruegsegger. All these men, incidentally, are members of the Pearl River staff, with the exception of Dr. Embil.

INTRODUCTION

The increased incidence of paralytic poliomyelitis in the United States during 1959, despite the availability and use of large quantities of formalin inactivated poliomyelitis vaccine, has served to heighten interest in the oral method of immunization with attenuated strains of polio virus. In a recent publication namely, the *British Medical Journal*, October 1959,[1] we briefly reviewed the literature dealing with the simultaneous application of multiple types of polio virus and summarized the results of a small scale trial of trivalent oral vaccine. Extensive field trials with this type of vaccine, both in the United States and in Latin America, as well as in Europe, are now in progress and it is estimated that these will comprise well over 1,000,000 persons by the end of this year.

This report summarizes the completed serologic study of paired sera obtained from three separate groups of volunteers in different locations: a series of 360 Cuban children, 123 members of a closed institution in New England, and 933 persons, most of whom are Lederle employees or members of their families. The trivalent oral vaccine used in these studies was prepared from the same virus strains previously described.[1] Seventy-nine per cent of the 1,416 volunteers of the present study received vaccine from a single lot; the remainder received vaccine from two other lots which were similarly prepared and administered. In all instances a single 2 ml. dose representing 1,200,000 tissue-culture doses ($10^{6.1}$ TCD_{50}) of each of the three virus strains was fed. Blood samples were collected at the time of vaccination and again four to seven weeks later. Antibody levels were determined by the metabolic inhibition test described earlier.[1] Since this is a very important point, I would like to state that in our case the paired sera were run simultaneously, after they had been inactivated at 56° for 30 minutes. We used four-fold serum dilution, starting with 1:4 and ending at 1024.

We used 1/4 cc. volume of serum, to which we added 100 to 300 tissue-culture doses of virus. These mixtures were held at room temperature for three hours, and then the cells were added. In our laboratory, which is air-conditioned and thermostatically controlled the year-round, the temperatures vary between 70 and 75° F. The cells were quantitated to contain, as nearly as possible, 150,000 primary monkey-kidney cells per 1/4 cc. volume always using Cynomolgus monkey-kidney cells.

Our tests were carried out in duplicate tubes, using the pH method. We always used positive and negative serum controls. I think it is quite important to point this out.

There were no instances either in those who were fed, or in their contacts, of illness or other untoward manifestations attributable to the vaccine and attention will therefore be confined to

consideration of the various response aspects indicated by the serologic analyses.

OBSERVATIONS

Group 1 was made up of 360 Cuban children whose ages varied from four to 18 years, 80 per cent of whom were between seven and 11 years old. These children were either occupants of welfare institutions or short-term residents in rural recreation camps. They represent a broad cross-section of the childhood population, and a survey for intestinal parasites conducted in one of the camps, revealed the fact that approximately 70 per cent were carrying two or more species of round worm, mostly *Trichuris* and *Ascaris*. In one camp 10 cases of varicella and in another five cases of rubeola occurred during the early post-feeding observation period. In a third group an influenza-like syndrome was prevalent, but in none of these illnesses did the course appear unusual. I might add that Dr. Embil saw each of these individuals personally.

Two hundred and eighty-four of these children possessed antibody to all three types of poliovirus at the time of oral vaccination. The remaining 76 children lacked antibody for one or more types of virus. Incidentally, in our tests we record only the original serum dilution. We did not take the dilution factor into consideration. We have stated that when they convert from negative to 1:4, this is believed to be a significant titer. Of course any titer greater than 1:4 is that much better. Sixty-three were negative for a single type, and collectively there were 91 seronegatives for one type or another among them. The distribution of these is presented in Table 1. You can see that we broke these down into the monovalent, homotypic negatives, the double negatives and the triple negatives.

Since polioimmunity occurs quite early in life in Cuba, most of these individuals were immune; but there were 76 persons showing the various antibody gaps, as designated. Thus, 76 persons showed a total of 91 antibody gaps.

Blood samples collected five to seven weeks after administration of trivalent oral vaccine showed that 65 of the 76 children (86 per cent), who previously had been without antibody for one or more types of virus, were now triple positive. The conversion rate for the 91 pre-vaccination negatives in terms of filled in antibody gaps was 88 per cent, which compares favorably with the 84 per cent conversion rate obtained previously in comparable Cuban children when the three virus types were fed separately.[2] Seven of the 11 unconverted negatives were for Type 2 virus, three were for Type 1, and one was for Type 3. You can see that after feeding the vac-

TABLE 1. POLIOVIRUS ANTIBODY STATUS

GROUP: 76 CUBAN CHILDREN DOSE: 2 ML TRIVALENT VACCINE ($10^{6.1}$ TCD_{50} EACH TYPE PER DOSE)							DATE: 1959
PRE-VACCINATION			POST-VACCINATION				
NEGATIVE TO TYPE	NO. OF PERSONS	TOTAL NO. NEGS.	RESIDUAL NEGATIVES TO TYPE 1	2	3	TOTAL	NO. OF COMPLETE CONVERSIONS
1	25	25	2			2	23
2	15	15		6		6	9
3	23	23			1	1	22
1 & 2	2	4					2
1 & 3	6	12	1			1	5
2 & 3	3	6					3
1, 2 & 3	2	6		1		1	1
TOTALS	76	91	3	7	1	11	65 = 86%

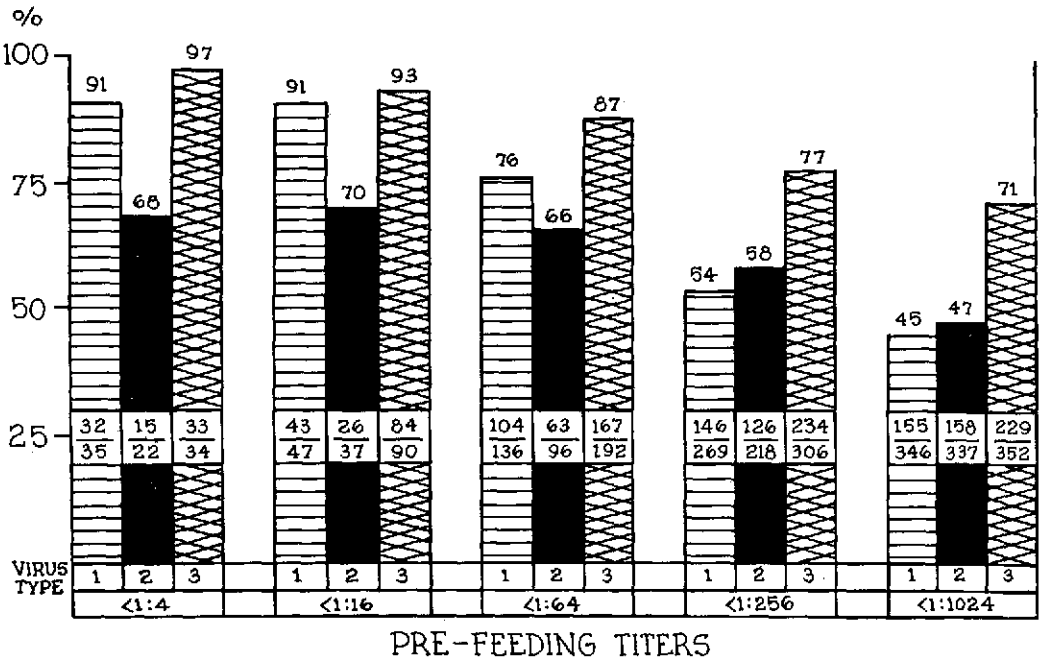


FIG. 1. Percentage of 360 Cuban children with four-fold or greater rise in antibody titers after taking 2ml. oral trivalent poliovaccine.*

* ($10^{6.1}$ TCD_{50} each type per dose.)

cine no triple negatives were left. Figure 1 shows the conversion and booster response rates in 360 Cuban children after ingestion of the trivalent oral vaccine. The same figures are shown on the bar graph indicating the response of those individuals who showed no detectable antibodies, at least by our own methods. Thirty-two out of 35 converted to Type 1, which is 91 per cent; 15 out of 22 to Type 2, which is 68 per cent; and 33 out of 34 to Type 3, which is 97 per cent.

Now, I should like to point out that these other figures are cumulative values. In other words, anything with an antibody titer of 1:8, but less than 1:16, is depicted here. As you can see, as you go across the chart, as the antibodies are found to increase, lesser responses to the vaccine are found. This is what you would expect.

Group 2 comprised 123 males residing in a closed institution in New England. They ranged in age from 17 to 70 years, but most were in their mid or late 20's. In this institution we believe there were plenty of chances for rebound infections. At the time of vaccination, the frequency rates of seronegatives among them were 31, 31, and 32 per cent, respectively, for poliovirus Types 1, 2, and 3. The distribution and grouping of these seronegatives is detailed in Table 2, and collectively they represent 115 antibody gaps in 67 of the 123 inmates. You can see that these are broken down into single negatives to Types 1, 2, and 3; double negatives to 1 and 2, 1 and 3, 2 and 3; and triple negatives.

Of the 123 individuals there were 12 triple negatives. In these columns you see the numbers of negatives left after the vaccine was fed; thus we had 100 per cent conversion to Type 1. Nine individuals failed to convert to Type 2, and one individual failed to convert to Type 3. We had left a single double negative, an individual that failed to respond. He was a double negative to Types 2 and 3. No triple negatives were left. Serum neutralization tests on specimens collected 31 days after feeding showed that 103 (89.6 per cent) of the 115 pre-vaccination negatives became positive as a result of oral vaccination. Ten of the 12 residual seronegatives were for Type 2 virus, and seven of these occurred among the 12 originally triple negatives. This points up to what we have been uniformly finding, that in the triple negative it is more difficult to get a positive

TABLE 2. POLIOVIRUS ANTIBODY STATUS

GROUP: 67 SERO NEGATIVES OF 123 PERSONS FED IN ST. JOSEPH'S ABBEY, WORCESTER, MASS. DATE: 1959

DOSE: 2 ML ORAL TRIVALENT POLIOVACCINE (7-1238-803)

PRE-VACCINATION			POST-VACCINATION					
			RESIDUAL NEGS. TO TYPE					
NEGATIVE TO TYPE	NO. OF PERSONS	TOTAL NO. NEGS.	1	2	3	2&3	TOTAL NO. NEGS.	NO. OF COMPLETE CONVERSIONS
1	10	10					0	10
2	8	8		1			1	7
3	13	13			1		1	12
1 & 2	10	20		1			1	9
1 & 3	6	12					0	6
2 & 3	8	16					0	8
1,2,&3	12	36		7		1	8	4
TOTALS	67	115	0	9	1	1	11	56 (83.6%)

conversion to Type 2. The conversion rates were 100, 74, and 95 per cent, respectively, for poliovirus Types 1, 2, and 3. This information and the booster response rates in persons possessing various levels of antibody before feeding are summarized graphically in Fig. 2.

Here you see pretty much the same thing. I do not know, but it may be because we may have introduced a bit more of Type 1 into this particular batch, since here we obtained a conversion rate of 100 per cent for Type 1, that is, 38 out of 38, 28 out of 38 for Type 2, and 37 out of 39 for Type 3.

These other charts are practically identical to what we saw previously in the Cuban data.

In this group we are talking about those individuals who had detectable antibodies at a level of less than 1:16. Here are indicated those people who had antibody levels of 1:32, but less than 64. These had antibody levels of 128 but less than 256, and here are those with antibody levels of 512 but less than 1024.

Some of these persons actually had antibody levels higher than 1024, but we did not carry our tests higher than that because we did not think it necessary.

These sera are on hand, and some day, when we have more time, we intend to find out just how high these antibody levels did go.

Here you see each of the percentage conversion rates and here you have the actual figures in terms of ratios.

Group 3, the largest of the three study groups, consisted of 933 persons all but 95 of whom were over 18 years of age. Included in the 933 are 188 persons who received the 2 ml. dose of trivalent oral vaccine described in our previous report contained in the *British Medical Journal* of October 1959.[2] With few exceptions this group represented Lederle Laboratories personnel and members of their families.

A single lot of trivalent vaccine was used to vaccinate 652 persons, including the 188 mentioned above, and 275 others received vaccine from a second lot. Six individuals were fed from a third lot.

Pre-vaccination sera showed that 485 persons, or 52 per cent of the 933 volunteers, were without antibody for one or more types of poliovirus and the distribution and combinations of antibody gaps are indicated in Table 3. Here you see the breakdown of the antibody negatives. We

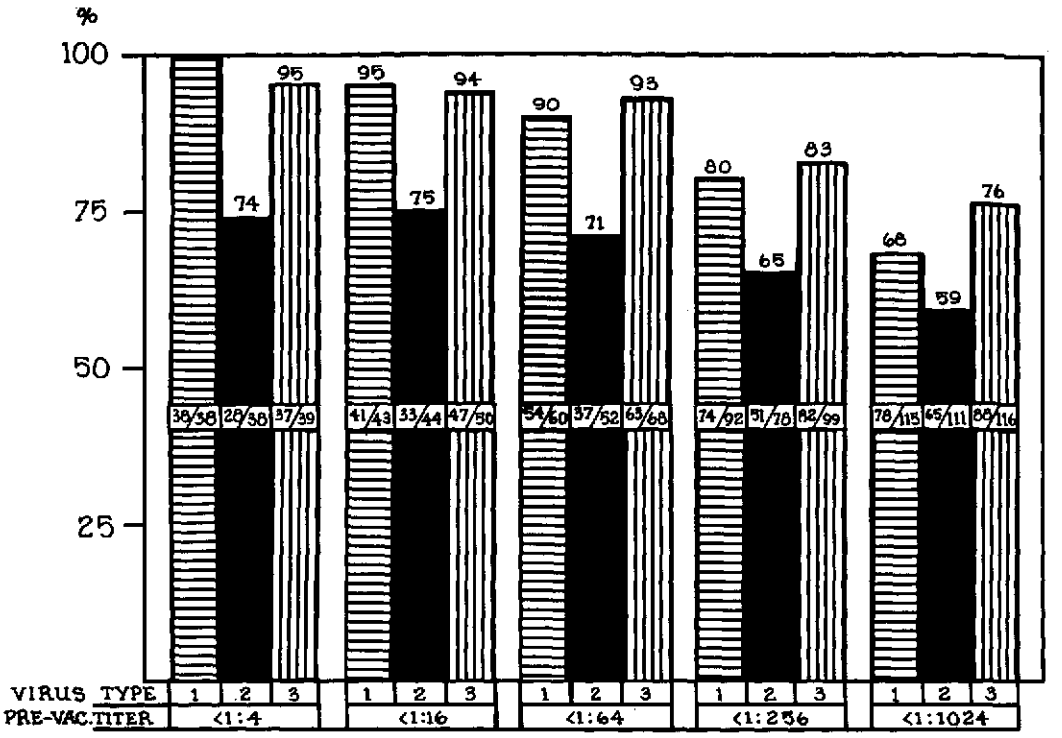


FIG. 2. St. Joseph's Abbey, Worcester, Mass., 1959—123 persons. Percent of persons with fourfold or greater rise in poliovirus antibody titer following ingestion of oral trivalent poliomyelitis vaccine, by virus type and pre-vaccination titer.
(2ml. vaccine, 7-1238-803)

TABLE 3. POLIOVIRUS ANTIBODY STATUS

GROUP: 485 LEDERLE PERSONNEL & FAMILIES DATE: 1959-60

DOSE: 2 ML ORAL TRIVALENT POLIOVACCINE ($10^{6.1}$ TCD_{50} EACH TYPE DOSE)

PRE-VACCINATION			POST-VACCINATION						
NEGATIVE TO TYPE	NO. OF PERSONS	TOTAL NO. NEGS	RESIDUAL NEGATIVES TO TYPE						NO. OF COMPLETE CONVERSIONS
			1	2	3	1&2	1&3	2&3	
1	101	101	7						94
2	58	58		15					43
3	130	130			7				123
1 & 2	45	90		6					39
1 & 3	53	106	3		2		3		45
2 & 3	37	74		4				2	31
1, 2 & 3	61	183	2	19	3	3		2	32
TOTALS	485	742	12	44	12	3	3	4	407 = 84%

had 101 individuals without antibody to Type 1, 58 to Type 2, and 130 to Type 3. We had 45 individuals who were double negatives to 1 and 2, 53 double negatives to 1 and 3, and 37 lacking antibody to Types 2 and 3; in this group, mostly all over 18 years of age, we found 61 triple negatives.

Incidentally, the manager of our Washington office, who is 54 years old, proved to be a triple negative. Curiously enough, he has four sons of high school and college age. They all had had four injections of Salk vaccine and three of the four were double negatives to Types 1 and 3 polioviruses.

Here you see what happened after vaccination. These totals at the bottom show the residual blanks. We failed to convert 12 to Type 1, 44 to Type 2, and 12 to Type 3 only. We missed three double negatives to 1 and 2, three double negatives to 1 and 3, and four double negatives to 2 and 3; but we had no triple negatives left.

There were 61 triple and 135 double negatives in group 3 at the time of feeding. Including an additional 289 persons who lacked only one type of antibody, there was a total of 742 negatives for one type or another variously grouped in 485 persons.

Serum samples collected four to five weeks after feeding the trivalent vaccine demonstrated that 407 (84 per cent) of the 485 volunteers who had been negative for one or more types of antibody had become positive for all three types. Out of the 485 antibody gaps, 407 were converted from negative to positive. Seventy-eight antibody gaps remained. In other words, 89 per cent of the total antibody gaps were filled in. Thus, 89 per cent of all pre-vaccination seronegatives converted. Type 2 accounted for 58 per cent of the unconverted negatives. The responses of the 933 volunteers of group 3 are presented in Fig. 3, where it is seen that the conversion rates were 93 per cent for both Types 1 and 3 and 75 per cent for Type 2. This figure also shows the rate of booster response among those who possessed

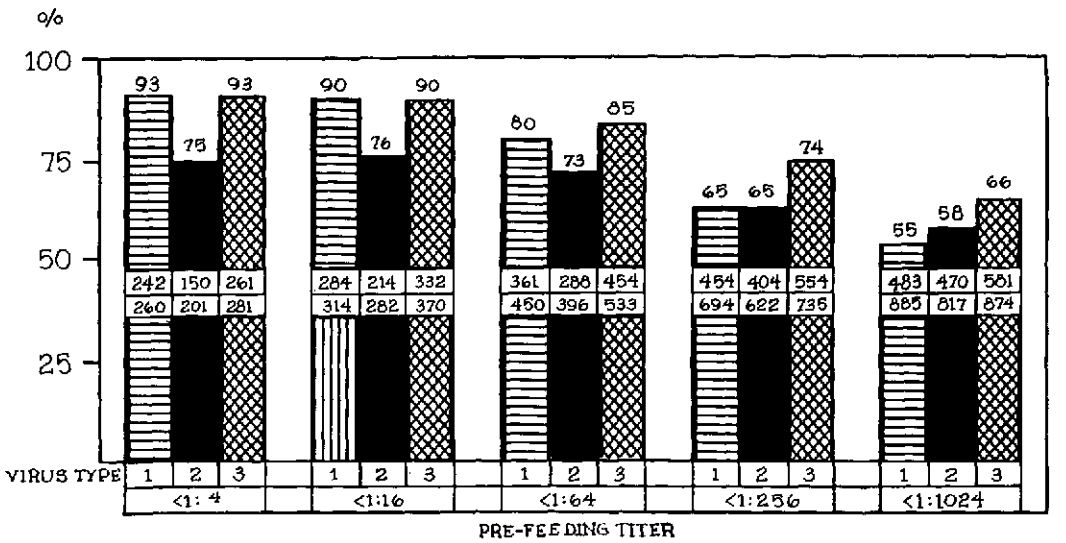


FIG. 3. Percentage of 933 Lederle employees with fourfold or greater rise of antibody titer after a feeding of 2.0 ml. oral trivalent poliomyelitis vaccine ($10^{5.1}$ TCD_{50} each type per dose. Lot nos. 7-1238-801, 7-1238-803 and 7-1238-804A).

pre-vaccination antibody titers. This chart is prepared the same as previous charts. These figures show those persons that had no detectable antibody, and here you see the conversion rates. These are the ratios.

These persons had antibody levels of less than 1:16. These persons had antibody levels of 1:32 but less than 64 and here are shown those with antibody levels of 128 but less than 256; and lastly, those who had antibody levels of 512 but less than 1024, are shown.

FACTORS RELATED TO SEROLOGIC RESPONSE

The influence of pre-existing antibody levels on response to oral trivalent poliovirus vaccine is illustrated in Fig. 4. Here is the antibody titer present at the time of feeding; you can see that the best responses were obtained to Types 1 and 3. One line represents the response to Type 1, another line represents the response to Type 3, and still another is the response to Type 2. Type 3 responses apparently are the best, which not only have been found by us but by all of our investigators as well. The curves are based on the serologic findings in those 1,416 volunteers comprising the three groups whose pre-vaccination antibody levels were less than 1:1024 and who showed a four-fold or greater rise in titer after vaccination. The depressing effect of increasing antibody concentration on the degree of booster response, illustrated here, is essentially the same as had been observed previously when the three viruses were fed separately.

The responses summarized in Fig. 4 are a composite of approximately 1,300 individuals of diverse experience with respect to the three types of poliovirus. The 933 volunteers of group 3 included 61 persons who were without demonstrable antibody to any type of poliovirus. All but five of these triple negatives (92 per cent), responded to both Type 1 and Type 3 virus when fed the trivalent vaccine. Thus, these triple negatives responded as frequently to these types as did all other Type 1 and Type 3 seronegatives in the entire study group. However, the response of the triple negatives to Type 2 virus was 60 per cent versus 74 per cent for those who lacked antibody for Type 2 only. This observation

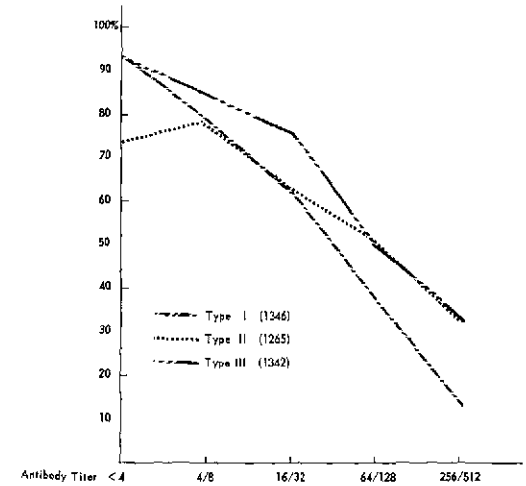


FIG. 4. Percent with a fourfold or greater response at various prevaccination antibody levels.

prompted an examination of the data relating to the responses of those double negatives who were without antibody to Types 1 and 2 or were negative to Types 2 and 3. It was found that among the 45 individuals who were negative for Types 1 and 2, the response rate was 87 per cent for Type 2, and that among 37 persons who were Type 2 and Type 3 negative, the response rate for Type 2 was 84 per cent.

The numbers in these groups are limited but the data suggest that simultaneous susceptibility to either one of the other two virus types, but not to both of them, enhances the response rate to the Lederle Type 2 strain. These observations may be indicative of the more subtle antigenic relationships within the poliovirus group. Gelfand and his associates [3] have recently reviewed these intragroup reaction patterns as shown by the responses to Salk vaccine, and their data, in addition to showing the inferiority of the Type 1 and 3 antigens in formalin inactivated vaccine, indicate that the lowest geometric mean titer attained for Type 2 antigen was found in children who possessed antibodies for both Types 1 and 3 prior to undergoing a full course of three Salk vaccine inoculations.

Age did not appear to be an important element in conditioning the response to the administration of the trivalent oral poliovirus vaccine, except possibly in the case of Type 2 virus. The conversion rates of 95 persons under 18 years of

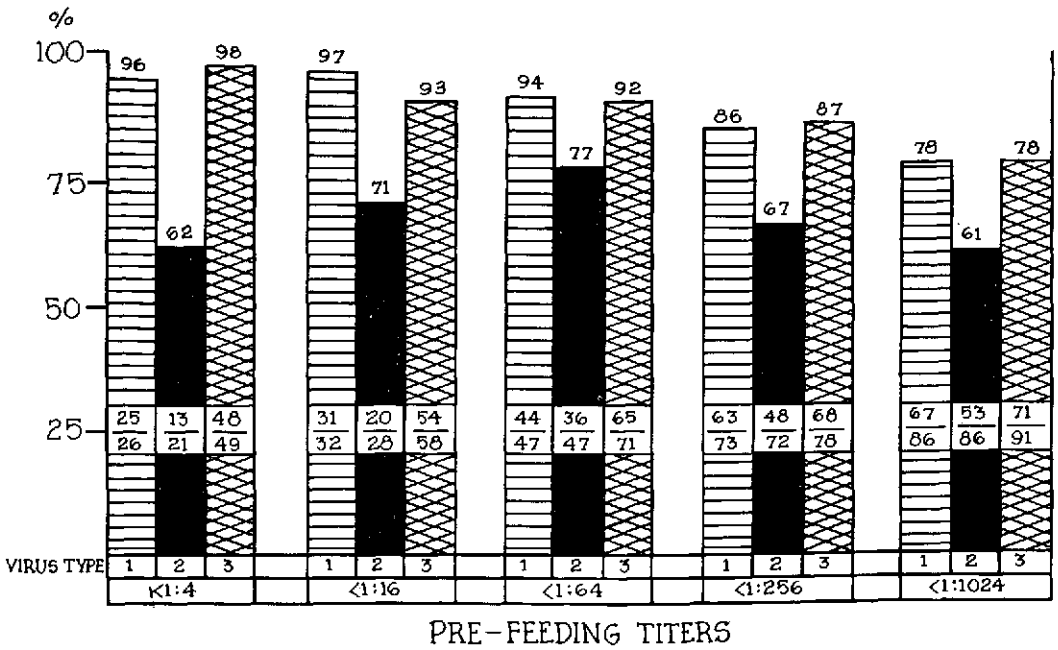


FIG. 5. Percentage of 95 children* under 18 with fourfold or greater rise in antibody titers after taking 2ml. oral trivalent poliovaccine†

* Of Lederle employees. † $10^{8.1}$ TCD_{50} each type per dose.

age in group 3 (shown in Fig. 5), were 96 and 98 per cent, respectively, for Types 1 and 3, and 62 per cent for Type 2. The geometric mean post-vaccination titers attained by those less than 18 years old, compared with those attained by the entire group, were 363 versus 173, 176 versus 203, and 174 versus 150 for Types 1, 2, and 3, respectively. Seventy-two of these children were between nine and 16 years of age and approximately 50 per cent of the group, had received three or more injections of Salk vaccine.

Prior history with respect to Salk vaccine was obtained for 922 of the 933 volunteers in group 3. The type-specific antibody status of these persons at the time of oral vaccination in relation to the number of Salk injections is presented in Table 4. It may be seen that approximately 48 per cent already possessed antibody for all three types of poliovirus and that lesser proportions were lacking in one or more types of antibody. Seventy per cent of the 922 had received no Salk injections. The data show that the percentage of persons without antibody for Type 1 or for Type 3, or for both of these types, is remarkably similar in both the Salk vaccinates and the non-vaccinates. On the contrary, the percentages in sero-negative combinations which include Type 2, as well as in Type 2 alone, decrease in size as the number of Salk injections increases. The total numbers of seronegatives, when segregated from their various combinations, are tabulated in Table 5 as per cent frequencies. Here it is manifest that the reduction in Type 2 negatives is far in excess of the decrease in negatives for Types 1 and 3. These are persons without vaccine; and these are persons with three or more injections of Salk vaccine. In a recent compilation of the cases of paralytic poliomyelitis in the United States for 1959,[4] the reports showed 940 of the 5,342 cases were in those who had received three or more injections of Salk vaccine. This is a frequency of 17.5 per cent, a rate which approximates the incidence of seronegatives for Types 1 and 3 in our data on Salk vaccinates.

That Salk vaccination did not interfere materially with responses to the trivalent oral poliovirus vaccine, was shown by the fact that the rates of conversion from seronegative to positive of the 154 persons who had received three or more injections of Salk vaccine, were 100 per

TABLE 4. POLIO ANTIBODY STATUS AND SALK HISTORY

GROUP: 922 LEDERLÉ EMPLOYEES & FAMILIES

DOSE: NONE TO FOUR SALK SHOTS DATE: 1959-60

NEGATIVE TO TYPE	TOTAL PERSONS		PERSONS WITH SALK SHOTS					
			NONE		1 OR 2		3 OR 4	
	No.	%	No.	%	No.	%	No.	%
0	441	47.8	269	41.1	61	53.5	111	72.0
1	100	10.8	76	11.6	14	12.3	10	6.5
3	129	13.9	90	13.7	19	16.7	20	12.9
1 & 3	53	5.7	34	5.2	8	7.0	11	7.1
2	58	6.3	52	7.9	4	3.5	2	1.3
1 & 2	45	4.9	45	6.9	0	0	0	0
2 & 3	37	4.0	36	5.5	1	.9	0	0
1, 2 & 3	59	6.4	52	7.9	7	6.1	0	0
TOTALS	922		654		114		154	

TABLE 5. FREQUENCY OF SERONEGATIVES IN SALK-VACCINATES AND NON-VACCINATES

NO. OF PERSONS	NO. OF SALK SHOTS	SERONEGATIVES (%) TO TYPE		
		1	2	3
654	0	31.7	28.3	32.4
114	1-2	25.4	10.5	30.7
154	3+	13.6	1.3	20.1

cent for Types 1 and 2 and 97 per cent for Type 3. Moreover, from 37 to 45 per cent of those who had pre-vaccination antibody titers in the range of 1:4 to 1:512 showed four-fold or greater booster responses.

DISCUSSION

The data summarized in the present report are essentially an extension of our earlier study of trivalent vaccine which began in May 1958 with the simultaneous feeding of three capsules, each containing one of the three poliovirus strains.[2] This initial test was followed by the feeding of a fluid vaccine that contained balanced amounts of each of the three types of virus.[1] The results of those efforts were such as to justify the hope that the oral vaccination procedure could be simplified and made more rapid and less expensive, without any appreciable sacrifice of either efficiency or safety. Results obtained since in more than 1,200 additional volunteers confirm the earlier findings and provide a substantial basis for the greatly expanded field trials that are now in progress.

The 88 per cent over-all conversion rate of seronegatives to seropositives, obtained in the present trivalent oral vaccine study, is comparable to, or slightly better than, rates reported for the separate feeding of the same strains of virus in children in Minnesota (85 per cent),[5] Colombia (83 per cent),[6] and Cuba (85 per cent).[2] It is noteworthy, too, that for the most part, these conversions are based on sera collected four to five weeks after a single feeding. The conversion rates for virus Types 1 and 3, epidemiologically the most important, were over 93

per cent. Conversion rates of this order were also obtained in the triple negatives in a similar short span of time, but comparable conversion rates were not attained in the Salk vaccine study of Gelfand et al.,[3] until primary immunization had been followed by a booster injection more than 11 months later. The purpose here is not to detract from the good that the Salk vaccine has accomplished, but only to compare the results given by a new method of vaccination with those of the old—for it should be remembered that we here are concerned with matters of life and limb as well as with pride and treasure.

The relatively poorer response rate to the Type 2 strain in the present trivalent vaccine can certainly be improved by additional research and development. However, the magnitude of its deficiency must be judged in the light of our knowledge regarding the relative minor importance of Type 2 virus as a cause of paralytic poliomyelitis. The ideal poliomyelitis vaccine is still a thing of the future, perhaps the somewhat distant future, but this need not and should not mean that progress must await perfection.

Many problems still remain in the field of oral poliovirus vaccination. It is the obvious hope of everyone that a single feeding may be all that is required. However, in areas where maternal antibody levels are high, repeated feedings of vaccine may be necessary in the newborn and very young. Optimum conditions regarding the relationship of diet and the physiologic state of the digestive tract are largely unknown. The duration of immunity has not been established, and this may be difficult to do in certain areas because of the frequent opportunities for natural booster exposures after vaccination. Until adequate supplies of vaccine are available, so that an organization equipped for mass application can move quickly into an epidemic area, it will not be known how effective oral vaccination can be in curbing an outbreak of poliomyelitis.

Everyone agrees as to the desirability of strain markers and identifying characteristics, but there is considerably less agreement as to the significance and stability of these signs. These are but some of the more obvious and important problems that await solution, but they are secondary to the main problem of getting an effective tool into the hands of those who can and wish to put it to work.

SUMMARY

The serologic responses of 1,416 volunteers who received trivalent oral poliovirus vaccine are presented and discussed. A single vaccine dose, consisting of approximately $10^{6.1}$ tissue-culture doses (1,200,000 TCD_{50}) of each of the three types of attenuated poliovirus suspended in 2 ml. of fluid was administered.

In the three groups which constituted the study population, the conversion rates from seronegative to seropositive status ranged from 91 to 100 per cent for Type 1 poliovirus; from 68 to 75 per cent for Type 2, and 93 to 97 per cent for Type 3. Eighty-eight per cent of total of 948 seronegatives responded to a single oral vaccination.

One of the study groups included 154 persons who had received three or more injections of Salk vaccine. Collectively they represented 52 seronegatives, all but one of which responded to oral vaccination.

Response rates to the trivalent oral poliovirus vaccine were comparable to response rates to the same strains fed separately.

REFERENCES

1. Cox, H. R., Cabasso, V. J., Markham, F. S., Moses, M. J., Moyer, A. W., Roca-García, M., and Ruegsegger, J. M.: Immunological response to trivalent oral poliomyelitis vaccine. *Brit. M. J.* **2:** 591-597 (October 3) 1959.
2. Embil, Jr., J., Castellanos, A., and Jiménez, R. M.: A clinical and serological study of the response of Cuban children to oral vaccination with attenuated poliovirus vaccines. In: *Live Poliovirus Vaccines, First International Conference.* Scient. Pub. no. 44 of the Pan American Health Organization, pp. 593-617, 1959.
3. Gelfand, H. M., LeBlanc, D. R., Fox, J. P., and Potash, L.: Studies on the development of natural immunity to poliomyelitis in Louisiana. III. Serologic response to commercially produced "Salk Vaccine" of children totally or partially susceptible to poliovirus infection. *Am. J. Hyg.* **70:** 303-311 (November) 1959.
4. U. S. Dept. of Health, Education and Welfare,

CDC Poliomyelitis Surveillance Report no. 193, (January 18) p. 4, 1960.

5. Barr, R. N., Bauer, H., Kleinman, H., Johnson, E. A., Martins da Silva, M., Kimball, A. C., and Cooney, M. K.: The use of orally administered live attenuated polioviruses as a vaccine in a community setting: A controlled study. *J. Am. M. Assn.* **170:** 893-905 (June 20) 1959.
6. Abad Gómez, H., Piedrahita, F., Solórzano, R., and Martins da Silva, M.: Community-wide vaccination program with attenuated poliovirus in Andes, Colombia. *J. Am. M. Assn.* **170:** 906-913 (June 20) 1959.

11. FURTHER EXPERIENCES WITH ORAL POLIOMYELITIS VACCINES IN MINNESOTA

1. A Comparison of Monovalent and Trivalent Vaccines
2. Observations on Pharyngeal Recovery of Poliovirus and Viremia*

HERMAN KLEINMAN, M.D., ROBERT N. BARR, M.D., HENRY BAUER, PH.D., ANNE C. KIMBALL, PH.D., MARION K. COONEY, M.S., JACOB E. BEARMAN, PH.D., AND WAYNE E. MATHEY, M.D.†

DR. KLEINMAN *(presenting the paper)*: You will notice that the paper I am about to present has two subtitles: one, "A Comparison of Monovalent and Trivalent Vaccines"; and two, "Observations on Pharyngeal Recovery of Poliovirus and Viremia."

In fact, it might actually have a third one, which I would like to put in parentheses, namely, that it is a study that did not quite come off. The reason for this is that as we went along in this work we found that some of the Type 2, or labeled Type 2, monovalent vaccine had gotten into them some Type 1 poliovirus vaccine, as a contaminant.

True enough, the amount of Type 1 vaccine that we found in our Type 2 was small, of the magnitude of five tissue-culture doses; nevertheless, some of our participants did actually get two doses of Type 1 vaccine, even though the adventitious dose was quite small.

Now, I can explain how we first suspected, then proved, and finally were assured, that this contaminant was a vaccine virus and not a wild virus, but I shall leave that for the discussion period, or for private conversations with any of you, if you wish.

Therefore, as I go on, when I use the term monovalent, will you kindly make the proper mental correction in your minds, particularly with respect to Types 1 and 2.

Permit me to go ahead and present the data with a minimum of *minutiae* and without any speculation. I think that you will find that they are interesting, even though they did not strictly accomplish their primary intended purpose.

INTRODUCTION

Previous experience has convinced these authors that the Cox strains of attenuated polioviruses are good antigens.[1] Equally convincing and impressive was the ease of administration afforded by the oral route. The ability to feed the three types of the vaccine viruses simultaneously, instead of separately at intervals, could only enhance the existing operational and administrative advantages of such an immunizing agent.

Cox has published the evidence that led him and his associates to the belief that simultaneous feeding was a practical possibility. He has also presented evidence to show that such a trivalent agent is effective.[2] One of the objectives of this study was to use the trivalent vaccine in a selected group and to compare its action to the effect of the monovalent vaccines used in members of a comparable group.

Also, since the total group was of such a special character (University students and their children), it was deemed likely that they would cooperate in some studies directed toward discovering some of the facets of the vaccine viruses'

* This is one of a series of studies being reported. Supported in part by grants from the Sister Elizabeth Kenny Foundation and the Lederle Laboratories, Division of the American Cyanamid Company, Pearl River, New York.

† Dr. Kleinman, Dr. Barr, Dr. Bauer, Dr. Kimball, Miss Cooney (Minnesota Department of Health); Dr. Bearman (School of Public Health, University of Minnesota); and Dr. Mathey (formerly, Medical Officer, U.S. Public Health Service).

behavior in the human body. In particular, it was thought that efforts to demonstrate the fed viruses in the blood and the pharynx would not be too burdensome to the participating population. Accordingly, procedures designed to implement these efforts were built into the over-all plan of the experiment. These special efforts were limited to children in this study but were tried elsewhere in a group of adults in a study which will be reported.[3]

1. A Comparison of Monovalent and Trivalent Vaccines

MATERIALS AND METHODS

The attenuated strains of poliovirus used in this study were derived from the same seed that was used to produce the vaccine for the 1958 Como Village study that was reported in 1959. They were also essentially the same strains that were used by Martins da Silva *et al.* in an earlier study which included only newborns and infants under six months of age.[4] From the same seed also came the strains that were and are continuing to be used in the various large scale vaccination operations in South and Central America.[5, 6]

However, the dosage form and the dose were different. The vaccine used in the 1958 Como Village study was adsorbed on granular gelatin and dispensed in hard gelatin capsules. The virus dosage for this preparation was 4.8 logs for Type 1, 5.1 logs for Type 2, and 5.3 logs for Type 3. For the present study, the vaccine viruses were suspended in a liquid, sweetened and pleasantly flavored with cherry. The dose for each virus type was 6.1 logs whether used as a monovalent vaccine or a trivalent vaccine. The drinking dose for both the monovalent and the trivalent types was 2 cc. The liquid dosage form has the obvious advantages of such preparations especially for children. In addition, the stabilizer used has been found to insure a good "shelf life". And finally, it is easier, in a liquid preparation, to regulate the repeatability of the same virus quantity from dose to dose.[7]

Population Characteristics and Location. The population chosen for this study consisted of married University of Minnesota students and their children, living in Grove East, a housing village maintained by the University for student use. The village was in the city of St. Paul. There were residential areas on two sides and on the other two there were the grounds of the St. Paul Campus of the University of Minnesota. The dwelling units were duplex back-to-back structures of the metal barracks type. The water supply and sewage facilities were those of the city itself. This area, which was not quite as congested as the Como Village area of the 1958 study [1] also offered much less communal activity. There was no cooperative store; there was no local student's union; and the meager meeting place was so inadequate as to be generally shunned. Although there was some visiting and exchange of baby sitters, families, in general, tended to keep to themselves. It was difficult to set up a community meeting of any kind.

The characteristics of the volunteering population with respect to age and vaccinal status with Salk vaccine are shown in Table 1. The mean age of the fathers was 27, and the mean age of the mothers, 26. This is comparable to the adult group in the Como Village study. The children in this study were a little older with a mean age of 3.3 years. This is almost a year older than the Como Village children, on the average. Coverage with Salk vaccine was better over-all than it had been in Como Village, and in addition, fourth doses now appeared. Two-thirds of the children, 41.5 per cent of the fathers, and 69 per cent of the mothers had received three or more doses of Salk vaccine. Only a bare 10 per cent had received no Salk vaccine at all. The total of 219 participants represented a little more than 40 per cent of the village's population.

Design. The operating plan for the study is displayed in Table 2. It is obvious that there are two groups of participants; one destined to receive monovalent vaccine sequentially in the order of Types 2, 1, and 3, and the other to receive a trivalent vaccine preceded by two doses of placebo. The members of these two groups were chosen in a purely random manner. The scheduling of the blood and stool samples in relation to the feedings can be appreciated by consulting the table. Throat swabs for pharyngeal virus recovery and capillary blood for

TABLE 1. PARTICIPANTS BY AGE AND VACCINAL STATUS (SALK)—GROVE EAST STUDY, 1959

	TOTAL NO.	MEAN AGE	SALK VACCINE STATUS				
			NONE	ONE DOSE	TWO DOSES	THREE DOSES	FOUR DOSES
Children	111	3.3 years	10	0	27	63	11
Fathers	53	27 years	8	6	17	21	1
Mothers	55	26 years	3	1	13	36	2

viremia studies were collected after the trivalent feeding in the trivalent group and after the Type 3 feeding in the monovalent group.

It should be noted here that the feeding unit was the family and not the individual. That is, all the participating members of a family always received the same type of feeding.

It is also apparent from Table 2 that there was a period from the first day through the 52nd when those in the trivalent group received placebo while those in the monovalent group were receiving Type 2 and Type 1 vaccine in that order. This period was a definite control period, and its principal use in this study was to enable one to critically compare any "reaction" illnesses that occurred in those who received vaccine virus with those that occurred in the participants who received placebo.

Only two persons (A.C.K. and J.E.B.) in the project team knew the identity of the individuals who comprised the monovalent and the trivalent groups. The participants thus never knew until the end whether and when they had received vaccine or placebo or whether the vaccine they did receive was monovalent or trivalent. The project team with the exceptions noted was in a like posi-

TABLE 2. OPERATING PLAN—GROVE EAST STUDY, 1959

DAY*	TRIVALENT GROUP	MONOVALENT GROUP
1 – 3	First Blood and Stool	First Blood and Stool
4 – 5	Placebo	Type 2 vaccine
21–23	Second Stool	Second Stool
24–25	Placebo	Type 1 vaccine
50–52	Third Stool	Third Stool
53–54	Trivalent vaccine	Type 3 vaccine
57–61	Throat swabs and blood for viremia	Throat swabs and blood for viremia
77–79	Fourth Stool	Fourth Stool
80–81	Second Blood	Second Blood

* Day 1 is February 22, 1959.

tion. There could therefore be no bias in the clinical interpretation of symptoms that were reported.

Two blood specimens drawn at about 80-day intervals as shown in Table 2 were collected from each participant. These were sent to the laboratory with a label that identified their origin and sequence by code number. The specimens in any one pair were always tested on the same day in the same run. But, when these pairs were actually tested, the original identifying labels had been replaced by a scrambled and random numbering system that made it impossible for the testing personnel to identify any one specimen as to its individual origin, its set origin, or its sequence within any one given set.

Two public health nurses were employed to offer a generalized public health nursing service to the participants for the duration of the study. The public health nurses visited each participant's home to complete the family and individual record forms. They assisted at bleeding

TABLE 3. OCCURRENCE OF SYMPTOMS BY CLINICAL TYPE AND BY EXPERIMENTAL GROUP DURING THE CONTROL PERIOD

PERIOD	RESPIRATORY		GASTRO-INTESTINAL (DIARRHEA)		FEVER ALONE		OTHER	
	VIRUS	PLACEBO	VIRUS	PLACEBO	VIRUS	PLACEBO	VIRUS	PLACEBO
			CHILDREN(1)					
2/22–2/28				2				
3/ 1*–3/7			1	1				2(4)
3/ 8–3/14		2			1			1(5)
3/15–3/21								
3/22–3/28	1						1(3)	
3/29*–4/4			5					
4/ 5–4/11	2	2						
4/12–4/18	2	1						
4/19–4/22	2							
Totals	7	5	6	3	1	0	1	3
			ADULTS(2)					
2/22–2/28								
3/ 1*–3/7		1						
3/ 8–3/14								
3/15–3/21								
3/22–3/28								
3/29*–4/4	1						1(6)	
4/ 5–4/11		3				1		
4/12–4/18			1					
4/19–4/22							1(6)	
Totals	1	4	1	0	0	1	2	0

* Indicates feeding dates.
(1) Total children in virus group, 60; total in placebo group, 51.
(2) Total adults in virus group, 53; total in placebo group 55.
(3) Pyelitis.
(4) One rash; one case of allergy.
(5) Rash.
(6) Abortion.

clinics, distributed stool specimen containers, and either fed or supervised the feeding of the liquid vaccine or placebo as the case might have been. These activities alone brought them into frequent contact with the participating families. In addition, they were subject to call for consultation on any problem that a family wished to discuss with them. Participants were encouraged to report illnesses or symptoms to the public health nurses.

Liaison was established with the physicians who ordinarily treated these people and who might therefore be consulted for something that occurred during the course of the study. Two physician members of the project team (W.E.M. and H.K.) were also available for consultation.

The above arrangements insured, so far as was possible, that the medical and nursing surveillance would be complete and continuous. The entire project was operated from an office close to the village site, especially rented for this purpose. The project's office telephone was connected in with the switchboard serving the Minnesota Department of Health.

EVALUATION OF SYMPTOMS OCCURRING DURING CONTROL PERIOD

The symptoms which occurred during the control period are set out week by week in Table 3. These data, of course, were recorded without knowing whether the complainant had received vaccine virus or placebo. It appears evident from the meager entries in the table, that for the most part, both adults and children remained well through the control period.

The respiratory symptoms in both adults and children appear to be concentrated in time suggesting that these individuals had picked up something that was "going around". Indeed, several of the entries represent multiple cases in one family. Of the five diarrheas recorded in the virus-fed during the week beginning March 29, four occurred in one family. The symptoms in this family did not occur soon after a feeding. Actually, the second feeding date had to be postponed for these individuals until the bowel disturbances had subsided. Certain of the communicable diseases, scarlet fever and chickenpox, did appear but not until the control period

TABLE 4A. ANTIBODY RESPONSES TO MONOVALENT ORAL POLIOMYELITIS VACCINE, GROVE EAST, 1959
CHILDREN

No. of Salk Doses	Type 1				Type 2				Type 3			
	Initial Titer <4		Initial Titer 4 or >		Initial Titer <4		Initial Titer 4 or >		Initial Titer <4		Initial Titer 4 or >	
	No Change	Conversion	No Change	Booster	No Change	Conversion	No Change	Booster	No Change	Conversion	No Change	Booster
0	0	3	0	1	2	2	0	0	0	1	0	3
1	0	0	0	0	0	0	0	0	0	0	0	0
2	0	15	0	1	0	11	1	4	0	16	0	0
3	0	20	4	7	0	6	7	18	1	16	7	7
4	0	3	3	3	0	1	3	5	0	4	2	3
Totals	0	41	7	12	2	20	11	27	1	37	9	13

TABLE 4B. ANTIBODY RESPONSES TO TRIVALENT ORAL POLIOMYELITIS VACCINE, GROVE EAST, 1959
CHILDREN

No. of Salk Doses	TYPE 1				TYPE 2				TYPE 3			
	Initial Titer < 4		Initial Titer 4 or >		Initial Titer < 4		Initial Titer 4 or >		Initial Titer < 4		Initial Titer 4 or >	
	No Change	Conversion	No Change	Booster	No Change	Conversion	No Change	Booster	No Change	Conversion	No Change	Booster
0	3	2	0	1	4	1	1	0	1	5	0	0
1	0	0	0	0	0	0	0	0	0	0	0	0
2	3	7	0	1	2	4	0	5	1	9	0	1
3	1	16	7	8	3	6	9	14	0	20	5	7
4	0	0	0	2	0	0	1	1	0	1	0	1
Totals	7	25	7	12	9	11	11	20	2	35	5	9

TABLE 5A. ANTIBODY RESPONSES TO MONOVALENT ORAL POLIOMYELITIS VACCINE, GROVE EAST, 1959
ADULTS

No. of Salk Doses	TYPE 1				TYPE 2				TYPE 3			
	Initial Titer ≤4		Initial Titer 4 or >		Initial Titer ≤4		Initial Titer 4 or >		Initial Titer ≤4		Initial Titer 4 or >	
	No Change	Conversion	No Change	Booster	No Change	Conversion	No Change	Booster	No Change	Conversion	No Change	Booster
0	1	1	1	2	1	1	0	3	1	3	1	0
1	1	0	0	1	0	0	2	0	0	0	2	0
2	1	2	12	3	3	2	10	3	4	3	10	1
3	2	5	14	7	0	3	20	5	2	4	18	4
4												
Totals	5	8	27	13	4	6	32	11	7	10	31	5

TABLE 5B. ANTIBODY RESPONSES TO TRIVALENT ORAL POLIOMYELITIS VACCINE, GROVE EAST, 1959
ADULTS

No. of Salk Doses	TYPE 1				TYPE 2				TYPE 3			
	Initial Titer < 4		Initial Titer 4 or >		Initial Titer < 4		Initial Titer 4 or >		Initial Titer < 4		Initial Titer 4 or >	
	No Change	Conversion	No Change	Booster	No Change	Conversion	No Change	Booster	No Change	Conversion	No Change	Booster
0	0	2	2	2	1	2	1	2	1	3	1	1
1	2	0	3	0	1	0	4	0	1	1	1	2
2	1	0	10	1	0	1	9	2	1	2	7	2
3	1	4	20	4	0	1	23	5	0	7	15	7
4	0	0	3	0	0	0	2	1	0	1	1	1
Totals	4	6	38	7	2	4	39	10	3	14	25	13

was over. Both of these specific diseases were common in the surrounding community at that particular time.

Certainly there were no untoward results noted as a consequence of consuming the vaccine viruses. Nor can it be said on the basis of the available data that the vaccine produced any type of "reaction" symptoms.

RESULTS

The results of antibody titrations before and after feeding the monovalent and trivalent vaccines to children are displayed in Tables 4a and 4b. Similar data for adults are in Tables 5a and 5b. The data in these tables are arranged to show conversions to specific antibody types in those who entered the study with titers of less than 1:4 and booster effects in those who began the study with demonstrable antibody to specific types. Those in whom no change occurred are also listed. In addition, the conversion and booster effects are related to the Salk status of the participants. One interesting fact emerges from the tables instantly. A little calculation shows that of those children who entered the study with three or four doses of Salk vaccine, 54 per cent had titers of less than 1:4 for Type 1, 21.6 per cent had titers of less than 1:4 for Type 2; and 56.7 per cent had titers of less than 1:4 for Type 3. It can also be seen how well these deficiencies were corrected in the children with either the monovalent or the trivalent vaccine. No further comment will be made on these tables. They are the master tables, so to speak, and can be studied at leisure.

A summary of these last four tables has been prepared and is presented as Table 6. In this table, the Salk status has not been considered and the figures for those who showed no change have not been included. Children taking the monovalent vaccine converted in the case of Type 1 to the extent of 100 per cent; to Type 2 there was a conversion rate of 90.0 per cent, and to Type 3, 97.4 per cent. Except for Type 3, the conversion rates in children who consumed the trivalent preparation were not quite so good being, for the three types respectively, 78.1 per cent, 55 per cent, and 94.6 per cent. It appears, therefore, that in children the monovalent vaccine is superior to the trivalent although for Type 3, both dosage forms appear to be almost equally effective. The conversion rates for Type 2, following the use of the trivalent preparation, were somewhat disappointing.

In adults, as can be seen, the conversion rates associated with both the monovalent and trivalent vaccines were not so good as the rates recorded for the children. Likewise, there was little difference in the efficiency with which the two vaccine dosage forms converted the participants with respect to Types 1 and 2. For Type 3, the trivalent form appeared to act somewhat better than the monovalent form. The difference, however, turns out to be not statistically significant. The booster effects on titers in those who entered the study with antibody to the various types can be read off from these same tables

At this point, however, a word of restraint is in order. Certain laboratory findings at the Minnesota Department of Health suggest that some of the monovalent vaccine, Type 2, was con-

TABLE 6. ANTIBODY RESPONSES AS CONVERSIONS OR BOOSTER EFFECTS WITH MONOVALENT OR TRIVALENT ORAL POLIOMYELITIS VACCINE, GROVE EAST, 1959

	TYPE 1		TYPE 2		TYPE 3	
	Conversion	Booster	Conversion	Booster	Conversion	Booster
Monovalent						
Children (60)	41/41=100%	12/19=63.2%	20/22=90.9%	27/38=71.1%	37/38=97.4%	13/22=59.1%
Adults (53)	8/13=61.5%	13/40=32.5%	6/10=60%	11/43=25.6%	10/17=58.8%	5/36=13.9%
Trivalent						
Children (51)	25/32=78.1%	12/19=63.2%	11/20=55.0%	20/31=64.5%	35/37=94.6%	9/14=64.3%
Adults (55)	6/10=60%	7/45=15.6%	4/6=66.7%	10/49=20.4%	14/17=82.4%	13/38=34.2%

taminated with Type 1 vaccine virus*. This would have the effect of making some of the first feedings in the monovalent group in reality a bivalent feeding composed of Types 1 and 2 vaccine virus. Therefore, even though the results in the monovalent group are within the compass of expectation; though they do not appear at all bizarre; and though they are in line with previous experience, still, strictly speaking, one cannot say that a comparison between the effects of monovalent versus trivalent vaccine with respect to inducing antibodies to Types 1 and 2 poliovirus has actually been consummated. The comparison with respect to Type 3, however, is completely valid.

Another way to gauge the effectiveness of the vaccine, either monovalent or trivalent, is to measure the degree of success attained in converting to triple antibody positives those, who before ingesting the vaccine, lacked demonstrable antibody to one or more poliovirus types. The data to show this type of effect is entabled for the monovalent group, children and adults, in Tables 7a and 7b. Tables 8a and 8b are similarly applicable to those who received the trivalent vaccine. A summary of these tables appears as Table 9. From this table, it appears evident that conversions to triple positive are better accomplished in children by the monovalent vaccine. On the other hand, to accomplish the same purpose in adults, the trivalent vaccine appears better. The efficiency of the trivalent vaccine in generating triple positives is the same for both adults and children although, as has been mentioned, the monovalent vaccine is better for children.

* A total of 113 participants received the monovalent vaccine. After the Type 2 feeding, Type 1 virus was isolated from 32 specimens; Type 2 was isolated from 11 specimens, and 70 were negative for virus isolation. A single Type 2 isolation occurred in the placebo group. After the Type 1 feeding, there were 43 isolations of Type 1, two of Type 2, and a single Type 3! The placebo group yielded one Type 1 and two Type 2's. Type 1 virus was thus appearing following a Type 2 feeding when it should have been absent. The negative results in the placebo group appeared to exclude a laboratory "pick-up" or an adventitious, wild Type 1. Additional laboratory studies eventually revealed that Type 1 virus was present in four out of the four vials of Type 2 vaccine that were subjected to such a search. The concentration of the Type 1 virus found was of the order of 5 TCD_{50} per ml.

The Lederle Laboratories reported confirmation of the above results, but they also reported that the unfilled pool of Type 2 vaccine was free of Type 1 virus.

Lederle Laboratories, on review of their own protocol kept at the time that the vaccines were being readied for shipment to Minnesota for the Grove East study, reported to the State Health Department the series of events that explained how this incident occurred. The filling machine first was used to fill placebo vials. Then vials were filled with monovalent, Type 1, vaccine with the same machine. Because of the pressure of time, the machine was flushed through with placebo instead of being sterilized and used to fill vials with Type 2 vaccine; the first 200 ml. being discarded. A different machine was used for the Type 3 vaccine.

TABLE 7A. ANTIBODY STATUS, BEFORE AND AFTER VACCINATION, OF THE 23 ADULTS LACKING POLIOVIRUS ANTIBODIES BEFORE INGESTION OF TRIVALENT ORAL POLIOMYELITIS VACCINE, GROVE EAST, 1959

Number of Persons Negative*, by Poliovirus Type										
Pre-feeding		Post-feeding								
Negative to Type	No. of Persons	1	2	3	1 and 2	1 and 3	2 and 3	1, 2 and 3	Total No. of Negatives	No. of Complete Conversions to Triple Positive
1	4	2							2	2
2	2		1						1	1
3	10			1					1	9
1 and 2	0								0	0
1 and 3	4					1			1	3
2 and 3	1								0	1
1, 2, and 3	2							1	1	1
Totals	23	2	1	1		1		1	6	17

* Titer of <1:4.

No. of persons with complete conversion 17/23 or 73.9%.

TABLE 7B. ANTIBODY STATUS, BEFORE AND AFTER VACCINATION, OF THE 45 CHILDREN LACKING POLIOVIRUS ANTIBODIES BEFORE INGESTION OF TRIVALENT ORAL POLIOMYELITIS VACCINE, GROVE EAST, 1959

Number of Persons Negative*, by Poliovirus Type										
Pre-feeding		Post-feeding								
Negative to Type	No. of Persons	1	2	3	1 and 2	1 and 3	2 and 3	1, 2 and 3	Total No. of Negatives	No. of Complete Conversions to Triple Positive
1	5	1							1	4
2	3		2						2	1
3	9								0	9
1 and 2	0								0	0
1 and 3	11								0	11
2 and 3	1								0	1
1,2, and 3	16	2	2		3		1	1	9	7
Totals	45	3	4	0	3	0	1	1	12	33

* Titer of <1:4. No. of persons with complete conversion 33/45 or 73.3%.

2. Observations on Pharyngeal Recovery of Poliovirus and Viremia

During the Grove East study, virus isolation was attempted from one pharyngeal swab collected from each of 66 children. These collections were staggered so that almost equal numbers had specimens taken for each of the days from the second to the eighth day after feeding. Among the 31 children who had received the trivalent vaccine, Type 1 was isolated from four children, Types 1 and 2 from one child, and Type 3 from eight children. The remaining 35 of the 66 children had received monovalent vaccine, in this case, Type 3. This virus type was isolated from the pharynx of three children—in one of them from a sixth day specimen and two from

TABLE 8A. ANTIBODY STATUS, BEFORE AND AFTER VACCINATION, OF THE 29 ADULTS LACKING POLIOVIRUS ANTIBODIES BEFORE INGESTION OF MONOVALENT ORAL POLIOMYELITIS VACCINE GROVE EAST, 1959

Number of Persons Negative*, by Poliovirus Type										
Pre-feeding		Post-feeding								
Negative to Type	No. of Persons	1	2	3	1 and 2	1 and 3	2 and 3	1, 2 and 3	Total No. of Negatives	No. of Complete Conversions to Triple Positive
1	7	4							4	3
2	3		2						2	1
3	10			5					5	5
1 and 2	2	1							1	1
1 and 3	2			1					1	1
2 and 3	3		1	1					2	1
1,2, and 3	2						1		1	1
Totals	29	5	3	7	0	0	1	0	16	13

* Titer of <1:4. No. of persons with complete conversion 13/29 or 44.8%.

TABLE 8B. ANTIBODY STATUS, BEFORE AND AFTER VACCINATION, OF THE 48 CHILDREN LACKING POLIOVIRUS ANTIBODIES BEFORE INGESTION OF MONOVALENT ORAL POLIOMYELITIS VACCINE, GROVE EAST, 1959

Pre-feeding		Number of Persons Negative*, by Poliovirus Type								
		Post-feeding								
Negative to Type	No. of Persons	1	2	3	1 and 2	1 and 3	2 and 3	1, 2 and 3	Total No. of Negatives	No. of Complete Conversions to Triple Positive
1	7								0	7
2	1								0	1
3	5								0	5
1 and 2	2		2						2	0
1 and 3	14			2					2	12
2 and 3	1								0	1
1,2, and 3	18								0	18
Totals	48	0	2	2	0	0	0	0	4	44

* Titer of <1:4. No. of persons with complete conversion 44/48 or 91.6%.

TABLE 9. CONVERSIONS TO TRIPLE POSITIVES IN PERSONS LACKING POLIOVIRUS ANTIBODY TO ONE OR MORE POLIOVIRUS TYPES BEFORE INGESTION OF MONOVALENT AND TRIVALENT VACCINE

	MONOVALENT	TRIVALENT
Adults	13/29 = 44.8%	17/23 = 73.9%
Children	44/48 = 91.6%	33/45 = 73.3%

eighth-day specimens. In general, most recoveries of virus were made from swabs collected on the fifth day. Next in order were three isolations each on the fourth and sixth days. The earliest swabs were taken on the second day after feeding and the latest on the eighth day after feeding.

These and other types of data are collected together in Table 10. For example, it can be seen that in all cases except one where virus was recovered from the pharynx, the pre-feeding titer was less than 1:4 for the homotypic virus. The one exception (case J.D.) had a pre-feeding titer of 1:16 for Type 3; Type 3 virus was recovered from the pharynx and the titer for Type 3 rose to 1:1024 after feeding. Also in this group of 16 cases, there was always a positive effect on the post-feeding antibody titer for the virus type isolated. All 15 cases who had pre-feeding titers of less than 1:4 converted; the one case that began with a titer of 1:16 showed a booster effect of up to a titer of 1:1024. Only one case (D.A.H.) had had a tonsillectomy and adenoidectomy done. All of the 16 in whom virus was demonstrated by pharyngeal swab were well on the day the specimens were collected and remained so.

Among the 50 remaining children from whom pharyngeal swabs were taken but which did not yield virus, there were 27 who had titers of less than 1:4 to Type 3 and 32 who had titers of less than 1:4 for Type 1. For Type 3, 24 of these 27 converted for a percentage of 88.9 and for Type 1, 30 out of the 32 converted for a percentage of 93.8. The finding of virus in the pharynx is therefore not a sine qua non for a conversion response. Or putting it conversely, the inability to demonstrate virus in the pharynx does not preclude a satisfactory antibody response.

TABLE 10. DATA ON PARTICIPANTS FROM WHOM POLIOVIRUS WAS RECOVERED FROM THE PHARYNX CROVE EAST, 1959

Case	Age	Sex	Salk Status	Day After Feeding	Type Isolated	Antibody Titers Before Feeding 1	2	3	After Feeding 1	2	3	T and A
Monovalent (Type 3)												
K.L.D.	3	F	3	8	3	<4	4	<4	64	64	16	No
D.K.	3	F	3	8	3	64	16	<4	1024	1024	64	No
V.L.	2	M	4	6	3	16	64	<4	256	1024	64	No
Trivalent												
K.A.G.	6	M	3	5	3	256	4	<4	1024	256	16	No
D.H.G.	9	M	3	5	3	256	4	<4	1024	64	64	No
G.R.G.	7	M	3	5	3	256	64	<4	1024	1024	16	No
D.A.H.	6	F	3	6	3	256	4	<4	256	16	64	Yes
G.J.H.	4	M	3	6	1	<4	<4	<4	1024	16	16	No
A.L.V.H.	2	M	3	5	3	<4	<4	<4	4	16	16	No
K.M.P.	5	M	3	7	1	<4	4	<4	16	64	16	No
L.C.	2	F	1	3	1	<4	<4	<4	16	64	16	No
L.S.C.	4	M	3	4	1 and 2	<4	<4	<4	16	256	4	No
S.H.	2	F	3	4	3	<4	<4	<4	1024	16	64	No
K.H.	<1	F	0	4	3	<4	<4	<4	<4	<4	16	No
J.O.	2	F	2	5	3	<4	64	16	<4	1024	1024	No
P.R.*	2	M	2	5	1	<4	<4	<4	64	<4	<4	No

* Type 1 Viremia also demonstrated.

Blood specimens for viremia studies were collected at the same time from these same 66 children by pricking the ear lobe and absorbing as much blood as could be collected on a strip of filter paper. The blood-soaked filter paper was placed immediately into monkey-kidney tissue culture tubes. Type 1 virus was isolated from the blood of only one child; from a specimen which had been collected on the fifth day after the ingestion of the trivalent vaccine. Type 1 virus had also been isolated from the pharynx of this child. The child, a two-year old boy, was well when the virus-containing specimen was collected and he continued in good health. Prior to feeding, he was a triple negative and after feeding he had a neutralizing titer to Type 1 poliovirus of 1:64.

LABORATORY METHODS

Antibody titrations. The preparation of tissue-culture tubes and poliovirus pools were the same as described for the Como Village study[1]. The neutralizing antibody test procedure was also the same except that tests were performed using five tissue-culture tubes per serum dilution. Serum antibody titers are reported as the 50 per cent end-point of the serum dilution which neutralized 100 TCD_{50} of the virus, as calculated by the method of Reed and Muench.

Pharyngeal swabs for virus isolation. Cotton tipped swabs were supplied in tubes containing 0.5 ml. of tissue-culture maintenance medium. After use, the swabs were returned to the laboratory in the original tubes in which they had been supplied. Upon receipt in the laboratory, the swabs were immersed in 2 ml. of tissue-culture maintenance medium, and allowed to stand at room temperature for one hour. The swabs were then withdrawn from the fluid and pressed against the side of the tube in order to express as much fluid as possible. Two monkey-kidney tubes, from which growth medium had been re-

moved, were then inoculated with one ml. each of the maintenance medium in which the throat swabs had been immersed. The inoculated tubes were incubated, observed for cytopathogenic effect, and cytopathogenic agents were identified in the usual manner.

Procedure for viremia demonstration. The sterilized ear lobe of the subject was punctured with the point of a number 11 Bard-Parker blade. Two strips of sterile filter paper (the size of litmus paper strips) were saturated with the blood and each strip was then immediately dropped into a monkey-kidney tissue culture tube. Tissue-culture tubes were incubated at 37° C. for seven days and observed daily for cytopathogenic effect. Transfer to a fresh monkey-kidney tissue culture tube was made if cytopathogenic effect was noted or at the end of seven days. The identification of the cytopathogenic agent was made in the usual manner.

COMMENT

Because of the incident mentioned in the section on "Results", one of the intents of this study was not fully realized. One cannot say absolutely that the effects of a trivalent vaccine have been compared with the effects of monovalent vaccines, Type 3 alone excepted. With respect to Type 3 in children, the monovalent, Type 3, vaccine and the trivalent form appear to act equally well—extremely well, in fact. In children, too, even though the results associated with the use of "monovalent vaccines, Types 1 and 2", might appear to be "too good", it is not too brazenly presumptuous to assume that for these types the monovalent and, as it turned out in some instances, a bivalent vaccine did act better than did the trivalent.

However, the trivalent vaccine on the basis of the results reported here, emerges as a promising antigen. This promise is justified when measured by the ability of this preparation to induce antibody conversions to specific poliovirus types as well as when measured by its ability to create triple positives out of those who, pre-feeding, showed a deficiency in antibody to one or more of the poliovirus types. The promise is indeed good enough to submit this type of vaccine to the attention of public health workers as a tool worthy of consideration in the field of preventive medicine.

The data on viremia and recovery of the viruses from the pharynx have been presented factually. There has been a little discussion relative to the relation of virus in the pharynx or blood and the associated antibody response. The important thing is that these phenomena do occur and that when they do the individual involved is subjectively unaware of the event. Likewise, the observer can find no objective clinical evidence to lead him to suspect that these phenomena are occurring.

It is increasingly evident that the attenuated poliovirus does everything that the wild poliovirus does without producing clinical illness. Such an agent, harmless to man, can be used not only to produce immunity against poliomyelitis, but, in addition, such an agent, sentiently used in man, can serve as the means of studying out the detailed pathogenesis of the disease itself.

REFERENCES

1. Barr, Robert N., Bauer, Henry, Kleinman, Herman, Johnson, Eugene A., Martins da Silva, Mauricio, Kimball, Anne C., and Cooney, Marion K.: Use of orally administered live attenuated polioviruses as a vaccine in a community setting: A controlled study. *J. Am. M. Assn.* **170:** 893-905 (June 20) 1959.
2. Cox, Herald R., Cabasso, Victor J., Markham, Floyd S., Moses, Max J., Moyer, Arden W., Roca-García, Manuel, and Ruegsegger, James M.: Immunological response to trivalent oral poliomyelitis vaccine. *Brit. M. J.* **2:** 591-597 (October 3) 1959.
3. Bauer, Henry, Barr, Robert N., Kleinman, Herman, Kimball, Anne C., Cooney, Marion K., Bearman, Jacob E., and Mathey, Wayne E.: Minnesota studies with oral poliomyelitis vaccines: 1. Efficacy of the vaccine in relation to its ingestion before and after food. 2. Observations on pharyngeal recovery of poliovirus. 3. Occurrence of viremia. In preparation.
4. Martins da Silva, Mauricio, McKelvey, John L., Prem, Konald A., Bauer, Henry, Cooney, Marion K., and Johnson, Eugene A.: Studies of orally administered attenuated live virus

poliomyelitis vaccine in newborns and infants under six months. *U. Minn. M. Bull.* **29:** 133-150 (December 15) 1957.

5. Cabasso, Victor J., Jervis, George A., Moyer, Arden W., Roca-García, Manuel, Orsi, Ernest V., and Cox, Herald R.: Cumulative testing experience with consecutive lots of oral poliomyelitis vaccine. *Brit. M. J.* (1959) In press.
6. Kleinman, Herman, Barr, Robert N., Bauer, Henry, Johnson, Eugene A., Martins da Silva, Mauricio, Kimball, Anne C., and Cooney, Marion K.: Field trials with the Lederle-Cox strains of live poliovirus vaccines: A short review. *U. Minn. M. Bull.* **31:** 263-282 (February 15) 1960.
7. Cox, Herald R.: Personal communication.

SIXTH SESSION

WEDNESDAY, 8 JUNE 1960, 2:00 P.M.

Chairman

DR. PIERRE R. LÉPINE

Chief, Virus Research Division

Institut Pasteur

Paris, France

TOPIC III. EFFICACY. (A) LABORATORY EVIDENCE *(continuation)*

(1) ANTIBODY RESPONSE

(c) Influence of Dosage and Regimen

Presentation of Papers by:

Dr. Henry Bauer

Dr. Juan Embil, Jr.

(DISCUSSION)

(2) INTERFERENCE BY HETEROTYPIC POLIOVIRUSES OR BY OTHER VIRUSES

Presentation of Papers by:

Dr. Albert B. Sabin

Dr. Manuel Ramos Alvarez

(DISCUSSION)

TOPIC III. EFFICACY. (B) FIELD EVIDENCE

Presentation of Paper by:

Dr. Marina K. Voroshilova

for

Dr. Mikhail P. Chumakov

(DISCUSSION)

TOPIC III. EFFICACY. (A) LABORATORY EVIDENCE *(continuation)*

12. MINNESOTA STUDIES WITH ORAL POLIOMYELITIS VACCINES

1. Efficacy of the Vaccine in Relation to Its Ingestion Before and After Food. 2. Observations on Pharyngeal Recovery of Poliovirus. 3. Occurrence of Viremia.*

HENRY BAUER, PH.D., ROBERT N. BARR, M.D., HERMAN KLEINMAN, M.D., ANNE C. KIMBALL, PH.D., MARION K. COONEY, M.S., JACOB E. BEARMAN, PH.D., AND WAYNE E. MATHEY, M.D.†

DR. BAUER *(presenting the paper)*:

INTRODUCTION

This is one of a series of studies with the Lederle-Cox oral poliomyelitis virus vaccines being made in Minnesota regarding safety, antibody response, and its relation to dose form, community spread, and course of infection in individuals fed the vaccine.

The purpose of this paper is to report the following: (1) efficacy of the trivalent vaccine in stimulating antibodies in relation to its ingestion before or after food; (2) the presence of virus in the oropharynx as related to the giving of the vaccine as a liquid which bathes the oropharynx or in a gelatin capsule to by-pass the pharynx; and (3) the frequency of the occurrence of viremia.

POPULATION CHARACTERISTICS

The study was conducted in the St. Cloud Reformatory for men because the regulated life of the inmates facilitated the carrying out of this particular study. Also, opportunity for transmission of virus between participants was limited because inmates spent a great deal of each 24 hours as "cell time" in individual cells. In Table 1 are shown the mean age, Salk-vaccine status of the inmates, the vaccine form and dose, as well as the objectives of the study. The mean age of the participants was 23.5 years. Immunization with Salk vaccine was virtually absent.

DESIGN AND OPERATION OF STUDY

The 165 volunteers were divided into five experimental groups, each group comprising one house designated respectively, House A, B, C, D, and E. The five groups are shown in Table 2. Group A was fed liquid vaccine about one hour before lunch and group D was similarly fed one hour after lunch. Group C was fed liquid vaccine in gelatin capsules (to by-pass the pharynx) about one hour after lunch. Group E was also fed vaccine in capsules, but about one hour before lunch. Group B (control group) was given liquid placebo. This group consisted of food handlers and was assigned placebo in order to obviate the spread of virus through food handling activities. In addition to this placebo bloc, placebo feedings were scattered throughout the

* This is one of a series of studies being reported. Supported in part by grants from the Sister Elizabeth Kenny Foundation and the Lederle Laboratories, Division of the American Cyanamid Company, Pearl River, New York.

† Dr. Bauer, Dr. Barr, Dr. Kleinman, Dr. Kimball, and Miss Cooney (Minnesota Department of Health); Dr. Bearman (School of Public Health, University of Minnesota); and Dr. Mathey (formerly Medical Officer, U.S. Public Health Service).

TABLE 1. ST. CLOUD REFORMATORY STUDY, 1959

PARTICIPANTS								VACCINE FORM AND DOSE	MAIN OBJECTIVES
	NO.	MEAN AGE	SALK VACCINE STATUS						
			NONE	ONE DOSE	TWO DOSES	THREE DOSES	FOUR DOSES	NO. UNK.	
Adults all male	165	23.5 years	156	2	–	1	–	6	Liquid—Trivalent 6.1 logs each type	Effect of feedings in relation to meals Pharyngeal recovery of virus Viremia

other four groups. Observation of all groups extended from day "O" through day "28." Stool specimens were collected from each participant on days, —1 or —2, and on day "O" the first blood for antibody titration was collected before vaccine or placebo was given to the participant. Blood specimens for viremia studies and throat swabs for isolation of virus from the pharynx were collected on the third, fifth, and seventh day after ingestion of the placebo or vaccine. Blood collections for viremia studies were made from one half of each group of participants on the third and seventh day, and from all participants on the fifth day after ingestion of vaccine. A second stool specimen was collected from each participant during the period, days 14-21, and blood specimens for antibody titrations were collected on the 28th day after the ingestion of placebo or vaccine. On day 28, which was July 13, all placebo-fed participants were given the liquid vaccine. Some of these received the trivalent vaccine in a single 2 ml. dose and some received each of the virus types in separate vials containing 6.1 logs of virus in 2 ml. of liquid. The individuals receiving the monovalent form of the vaccine drank the individual virus types in rapid succession. Blood for antibody titrations were collected from this group 30 days after ingesting the vaccine.

Only one person on the project team knew the identity of the placebo control and vaccine virus-fed individuals. Although members of the project team responsible for feeding the vaccine knew that group B was receiving placebo, the participants, the reformatory physician, other reformatory personnel, and the laboratory personnel doing the antibody titrations and virus isolation did not have this knowledge. Accordingly, there

TABLE 2. ST. CLOUD REFORMATORY STUDY, 1959

	OPERATING PLAN				
	A	B	C	D	E
CELL HOUSE	(LIQUID (BEFORE LUNCH)		(CAPSULES (AFTER LUNCH)	(LIQUID (AFTER LUNCH)	(CAPSULES (BEFORE LUNCH)
Day —1 and —2	Stool	Stool	Stool	Stool	Stool
Day 0	Blood	Blood	Blood	Blood	Blood
Day 0	Vaccine*	Placebo	Vaccine*	Vaccine*	Vaccine*
Day 3	Throat Swab	Throat Swab	Throat Swab	Throat Swab	Throat Swab
Day 3	Blood on half of participants	Blood (token)	Blood on half of participants	Blood on half of participants	Blood on half of participants
Day 5	Throat Swab	Throat Swab	Throat Swab	Throat Swab	Throat Swab
Day 5	Blood	Blood (token)	Blood	Blood	Blood
Day 7	Throat Swab	Throat Swab	Throat Swab	Throat Swab	Throat Swab
Day 7	Blood on other half of participants	Blood (token)	Blood on other half of participants	Blood on other half of participants	Blood on other half of participants
Day 14—21	Stool	Stool	Stool	Stool	Stool
Day 28	Blood	Blood	Blood	Blood	Blood
Day 28	Vaccine feeding to all placebo-fed participants.				
Day 58	Blood specimen on placebo-fed participants who were fed vaccine on day 28.				

* Some placebo feedings were scattered among the participants in these cell houses.

could not be bias in the interpretation of the symptoms that were reported or in the evaluation of laboratory findings by laboratory personnel. Blood specimens, throat swabs, and stool specimens were coded before being sent to the laboratory for testing. The blood specimens for antibody titrations were assigned numbers from a table of random numbers, and it was impossible for the testing personnel to identify any one specimen as to its individual origin, its set origin or sequence within any given set. However, the blood specimens were so arranged that all specimens from the same person were tested on the same day and in the same test run.

LABORATORY METHODS

Except for antibody titrations, detection of virus in blood and pharynx, the laboratory procedures, and materials used for virus isolation and identification were the same as those described in a previous publication.[1] To determine the antibody titer, six four-fold dilutions of serum and five tissue-culture tubes per dilution were used. Accordingly, a fourfold rise in antibody titer by this procedure was significant. Pharyngeal swabs (cotton tipped) supplied in 0.5 ml. of tissue-culture maintenance medium were used to collect specimens from the pharynx of the participants. The swabs were returned to the laboratory in the original tube on the day collected. Upon receipt in the laboratory, the swabs were immersed in 2 ml. of tissue-culture maintenance medium and allowed to stand at room temperature for one hour. The swabs were then withdrawn from the fluid and pressed against the side of the tube to express as much fluid as possible. Two monkey-tissue culture tubes from which growth medium had been removed were inoculated with 1 ml. each of the maintenance medium in which the pharyngeal swabs had been immersed. Whole blood specimens for viremia studies were collected by venipuncture and brought to the laboratory on the day of collection. The serum was drawn off of the clot and stored at —20° C. Attempts to isolate virus from all specimens were made by inoculating 0.2 ml. of serum into each of two monkey-kidney tissue culture tubes. A passage of tissue-culture fluid to fresh tissue-culture tubes was made from all inoculated tissue-culture tubes which showed cytopathogenic effect and also from those which showed no cytopathogenic effect on the seventh day. In an effort to increase the number of virus isolations, serum specimens from sixty-six persons lacking antibody to one or more types of poliovirus were retested by inoculating 2.0 ml. of serum onto a HeLa cell sheet in a 200 ml. donor bottle containing approximately 7,000,000 HeLa cells. An adsorption period of one hour at room temperature was allowed. The bottles were tilted at 10-minute intervals to distribute the serum over the cell sheet. Following the adsorption period, the serum was removed and 10 ml. of maintenance medium was added. The bottles were incubated at 37° C. for seven days. Microscopic examination of the cell sheet for cytopathogenic effect was made daily. The tissue-culture fluid was removed from the bottle for transfer to HeLa cell tubes when cytopathogenic effect was observed. If no cytopathogenic effect was observed at the end of the seventh day of incubation, the tissue-culture fluid was also transferred.

EVALUATION OF SYMPTOMS DURING THE CONTROL PERIOD

The recording and evaluating of illnesses and complaints during the control period was done by the institution's medical staff. There was neither an increase in the frequency of visits to the infirmary nor were visits made for qualitatively different reasons than usual.

LABORATORY RESULTS

The initial antibody status of participants in the various groups under study are shown in Table 3. An analysis of the data shows that there is no marked variation in the proportion of individuals with single and double antibody titers of less than four. Accordingly, it is considered justifiable to use these groups to study the effect of dosage form on the antibody response. Table 4 shows the number of blanks filled in and the number of persons who converted to triple positives by groups. The per cent of blanks filled in varied from 57.9 to 81.2 per cent. No correlation could be found between type and time of feeding and antibody response. Seventy-three per cent of the Type 1 blanks were filled in, 62

TABLE 3. INITIAL ANTIBODY STATUS OF PARTICIPANTS

ANTIBODY TITER LESS THAN 4, BY TYPES	CELL HOUSE GROUPS						
	A	C	D	E	B CONTROL	A C D E CONTROL	TOTAL
I	3	2	2	3	5	3	18
II	2	5	3	3	2	1	16
III	5	4	10	9	8	13	49
I and II	0	1	1	1	1	1	5
I and III	2	0	1	3	5	5	16
II and III	1	3	1	0	2	3	10
I, II, and III	0	0	2	3	1	2	8
Total persons titer less than 4, one or more types	13	15	20	22	24	28	122
Total persons triple positive	6	4	6	9	10	8	43
TOTAL	19	19	26	31	34	36	165

per cent of the Type 2, and 61 per cent of the Type 3 blanks were filled in. The per cent of persons who converted to triple positive varied from 45 per cent to 78.2 per cent.

Virus was isolated only from the throat swabs collected from persons in Houses A and D who were fed liquid vaccine with the exception that Type 1 virus was isolated from the pharynx of only two persons who swallowed the vaccine in gelatin capsules. These two persons had a pre-vaccine antibody titer of less than four for all three types of virus. Pharyngeal infection was not detected in the placebo control groups. It is quite clear from Table 5 that isolation of virus from the pharynx was much more frequent when the antibody titer was less than four for the viruses isolated. Also, Type 2 virus was isolated from the pharynx less frequently than virus Types 1 and 3. Table 6 is essentially the same as Table 5, except that persons with antibodies to all three types are shown. The presence of antibodies to two or more poliovirus types does not

TABLE 4. ANTIBODY RESPONSES—CONVERSIONS OF BLANKS AND CONVERSION TO TRIPLE POSITIVES

HOUSE	NUMBER FED	TYPE AND TIME OF FEEDING	*CONVERSIONS OF ANTIBODY TITER FROM LESS THAN 4 TO ANY TITER, ONE OR MORE TYPES	**CONVERSION TO TRIPLE POSITIVES
A	19	Liquid before lunch	11/16 = 68.8%	8/13 = 61.5%
C	19	Capsules after lunch	12/19 = 63.2%	9/15 = 60.0%
D	26	Liquid after lunch	16/27 = 59.3%	9/20 = 45.0%
E	31	Capsules before lunch	19/32 = 59.4%	14/22 = 63.6%
Control Group B	34	Liquid after lunch	26/32 = 81.3%	18/23 = 78.3%
Control Group A C D E	36	Liquid after lunch	22/39 = 56.4%	14/28 = 50.0%
Total	165		106/165 = 64.2%	72/121 = 59.5%

BLANKS FILLED, BY TYPES*

I	II	III
34/46 = 73.9%	24/39 = 61.5%	48/80 = 60.0%

* Numerator: blanks filled in.
Denominator: blanks to be filled in.

** Numerator: total persons converted to triple positive.
Denominator: total persons lacking one or more types.

always prevent virus from multiplying in the pharynx. The presence of virus in a stool specimen collected on the fourteenth day after ingestion of the vaccine could not be predicted from the pre-vaccine antibody titer; however, there seemed to be a trend that virus isolation was more frequent when the antibody titer was less than four for the poliovirus type isolated.

A summary of the laboratory results obtained in eight persons for whom viremia was demonstrated is shown in Table 7. Viremia and its association with dose form, time of ingestion of vaccine in relation to lunch, pharyngeal infection, gut infection, pre-vaccine, and post-vaccine antibody titers are all shown in Table 7. Type 1 virus was isolated from seven persons and Type 3 poliovirus from one person. Virus was isolated more frequently from 2.0 ml. of serum than from 0.2 ml. of serum. In the case of specimens Nos. 491, 511, and 576, from which virus was isolated from 0.2 ml. of serum, isolation of virus was attempted from the three specimens from serum dilutions 1:100 and 1:1000. In specimen 491, Type 1 virus was detected in the 1:1000 serum dilution. No virus was detected in diluted serum specimens Nos. 511 and 576. It is interesting to note that specimen 576—which is person 576—reported that he had three doses of Salk vaccine. His antibody titer was less than four for the three poliovirus types. In the majority of instances, the virus isolated from the blood serum was also detected in the oropharynx and the stool specimen. Antibody conversion occurred for the virus isolated in all instances except one. The dosage form, ingestion of the vaccine before or after lunch, appeared to have no relationship to viremia.

As shown in Table 8, the frequency of virus isolation from the blood serum was greater from those persons whose pre-vaccine antibody titer

TABLE 5. VIRUS ISOLATION FROM PHARYNX OF PERSONS WITH PREVACCINE ANTIBODY TITER OF LESS THAN 4 TO ONE OR MORE TYPES. HOUSES A AND D—RECEIVED UNENCAPSULATED VACCINE

TITER OF LESS THAN 4		VIRUS ISOLATED								NUMBER OF PERSONS FROM WHOM VIRUS WAS ISOLATED
TYPE	NUMBER OF PERSONS	I	II	III	I AND II	I AND III	II AND III	I, II, AND III	TOTAL NUMBER OF ISOLATES	
I	5	# X 0 # X 0 # X							8	3
II	5		0						1	1
III	15			# X 0 X 0 X 0 X 0 X 0	X	# X 0 # #			23	7
I and II	1	0							1	1
I and III	3	X 0		# X 0					5	2
II and III	2			X 0 X					3	2
I, II, and III	2		0		# 0 #			X X	13	2
Total	33								54	*18

Summary showing day and number isolated by virus types

	DAY	I	II	III	TOTAL ISOLATED
#	3	8	2	5	15
X	5	8	3	11	22
0	7	6	3	8	17
	Total	22	8	24	54

* Number of persons from whom virus was isolated: 18/33 or 54.5%.

TABLE 6. VIRUS ISOLATION FROM PHARYNX OF PERSONS WITH PREVACCINE ANTIBODY TITER OF 4 OR GREATER TO ONE OR MORE TYPES. HOUSES A AND D—RECEIVED UNENCAPSULATED VACCINE

TITER GREATER THAN 4		VIRUS ISOLATED								NUMBER OF PERSONS FROM WHOM VIRUS WAS ISOLATED
TYPE	NUMBER OF PERSONS	I	II	III	I AND II	I AND III	II AND III	I, II, AND III	TOTAL NUMBER OF ISOLATES	
I	2			X 0 X					3	2
II	3	X 0		# X 0					5	1
III	1	0							1	1
I and II	15			X 0 # X 0 X 0 X 0 X 0	X	# X 0 # #			23	7
I and III	5		0						1	1
II and III	5	# X 0 # X 0 # X							8	3
I, II, and III	12	0	# X 0 #	X X X	0				10	4
Total	43								51	*19

Summary showing day and number isolated by virus types

	DAY	I	II	III	TOTAL ISOLATED
#	3	6	2	5	13
X	5	6	2	12	20
0	7	7	3	8	18
	Total	19	7	25	51

* Number of persons from whom virus isolated: 19/43 or 44.2%.

was less than four to all three types of virus. Type 1 virus was isolated from four of five persons with an antibody titer of less than four to all three types of poliovirus (a ratio of 1:1.25), from two of nine persons with antibody titers of less than four for poliovirus Types 1-2 and 1-3 (a ratio of 1:4.5), and from one of 10 persons with an antibody titer of less than four to Type 1 poliovirus (a ratio of 1:10). Type 1 virus was isolated from the blood serum of seven out of 24 persons (a ratio of 1:3.4) with an antibody titer less than four for Type 1 where this titer occurred singly or in combination with poliovirus Types 2 and 3. Poliovirus Type 3 was isolated from the blood serum of only one out of 44 persons whose antibody titer was less than four for Type 3 singly or in combination with the other two types of poliovirus. No Type 2 virus was detected in the blood serum specimens tested.

DISCUSSION AND CONCLUSIONS

The dose form and the ingestion of trivalent vaccine before or after a meal does not appear to influence the antibody response. The high conversion rate (81.2 per cent) experienced by the participants in House B, the placebo-fed (control group), is unexplainable at the present time. Participants in this house were given unencapsulated vaccine approximately one month later, July 13, than participants in other houses, but at the same time as placebo-fed (control) participants in Houses A, C, D, and E whose antibody conversion rate (57.9 per cent) was comparable to participants who received the vaccine approximately one month earlier. Two persons in House B and two persons among the placebo-fed groups in Houses A, C, D, and E showed an antibody conversion during the control period

TABLE 7. SUMMARY OF LABORATORY RESULTS OBTAINED IN THE 8 PERSONS IN WHOM VIREMIA WAS DEMONSTRATED. A TOTAL OF 95 PERSONS TESTED

SPECIMEN NUMBER	PREVACCINE ANTIBODY TITER			POLIO TYPE ISOLATED FROM SERUM	DAY OF SPECIMEN						POSTVACCINE ANTIBODY TITER			PHARYNGEAL ISOLATE TYPES	STOOL ISOLATION TYPES	DOSE FORM TIME OF DOSE
					SIZE OF INOCULUM AND RESULTS											
					3		5		7							
	I	II	III		0.2 ml	2.0 ml	0.2 ml	2.0 ml	0.2 ml	2.0 ml	I	II	III			
411	<4	4	4	I			−	+	−	+	16	64	64	I	0	Liquid before lunch
444	<4	<4	<4	I	−	+	−	−			<4	<4	4	0	III	Capsule before lunch
491	<4	<4	64	I	−	−	+	+			16	<4	1024	I	I	Liquid after lunch
511	<4	<4	<4	I	+	Not Done	+	Not Done			64	<4	4	I, II, III	I, III	Liquid after lunch
516	<4	16	<4	I	−	+	−	−			4	256	<4	I	I, III	Liquid after lunch
565	16	64	<4	III			−	−	−	+	64	256	4	0	0	Capsule before lunch
576	<4	<4	<4	I			+		−	+	64	<4	<4	I	I	Capsule before lunch
583	<4	<4	<4	I	−	+	−	+			4	256	64	I	I	Capsule before lunch

indicating there was very little spread of virus during the control period.

The pre-vaccine antibody titers measured in this study were in all probability acquired in natural infection since only three of 165 participants gave a history of having had some Salk vaccine.

The data indicates that adults with a mean age of 23 years and with antibody titers encountered in this study could expect, following one dose of the trivalent vaccine used in this study, an antibody conversion rate of 57.9 to 81.2 per cent. Accordingly, it appears that more than one dose of triple vaccine will be necessary to assure that an adult has developed antibodies to all three types of virus. The effectiveness of multiple feedings of trivalent vaccine requires additional study to determine the optimum number of doses which should be routinely administered to the adult population.

There appears to be a relationship of the presence of circulating antibodies and the resistance of the pharynx to infection as indicated by the greater isolation rate from persons lacking antibodies to one or more poliovirus types. This relationship is not absolute since poliovirus was occasionally isolated from the pharynx in the presence of homotypic antibodies.

Because only one stool specimen was collected 14 days after feeding, our evidence of relationship of circulating antibodies to virus isolation is not as distinct, but the same relationship as noted with pharyngeal isolation and presence of antibodies appears to be operative.

Demonstrable viremia is strikingly more frequent in persons with an antibody titer of less than four for all three types than in persons who had antibodies present to one or more types of poliovirus. Of importance from the standpoint of methodology is the increased number, i.e, nine virus isolations when 2 ml. of blood serum was inoculated onto sheets of HeLa cells in 200 ml. bottles as compared to four virus isolations when 0.2 ml. of blood serum was inoculated into each of two monkey-kidney tissue culture tubes. It is possible that if larger quantities of blood serum

TABLE 8. VIREMIA IN RELATION TO PREVACCINE ANTIBODY STATUS

ANTIBODY TITER LESS THAN 4, BY TYPES	NUMBER OF PERSONS	TYPE I VIRUS ISOLATED	RATIO
I, II, and III	5	4	1 : 1.25
I and II I and III	9	2	1 : 4.5
I	10	1	1 : 10
TOTAL	24	7	1 : 3.4

ANTIBODY TITER LESS THAN 4, BY TYPES	NUMBER OF PERSONS	TYPE III VIRUS ISOLATED	RATIO
I, II, and III	5	0	0
I and III II and III	11	0	0
III	28	1	1 : 28
TOTAL	44	1	1 : 44

or perhaps whole laked blood could be used as inocula, a larger number of virus isolations would have been accomplished. No clinical evidence of illness was present at the time of viremia in the eight people from whose blood serum virus was isolated. It is of interest that Type 1 poliovirus was isolated from seven persons and Type 3 virus was isolated only once. In no instance was Type 2 poliovirus isolated from the blood. Whether this represents a strain difference or a type difference remains to be studied. Approximately one and three quarter million people have ingested the oral poliomyelitis vaccine virus strains reported in this study. On the basis of the data presented, it is reasonable to suppose that thousands of persons who have ingested the vaccine have had a viremia with one or perhaps more of the vaccine virus types, yet illnesses among these vaccinees which can be causally associated with the vaccine, if any, have not been proven.

REFERENCE

1. Barr, Robert N., Bauer, Henry, Kleinman, Herman, Johnson, Eugene A., Martins da Silva, Mauricio, Kimball, Anne C., and Cooney, Marion K.: Use of orally administered live attenuated polioviruses as a vaccine in a community setting: A controlled study. *J. Am. M. Assn.* **170**: 893-905 (June 20) 1959.

13. USE OF ATTENUATED LIVE POLIOVIRUS VACCINE IN CUBAN CHILDREN

JUAN EMBIL, JR., M.D., LUIS GERVAIS, M.D., CARLOS HERNÁNDEZ MIYARES, M.D., AND GUSTAVO CARDELLE, M.D.*

DR. EMBIL *(presenting the paper)*: The present summary is a progress report on a series of studies in the oral application of live poliovirus vaccine in Cuban children. More than 10,000 have been vaccinated to date and summaries have been presented as serologic data became available. Four groups were discussed at the First Conference last year [1] and these, together with two additional groups, are the subject of another report.[2] Other groups totalling 360 children, all of whom received a trivalent liquid vaccine prepared from the Lederle strains of attenuated polioviruses, are included in a separate report.[3] The present report concerns three additional groups of children who received vaccine in the form of monovalent capsules and two other groups that were given the trivalent liquid vaccine. The vaccines were provided by Lederle Laboratories of Pearl River, N. Y., and the methods by which the serologic data were obtained have been previously described.[3]

* Dr. Embil (Director, Viral and Rickettsial Research Department, Municipal Children's Hospital, Havana, Cuba); Dr. Gervais (Assistant Director, Viral and Rickettsial Research Department, Municipal Children's Hospital, Havana); Dr. Hernández Miyares (Medical Director, "Colonias Infantiles" of the Republic of Cuba, Havana); and Dr. Cardelle (Pediatrician, Sanatorio Covadonga, Havana).

MONOVALENT VACCINE CAPSULES

Two groups (7 and 8) of children totalling 183, who were residents in a rural recreation camp, were fed monovalent poliovirus vaccine capsules at seven-day intervals in the order Types 2, 3, and 1. The first dose consisted of two capsules of Type 2 virus representing approximately 5.5 logs of virus; the second feeding was two capsules of Type 3 vaccine representing approximately 5.8 logs of virus; and the final feeding was a single capsule of Type 1 virus, roughly 4.8 logs. The children ranged in age from five to 13 years and the median age was between eight and nine years. Blood samples were collected at the time of the initial feeding and again 30 days after the last feeding.

The pre- and post-feeding type-specific seronegatives found in these 183 children are summarized in Table 1. The total seronegative conversion rate was 68 per cent, which is relatively

TABLE 1. MONOVALENT CAPSULES, 7 DAY INTERVAL

SUMMARY OF SEROLOGIC DATA (GROUPS VII AND VIII)

DISTRIBUTION OF SERONEGATIVES BEFORE AND AFTER VACCINATION				SERONEGATIVE CONVERSION RATES		
PRE	PERSONS	SERONEGATIVES PRE	SERONEGATIVES POST			
TYPE I	12	12	10	TYPE I	7/17	41%
TYPE II	5	5	1			
TYPE III	12	12	2	TYPE II	5/6	83%
TYPE I, III	5	10				
TYPE II, III	1	2	—	TYPE III	16/18	89%
	35	41	13	TOTAL	28/41	68%

TABLE 2. MONOVALENT CAPSULES, 15 DAY INTERVAL

SUMMARY OF SEROLOGIC DATA (GROUP XI)

DISTRIBUTION OF SERONEGATIVES BEFORE AND AFTER VACCINATION						
PRE	PERSONS	SERONEGATIVES PRE	SERONEGATIVES POST	SERONEGATIVE CONVERSION RATES		
TYPE I	9	9	3	TYPE I	7/10	70%
TYPE II	1	1	0			
TYPE III	10	10	4	TYPE II	3/3	100%
TYPE I,III	1	2				
TYPE II,III	2	4		TYPE III	9/13	69%
	23	26	7	TOTAL	19/26	73%

low when compared with the rate of 92 per cent for a total of 361 children (groups 3, 5, and 6), who also were fed at seven-day intervals and who were included in a previous report.[2]

The above findings for the seven-day interval feeding may be compared with those in another group (11) of 146 children who were fed monovalent capsules at 15-day intervals. These subjects ranged in age from nine to 23 years with a median age of 13. They were residents of a Cuban equivalent of Father Flannagan's Boystown. The boys were given the same lots of vaccine in the same order and dosage as described above for the seven-day interval feedings. Blood samples were taken at the time of the initial feeding and again 35 days after the final feeding.

The pre- and post-vaccination type-specific seronegatives found in these 146 children are summarized in Table 2. The total seronegative to positive conversion rate was 73 per cent vs. 68 per cent for the seven-day feeding schedule.

TRIVALENT FLUID VACCINE

Two groups (14b and 22) of children were given a single feeding of trivalent fluid poliovirus vaccine which contained approximately $10^{6.1}$ TCD_{50} of each of the three types of poliovirus per dose. The children were from two to 12 years of age and the median was approximately nine years. Only three of the 167 children were under six years of age and all were residents of a

TABLE 3. TRIVALENT LIQUID

SUMMARY OF SEROLOGIC DATA (GROUPS XIV_B AND XXII)

DISTRIBUTION OF SERONEGATIVES BEFORE AND AFTER VACCINATION						
PRE	PERSONS	SERONEGATIVES PRE	SERONEGATIVES POST	SERONEGATIVE CONVERSION RATES		
TYPE I	9	9	1	TYPE I	14/15	93.4%
TYPE II	9	9	8			
TYPE III	9	9	1	TYPE II	6/14	42.8
TYPE I,II	3	6				
TYPE I,III	2	4		TYPE III	12/13	92.3
TYPE II,III	1	2				
TYPE I,II,III	1	3		TOTAL	32/42	76.2
	34	42	10			

recreation camp. Blood samples were collected at the time of vaccination and again seven weeks after vaccination.

The pre- and post-vaccination type-specific seronegatives found in these 167 children are tabulated in Table 3. The seronegative to positive conversion rate for all three poliovirus types was 76 per cent. It thus appears that under our test conditions, the trivalent fluid vaccine was associated with conversion rates which are comparable to those obtained with the monovalent capsule feedings at both the seven- and 15-day intervals.

COMMENT

Of the 496 children included in the monovalent and trivalent vaccine trials described above only 92 were without antibody for one or more types of poliovirus at the time of vaccination. Collectively these 92 children represented a total of 109 type-specific seronegatives, which is indicative of a relatively high rate of natural immunization among the Cuban children under observation. Seronegative conversion rates calculated from data obtained in such a population are liable to considerable variation unless much larger numbers of children are studied than were involved in the present trials. A criterion which is more comprehensive and informative than either conversion rates or geometric mean titers for expressing poliovirus immunity has been suggested and is described in the appendix. This is the seronegative index which is intended to express the relative hazard of paralytic poliomyelitis in an adequately sampled community. It assumes that the number of seronegatives in the study population is indicative of the chances of paralytic disease when virulent strains of virus are introduced. The maximum hazard would be 100 per cent in a population of triple-negatives. Natural infections reduce this maximum hazard progressively so that the ratio of seronegatives to seropositives at any given time is a measure of the poliomyelitis potential. In Table 4 the seronegative indices before and after vaccination are presented for several of the Cuban oral poliovirus vaccination trials.

Here it may be seen that in spite of varying type-specific and total seronegative conversion rates, the net residual poliomyelitis potential in these vaccinated children has been reduced to the low level of an adult population in which paralytic poliomyelitis is most infrequent.

CLINICAL OBSERVATIONS

As we have reported in connection with the trials described earlier, there have been no instances of illness that can be attributed to the vaccine among children who have been fed the attenuated strains of poliovirus in the present trials. There are, however, several observations regarding pre-existing and concurrent conditions that are of interest in relation to the administration of oral poliovirus vaccine.

TABLE 4. SERONEGATIVE INDICES IN VARIOUS POLIOVIRUS VACCINATION TRIALS IN CUBA

TRIAL GROUPS	TYPE VACCINE	FEEDING INTERVAL	NUMBER CHILDREN	TYPE SPECIFIC SERONEGATIVES		SERONEGATIVE INDICES	
				PRE	POST	PRE	POST
7,8	MONO	7	163	41	13	7.46	2.36
3,5,6*	"	7	361	76	6	7.02	0.60
11	"	15	146	26	7	5.9	1.6
4*	"	21-35	105	15	5	4.7	1.6
2*	"	0	62	36	4	19.3	2.1
14a-18**	TRIVAL.	0	360	91	11	8.4	1.0
14b,22	"	0	167	42	10	8.4	2.0
TOTAL			1384	327	56	7.87	1.34

* Previously reported (3).
** Previously reported (4).

Intestinal Parasitism. A parasite survey was conducted in one of the recreation camps and the findings of this study have been correlated with the poliovirus antibody studies. Among 22 children who carried *Giardia lamblia* and were undergoing atabrine treatment there were three type-specific seronegatives for Type 1, of whom two became positive, and one seronegative for Type 3, who also converted. Of the remaining 18 *Giardia*-infested children, all of whom were triple-positives, 17 experienced four-fold or greater booster responses for one or more types of poliovirus.

Sixty-seven of the children were infested with *Trichuris trichiura, Ascaris lumbricoides,* or *Endamoeba coli,* and various combinations of these parasites. In this group of children there was a total of 16 type-specific seronegatives including all three virus types, and 13 of the 16 converted: six for Type 1, six for Type 3, and one for Type 2; one seronegative for each virus type failed to convert. Among the remaining triple-positive children who were parasitized, all but 12 manifested four-fold or greater booster responses to one or more types of poliovirus.

Mumps. Seventeen children were given oral poliovirus vaccine while undergoing an attack of infectious parotitis. Among them were one child who was negative for Type 1 and another who was negative for Type 3 polioviruses; both converted. Of the remaining 15 triple-positive children, eight manifested booster responses to one or more poliovirus types.

Influenza. There was a single type-specific seronegative among six children who suffered attacks of influenza during the oral vaccination program. This single Type 3 seronegative converted and three of the other five children showed booster responses for one or more types of virus.

Herpangina. During the winter of 1959-1960, a number of clinically typical cases of herpangina were admitted to the Sanatorio Covadonga, of Havana. When afebrile but while ulcers were still present on the anterior pillars, five of these children, whose ages varied from 14 months to five years, were fed a dose of trivalent oral poliovirus vaccine after the collection of stool and blood specimens. Laboratory examination of stools is not yet completed. To date, three non-polio enteric viruses, as yet unidentified, have been isolated either in day-old mice or in tissue culture from the pre-vaccination stools and Type 3 poliovirus has been recovered from several of the post-vaccination stools.

The serologic evidence obtained from the pre- and post-vaccination sera of four of the five children, of whom one was a triple negative, shows that six of the seven type-specific seronegatives converted and a booster response from 1:64 to 1:1024 was shown in one of the children. The triple-negative child converted for all three virus types.

When the blood and stool investigations associated with these children are completed and the non-polio enteric viruses identified, this study will be the subject of a separate report.

DISCUSSION

During the past two years, we have administered the Lederle attenuated poliovirus strains to approximately 10,000 children in various parts of Cuba. The bulk of these children were between six and 12 years of age. Our studies have included the administration of the vaccine in capsular and fluid form. Monovalent vaccine has been given at intervals of seven, 15, and 21 days, and simultaneously. Trivalent liquid vaccine has been given in a single dose. There have been no instances of nervous-system disease among any of these vaccinated children and we have observed no unfavorable side effects attributable to vaccination. On the other hand, in spite of nutritional problems, heavy parasitic infestations, and concurrent virus infections including rubeola, rubella, varicella, influenza, mumps, and herpangina, the responses in these children have been good and none of these various complicating conditions has either been augmented by vaccination or been shown to contraindicate vaccination.

The speed, simplicity, and effectiveness of the trivalent vaccination under Cuban conditions obviously makes it the method of choice. By reducing the residual collective poliomyelitis susceptibility of children to a level comparable to that of the adult population, as indicated by the seronegative index, we believe that poliomyelitis as a public health problem in Cuba will disappear.

APPENDIX

THE SERONEGATIVE INDEX: A METHOD OF EXPRESSING POLIOVIRUS IMMUNE STATUS

$$\frac{\text{Observed total of type specific seronegatives}}{\text{Number in population sample X3}} \times 100 = \text{Seronegative Index}$$

Theoretically, each individual is a potential victim for paralytic attack by each of the three types of poliovirus. Hence, the maximum number of chances of paralytic disease in a population is three times the number of persons in the group. The actual number of seronegatives observed in the population sample is a measure of the extent to which the maximum hazard has been reduced by natural exposure and infection. Thus, in dividing the observed number of type-specific seronegatives by the theoretical maximum and multiplying the result by 100, one obtains the per cent of the theoretical number of paralytic chances which still remain in a community that has been adequately sampled. A convenient term for the figure thus obtained is the seronegative index.

Epidemiologically, such an index conveys more information about the population under investigation than the geometric mean serologic titer which varies with antibody levels, but does not indicate concisely the changes in the number of susceptibles. Conversion rates, on the other hand, do not by themselves reveal the relationship of the seronegatives to the immune status of the community as a whole.

The seronegative index affords a simple means of comparing populations, age or economic groups and methods of immunization with one another. It may also prove useful in determining when and where to initiate immunization measures. For example, in Dr. Cox's report [3] on 933 Lederle employees who received the trivalent vaccine, there were 742 type-specific seronegatives in the pre- and 88 in the post-vaccination specimens. The pre- and post-vaccination indices based on these data are 26.5 and 3.14. These indices may be compared with those based on the pre-vaccination serologic data reported from Medellín, Colombia in 1959 by Dr. Abad Gómez and his co-workers [4] at the First International Conference on Live Poliovirus Vaccines. The data and indices are tabulated by age below.

PRE-VACCINATION

Poliovirus Seronegative Indices by Age in Inter-epidemic Medellín, Colombia, 1959

AGE IN YEARS	NUMBER PERSONS	SERONEGATIVES 1	2	3	TOTAL NEGATIVES	SERONEGATIVE INDEX
<1	41	26	33	38	97	79.0
1	66	35	49	44	128	64.6
2	91	41	47	49	137	50.0
3	84	22	31	32	85	33.7
4	96	15	21	22	58	20.1
5 to 9	389	34	49	50	133	11.4
10 to 15	147	5	7	15	27	6.1
Cord-bloods	133	18	15	29	62	14.0

F. S. MARKHAM

REFERENCES

1. Embil, Jr., J., Castellanos, A., and Martín Jiménez, R.: A clinical and serological study of the responses of Cuban children to oral vaccination with attenuated poliovirus vaccines. In: *Live Poliovirus Vaccines, First International Conference.* Scient. Pub. no. 44 of the Pan American Health Organization, pp. 593-617, 1959.
2. ————: Response of Cuban children to oral vaccination with living attenuated poliovirus vaccines—A clinical and serological study. *Brit. M. J. 1:* 1157-1162 (April 16) 1960.
3. Cox, H. R., Cabasso, V. J., Embil, Jr., J., Markham, F. S., Moses, M. J., Moyer, A. W., Roca-García, M., and Ruegsegger, J. M.: Recent experience with the Lederle trivalent oral poliomyelitis vaccine. (See this volume pp. 330-340).
4. Abad Gómez, H., Gaviria, D., Piedrahita, F., Caldós, M., and Martins da Silva, M.: Vaccination of 133,000 children under 10 years of age with live attenuated poliovirus in Medellín, Colombia — Preliminary report. In: *Live Poliovirus Vaccines, First International Conference.* Scient. Pub. no. 44 of the Pan American Health Organization, pp. 458-463, 1959.

DISCUSSION

CHAIRMAN LÉPINE: The two papers given at the close of the fifth session and the two just presented are now open for discussion.

DR. PAUL: These reports have been valuable in bringing out certain points as to how one may hope to evaluate the significance of the results.

Perhaps this is not exactly the time and place, but I am thinking of those unfortunate people to whom the task has been assigned of setting standards of effectiveness for an oral vaccine and for the measurement of the viremia it might produce.

We note that there are two results available here derived from testing: (a) a small amount of blood (0.2 ml.) and (b) a larger amount of blood. In our own laboratory we have tested over 100 samples with monovalent and triple vaccines made from the Sabin strains, using the smaller amount of blood—with negative results. But I do not believe that our results could be called significant.

In the assessment of antibody responses, I may have misunderstood Dr. Cox's questions as to whether or not a four-fold increase in antibody represents a rise from a titer of less than 1:4, or whether it is from less than 1:8 or 1:16. It is difficult for me to regard the former as a four-fold increase. Sooner or later we should all settle on a standard as to what is a four-fold increase.

Another question in these results concerns the matter of heterotypic antibody responses, which might be resolved, as Dr. Sabin has long preached, by confining the tests to the use of triple-negative subjects on which to assess the "efficacy" of the vaccine. Perhaps all of these things deserve some joint consideration as we try to evaluate what has been presented to us today. In any event, the new data are of great value.

CHAIRMAN LÉPINE: Dr. Cox, do you wish to answer Dr. Paul?

DR. COX: I do have correlation square data on all the tables that I showed. Perhaps Dr. Paul did not comprehend my remarks, that we considered positive conversions to be those which shifted from less than 1:4 to 1:4 or greater. We considered four-fold increases or greater as boosters. We do not take into consideration the dilution factor, such as Dr. Gelfand and his workers have reported. In recording our data, we use only the original serum dilutions, such as 1:4, 1:16, 1:64, 1:256, etc.

If Dr. Paul would like to see the actual data concerning the conversion square charts, I have these slides with me and would be very happy to make them available.

DR. PLOTKIN: I should like to ask Dr. Kleinman two questions.

First, what would be his opinion concerning the relative value of monovalent and trivalent vaccine fed to triple-negative infants or children?

Second, how many of the women in his study were pregnant at the time of virus feeding? This question is in relation to the two abortions which he reported. Were these two women excreting virus at the time of the abortions? Was any attempt made to isolate virus from the aborted fetus?

CHAIRMAN LÉPINE: The question will be answered subsequently.

Dr. Skovránek.

DR. SKOVRÁNEK: I should like to say a few words in connection with Dr. Kleinman's and Dr. Bauer's paper dealing with viremia, after the feeding of attenuated polioviruses.

In Czechoslovakia, as a part of the study concerning some problems of immunization of infants with live poliovirus, an attempt was made to demonstrate the appearance of virus in blood after feeding different doses of Type 1 attenuated poliovirus (Sabin, LSc virus).

For this study, a total of 46 infants were selected from five children's homes; 42 of these were triple negatives aged five to 10 months. Consecutive blood and stool specimens, and throat swabs were taken from each child.

Blood specimens were taken as follows: (1) immediately before feeding with virus; (2) the second specimen was drawn about six to eight hours after administration of virus; (3) eight additional samples were taken at approximately 12-hour intervals, on four and a half to five consecutive days; (4) four to six weeks after the Type 1 virus was received, further blood specimens were drawn from each child, who was then immediately fed Type 3 attenuated poliovirus.

Blood samples of 5 to 6 ml. were drawn from head veins of the children. The serum and the clot were investigated separately. If necessary, Dr. Vonka can give more details about the method used.

The available results show that no virus was found in any blood sample taken in the course of four and a half to five days after feeding with about one million TCD_{50} of LSc attenuated poliovirus strain.

More detailed information on this investigation may be obtained from Zácek and his co-workers in our Institute for Sera and Vaccines in Prague. The results will be published as soon as possible.

As you can see, there is evidence of a marked difference between the results presented by Dr. Kleinman and our results. However, it may be because we finished the samplings too early.

Finally, as an officer of the Public Health Service, I should like to reiterate the philosophical remark of Dr. Kleinman, who is also a public health officer: that the main purpose in this study of viremia is to determine not whether it occurs or not, but whether it is harmless for human beings or not.

DR. KLEINMAN: In reply to Dr. Plotkin's question, I am sure he knows that on the basis of our study we cannot say definitely whether the monovalent is better than the trivalent vaccine. For Type 3, there appears to be little difference in the efficiency of a monovalent preparation as compared to a trivalent preparation.

The point that I wanted to make, from the public health standpoint, was that the trivalent preparation does appear to be a promising public health and immunological tool, with its greatest deficiency appearing in response to the Type 2 component.

I am sorry I do not have the answer to Dr. Plotkin's second question. I regret that I do not have my original data sheets with me, so I cannot say exactly how many of the women were pregnant during this study. As I recall, it was certainly not over one dozen. It is very difficult to estimate at any one point how many given women are pregnant. They do not always tell you; they do not always know. Likewise, because of the lack of these data sheets, I do not know whether the women were excreting or not. As far as I know, no attempt was made to isolate virus from the products of conception.

DR. PLOTKIN: The reason for my question was that we have been unable, in triple-negative infants, to obtain uniform success with trivalent vaccine in contrast to success with monovalent vaccine, admittedly with different strains. However, and this point has been implied by Dr. Paul already, when one looks at the data of Dr. Cox and Dr. Kleinman, the response of those who were triple negative before ingestion of trivalent vaccine is certainly inferior to the results in the same type of people using monovalent vaccine.

For example, in Dr. Cox's paper there was conversion from triple negative to triple positive in 49 per cent of triple negatives. In Dr. Kleinman's study, whereas children fed monovalent vaccines converted in 18 out of 18 cases, those fed trivalent vaccine converted to triple positive in only seven out of 16 cases. Consequently, I think that there is a difference between monovalent and trivalent vaccine and that the difference can be shown by isolating the triple-negative children from the others.

DR. PREM: Yesterday I reviewed the data I have concerning the three abortions that I reported among the pregnant women who had been vaccinated with either monovalent or trivalent vaccine. Two of these three were women who were reported in Dr. Kleinman's study presented at the fifth session.

Two of these three women had negative antibody titers before feeding. They responded to feeding with significant antibody increases. This negative pre-feeding status suggests that virus excretion in the stools and viremia did occur if the viremia data presented by Dr. Bauer can be applied—that is, viremia is present pre-

dominantly in those individuals who have no measurable antibody titer before feeding.

DR. MELNICK: I should like to ask the people who have discovered viremia in their vaccinees whether they have done any studies on the virus recovered from the blood, to see whether or not it differs in any way from the virus originally present in the vaccine.

DR. SABIN: I have previously reported that in studies on three strains, viremia was detected in only two persons with the Type 2, P-712, Ch,2ab strain. In those two, the virus that was isolated from the blood was tested intracerebrally in monkeys using very large doses; as regards neurovirulence, at least, there was no difference in the properties.

DR. MURRAY: I wish to add one comment to what Dr. Paul said earlier. This concerns our evaluation of antibody rises in individuals who already have antibody.

In the end, I think the definitive analysis of the serological effectiveness of these vaccines must depend upon the results with triple negatives, or at least homotypic negatives. The dynamics—and even the phenomena themselves—of the rises in those who already have antibody are different, and it is very difficult to equate these.

I have just one additional comment on this question of four-fold antibody rises. Presumably, this is selected to eliminate experimental variation in individual tests. I think that by doing so, if we can neglect the falloff of the antibody during the interval the vaccine was being administered, we may actually be introducing some bias in an upward direction unless we correct it for the number of antibody falls as well.

We have noticed that in tables which have been presented here there are a number of falloffs which are 1/8. I saw a few of 1/16 and even 1/32. This bias may not be important in demonstrating a trend but certainly it may be of some importance when we come to calculate exact percentages.

DR. BODIAN: I think this point is important enough so that it should perhaps be stated in several ways. It seems to me that the possibility of heterotypic responses in all of those individuals who were discussed in relation to conversion this morning has to be taken into account, and certainly the low rate of conversion in triple negatives leads to the suspicion that many of the conversions which were included were transient heterotypic responses. So I think that Dr. Cox ought to clarify the assumption about the rate of conversion when serum is taken at one month, when in fact his results in triple negatives suggest that some of those conversions were fictitious.

DR. LANGMUIR: I should like to refer briefly to Dr. Cox's paper in connection with the data he presented on the proportion of sero-negatives to type in relation to Salk vaccination for his rather unusual population, namely, a large group of adults, some 600 I believe, and a small group of children, about 98, I believe, of Lederle employees.

The laboratory tests shown are the result of two phenomena: immunization with Salk vaccine and natural immunity. I do not believe this is a valid table until it is broken down by age groups.

Also, he raised the point of the 13 per cent Type 1 seronegatives, and pointed out how close this was to the proportion of vaccine failures that we have reported in the United States this year. Again, this must be carefully related to age. I do not think any blanket over-all comparison is valid.

DR. HORSTMANN: While Dr. Cox is answering the questions, I wonder if he would tell us exactly what he means by a "booster" effect? Does he imply true infection in this case and, if so, how does he rule out heterotypic antibody responses?

DR. COX: In the tables I showed this morning, we did not carry out excretion studies. Accordingly, our data represent purely a serological study.

It is true that we do not have the data on the above children to present to you, although we are in the process of carrying out studies along these lines.

I do have available conversion square charts, if anyone wishes to see them, showing how the booster responses were calculated on the basis of serological results.

There were a few persons whose antibody titers did drop, but the great majority showed an antibody rise of fourfold or greater. In calculating booster effect, we counted only antibody increases of fourfold or greater. As I explained previously, our tests were carried out using two tubes per dilution and fourfold dilutions.

I think that our tests are, with minor differences, rather comparable to the New Haven test. We count only the original serum dilution in recording our results. We now have studies in progress correlating virus excretion with antibody rise.

If you wish to see the conversion charts, I shall be glad to have them projected, provided the Chairman wishes to do so.

At this point I should like to ask Dr. Langmuir how he accounts for the fact that 47 per cent of all paralytic cases in Massachusetts last year occurred in triple vaccinates. I think that is something that seriously needs to be considered and explained. How does he explain the fact that 47 per cent of all paralytic cases in Massachusetts in 1959 had received three or more injections of Salk vaccine? Most of the cases were due to Type 3.

I think that there is an immunological explanation for this occurrence which I reported on in 1954 and I believe that there may be a valid explanation for this occurrence.

DR. LANGMUIR: There is no question that the Massachusetts experience in 1959, in my judgment at least, must be classed as a vaccine failure. This is a unique experience in that the cases of polio, 137 paralytic cases, have occurred across the state in an amazingly uniform manner without apparent concentration. Out of 55 virus isolations from these cases, 51 have been Type 3. We have no comparable pattern anywhere else in the country, either in 1959 or in the past three years. Polio elsewhere has been very largely Type 1 and very largely concentrated in unimmunized, residual, isolated ethnically, and distinct groups in several cities of the country.

The most logical explanation that I know of for the Massachusetts story is that during the period when Massachusetts was actively engaged in its early immunization program—which was different from that of almost all other states of the country because that state was late in embarking on this program—there may well have been issued vaccines of somewhat lower potency because in Type 3, potency was a very real problem for a certain period of time. We are now investigating this, but whether or not we can identify the actual lot numbers given in 1956 and 1957 is still somewhat problematic.

DR. COX: I am not claiming that I can prove the facts causing this occurrence, but I should like to quote from a report that I published in 1954, in which I stated that "extreme caution must be taken to prove that a killed vaccine contains a sufficient amount of antigen to make it a good immunizing agent, rather than a sensitizing agent."

In studies carried out with killed lymphocytic choriomeningitis, Rocky Mountain spotted fever, and epidemic typhus vaccines, as well as Japanese B vaccines, we found that preparations which do not contain enough antigen to produce a good immunogenic vaccine actually sensitized the vaccinated animals and made them more susceptible to challenge than the non-vaccinated controls. We actually experienced this for a period of about one year when I was in the Public Health Service, developing the spotted fever and typhus vaccines. Later on we found the same thing to be true, at Lederle, with killed Japanese B vaccines.

Frankly, I do not know of any good killed vaccine that is a good immunizing agent, unless it has 10^8 virus particles (one hundred million), and unless it is Rocky Mountain spotted fever. Thus, if adequate quantitative studies are not carried out in proposed killed vaccine preparations, more harm than good could possibly result from their use. I do not say that this is the explanation for the Massachusetts experience. However, it should be kept in mind.

CHAIRMAN LÉPINE: Thank you Dr. Cox. I suggest that we return to the question of live virus vaccine.

DR. BODIAN: I should like to ask Dr. Cox if he agrees that some of the conversions that he was tabulating could have been due to transient heterotypic responses.

DR. COX: I believe I stated in my paper that there was no doubt that good antibody responses were obtained against Types 1 and 3, or against Types 1 and 3 combined. However, the poorest conversion rates for Type 2 were obtained in the triple negatives. We have made no claim that all the Type 2 responses were entirely due to the feeding of the Type 2 component. All we have done is show the results we obtained serologically on blood taken anywhere from four to seven weeks later. These people are still available for follow-up studies. Of course, it is quite difficult to claim definitely that they may not have had wild virus booster infections in the meantime.

If this is a problem, we know that if you feed trivalent vaccine twice you fill in some of the unfilled antibody gaps that were missed on the first feeding. So we do not make the claim that we can completely immunize the entire population with a single feeding.

However, we are firmly convinced that the trivalent vaccine has much merit. People get broader antibody coverage across the board when they are fed trivalent vaccine than when they are fed monovalent vaccines. Like everything else that has ever been done, improvements are made as time goes on. At the present time, we believe that trivalent vaccine fed on two occasions would do a better job than trivalent vaccine fed a single time. However, this is not the time or place to argue about this; I believe this discussion can be carried on at some other time.

DR. VAN ROOYEN: I have listened with great interest to the criteria of conversion as indicated by antibody response. When an individual is vaccinated against smallpox, nobody carries out antibody titration to determine whether or not the individual is successfully vaccinated. It is customary to observe the lesion and to satisfy oneself whether or not the individual is protected according to the dermatological response.

In poliomyelitis, I believe that infection of the gut takes place, and subsequently, the receptivity or otherwise of the intestinal wall decides whether or not that particular section of gut has been immunized against wild virus.

Speaking for myself, I feel that the capacity of the intestine to reject wild virus is a better yardstick of immunity than the serological antibody response, particularly in adults.

The other point I should like to make is that Dr. Cox probably is going to receive much advice on how to prepare his own vaccine and to this I should like to add still more. As far as Nova Scotia, Canada, is concerned, the three strains of Cox virus as now present in the vaccine are well balanced to suit our area, where a high percentage of Type 2 antibody prevails among the population. Therefore, I feel that these agents should be harnessed and fed together as a team, so that each virus type could be encouraged to proliferate in the gut, in approximately the same relative proportion as its epidemiological distribution. Thus, the best advice I can give Dr. Cox is to leave the present Cox vaccine unchanged.

The third point I wish to make is to ask Dr. Langmuir if he would deal with another unique situation, namely, if he could explain why some 25 per cent of a total of 147 paralytic cases of poliomyelitis, due to Type 1 in Newfoundland, occurred in young children who had received three doses of Salk vaccine.

DR. LANGMUIR: We had a large number of triple vaccinated cases and even a small proportion, roughly 3 per cent of our reported cases, quadruply vaccinated in this country. Except for the Massachusetts story, these have been concentrated in crowded slums, isolated, ethnically distinct groups, the groups consistently throughout the country least well vaccinated. I suspect that the same thing was true in Newfoundland, that you had a sharp epidemic in unvaccinated population groups if it was a Type 1 epidemic.

If we take surveys, such as in the Des Moines epidemic, which was studied most intensively, we can relate the cases and get specific attack rates by vaccine doses and by social economic class. From this we can get corrected estimates of effectiveness which indicate that Salk vaccine, three or more doses, as given over the past four years in this country, is in the range of 80 per cent effective. This I believe is a conservative estimate.

I cannot yet give a carefully evaluated estimate of the effectiveness of four doses, but in my judgment, I believe it will come out in the range of 90 per cent or more.

I also am well aware that much of the vaccine that was distributed over the period from 1956 to 1958 was not as high in its potency as that which is now being generally distributed. So, without question, we have had a very real period of growing pains in the development of the Salk vaccine program in this country.

I suspect that the answer for Dr. Van Rooyen is again the question: How potent was the vaccine used and what was the distribution of cases in relationship to the vaccination in the population?

DR. ANDERSON: Dr. Langmuir made a statement regarding the concentration of cases in certain ethnic and various other adjective-described groups. Without questioning the fact that a number of episodes of that kind have occurred, I do not think we should leave the impression that they have been confined to that group. We had, for example, in Minnesota last year, over 200 cases of paralytic polio (the isolations that were made were Type 1 virus), 22 per cent of which were in persons who had had three or four doses of Salk vaccine. There was no concentration in ethnic or socio-economic groups. It was a cross-section of the community. I am certain that this holds in a great many other places.

DR. VAN ROOYEN: I must reply to Dr. Langmuir's statement. Newfoundland is not a slum area. The country tends to be underpopulated rather than overpopulated. With regard to Dr. Langmuir's figures regarding the efficacy of Salk vaccine and the incidence of paralysis in those who have received three and four shots of vaccine, I wonder whether this difference between three doses and four doses of Salk vaccine is not due to bias in the judgment of physicians who are reluctant to make a clinical diagnosis of poliomyelitis (without isolation of virus) in any child who has received three or four doses of Salk vaccine.

I would suggest that in the compilation of data on paralytic poliomyelitis Dr. Langmuir introduce two further data columns, one stating if virus was isolated and the other if the clinician was aware of the Salk vaccine status of the patient before making a clinical diagnosis.

CHAIRMAN LÉPINE: We shall now proceed with Dr. Sabin's paper on the "Effects of Rapid Mass Immunization of a Population with Live, Oral Poliovirus Vaccine under Conditions of Massive Enteric Infection with other Viruses." This will be followed by Dr. Ramos Alvarez's paper on the "Use of Sabin's Live Poliovirus Vaccine in Mexico. Results of a Large-Scale Trial."

14. EFFECTS OF RAPID MASS IMMUNIZATION OF A POPULATION WITH LIVE, ORAL POLIOVIRUS VACCINE UNDER CONDITIONS OF MASSIVE ENTERIC INFECTION WITH OTHER VIRUSES*

ALBERT B. SABIN
AND
M. RAMOS ALVAREZ, J. ALVAREZ AMÉZQUITA, W. PELON, R. H. MICHAELS, I. SPIGLAND, M. KOCH, J. BARNES, AND J. RHIM

The Children's Hospital Research Foundation, University of Cincinnati College of Medicine, Cincinnati, Ohio; The Children's Hospital, Mexico City, Mexico; and the Ministry of Public Health and Welfare of Mexico

DR. SABIN *(presenting the paper)*: In the absence of enteric infection with other viruses, the feeding of a single type of live poliovirus vaccine to children, who had not previously been infected with the same type of poliovirus, usually results in viral multiplication in the intestinal tract, antibody formation, and complete or partial resistance to reinfection. The use of live poliovirus vaccine in areas with climatic and hygienic conditions which permit extensive dissemination of naturally occurring polioviruses and other enteric viruses throughout the year has been complicated by the problem of viral interference,[1, 2] which we have attempted to overcome by the tactics to be described in this communication. The studies of Ramos Alvarez and his associates[3] in Mexico and of Plotkin and Koprowski[4] in the Belgian Congo, have shown that vaccination programs in large cities that are spread out over a period of many weeks and include only a small fraction of the susceptible population, not only fail to immunize a considerable proportion of the vaccinated children but also have little or no influence on the dissemination of the ever-present, naturally occurring paralytogenic strains of poliovirus. Accordingly, it seemed necessary to determine whether or not at least temporary dominance over the naturally occurring polioviruses and other interfering enteric viruses could be achieved by feeding a mixture of all three types of poliovirus vaccine to almost all susceptible children within a period of a few days. The reason for using a trivalent mixture instead of feeding the three types separately at intervals of four to six weeks, which I regard as optimum during the cold months of the year in temperate zones, was to implant all three types of the vaccine strains in the largest number of children, and then let nature help in their further dissemination in the community.

This study was carried out in Toluca, Mexico, in August 1959. Dr. Ramos Alvarez and his associates in Mexico were responsible for the field work and the collection of specimens, and all the virologic and serologic work was carried out in Cincinnati. Toluca, located at about 19° N. latitude, has a total population of about 100,000. A serologic survey of the population just before initiation of the vaccine program indicated that 90 to 100 per cent of the children become immune to all three types of poliovirus during the first four years of life (Fig. 1). According to available data for the past five years, this city has been paying an average price of 14 paralytic cases per year for this naturally acquired immunity. Twenty cases of paralytic poliomyelitis had already been reported in 1959, when our study began during the second week of August. Rectal swabbings from 1,892 persons —newborn to 10 years of age and mothers—obtained at random in different parts of the city within a few days before the vaccination program, were each tested in monkey kidney and human epithelioma (Hep.-2) tissue cultures, as well as in newborn mice. The results, summarized in Fig. 2, indicate that the special methods used revealed a much higher incidence of natural

* This study was aided by a grant from The National Foundation.

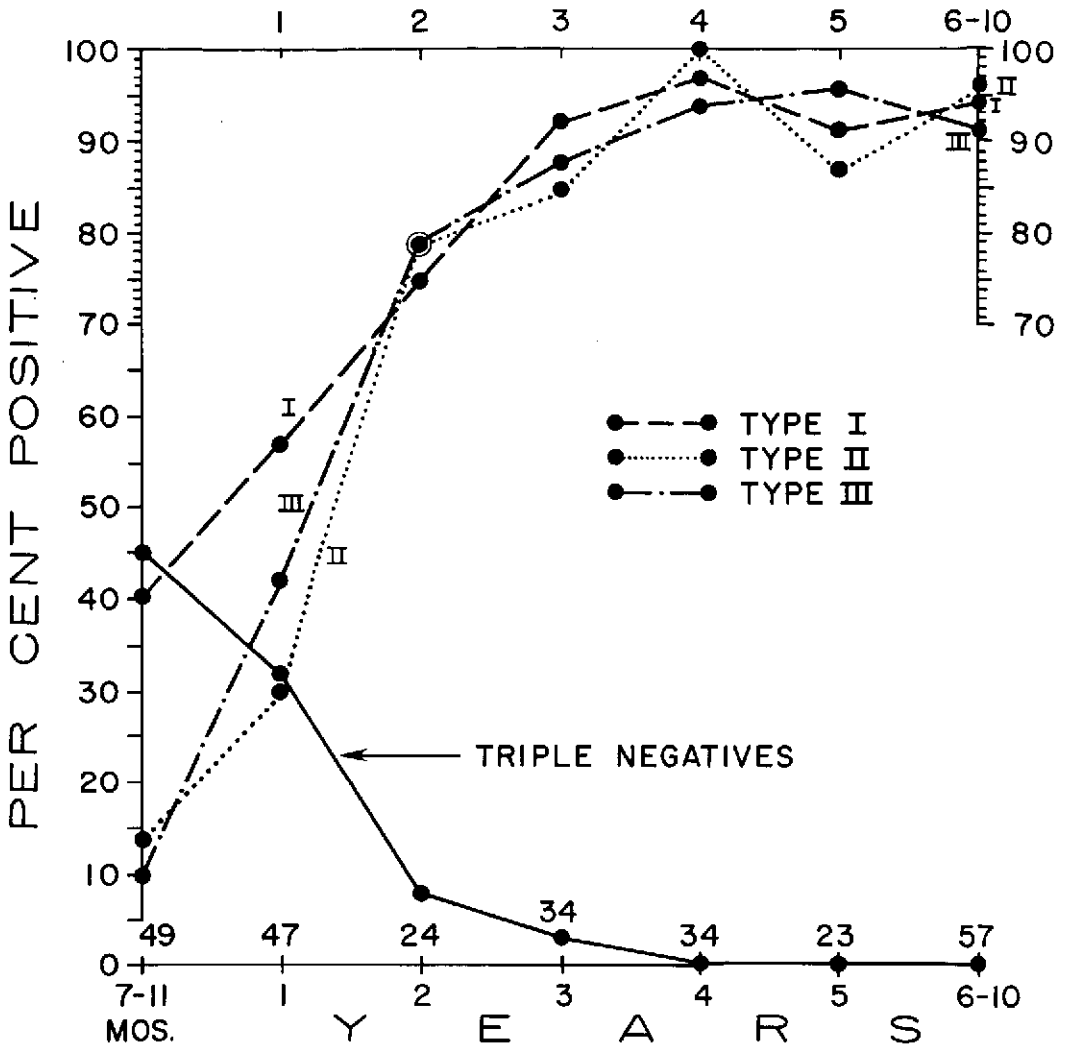


FIG. 1. Polio antibodies in children in Toluca, Mexico just before feeding vaccine.

enteric viral infection than has ever been demonstrated before, beginning during the first days after birth and reaching a peak of 72 per cent during the first year of life. Beginning with one-month old children, polioviruses were isolated from every age group tested, with a peak rate of 14 to 15 per cent between seven months and two years of age. Surprising was the high infection rate among the mothers of these young children: 5 per cent for polioviruses and 16 per cent for other enteric viruses.

It was in this setting that a single dose of trivalent poliovirus vaccine ($10^{5.2}$ to $10^{5.6}$ TCD_{50} of each type) was fed to a total of 26,033 children under 11 years of age—86 per cent of all the children of this age group in Toluca. The actual proportion of children of each age group who received the vaccine is shown in Table 1. Only four days were required to feed the vaccine to 97 per cent of the children who received it. To determine how effectively the attenuated polioviruses competed with the other enteric viruses, and how long and extensively polioviruses continue to disseminate in such a community after this rapid and massive artificial seeding, over 3,139 rectal swabbings were obtained during the subsequent three months and tested for virus. A total of 2,711 viruses were recovered from the

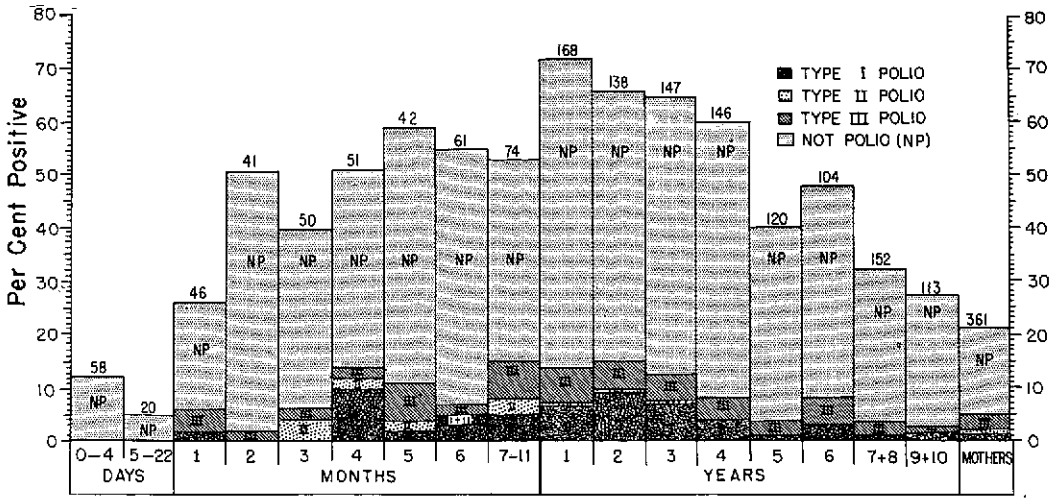


FIG. 2. Incidence of various types of poliovirus and other enteric viruses (NP) in children from birth to 10 years of age and in their mothers.
Toluca—August '59—just before feeding vaccine.

5,031 pre- and post-vaccination specimens, and the employment of special methods permitted us rapidly to classify them as polioviruses or non-polioviruses. The results shown in Fig. 3 for a group of 274 children who were tested repeatedly over a period of three months, and in Fig. 4 for hundreds of different children randomly chosen before and at monthly intervals after vaccination indicate the following:

(1) while the total incidence of enteric viruses varied little or not at all during the three months, the polioviruses became dominant during the first three weeks after vaccination—the isolation rates being as high as 40 to 70 per cent in the different age groups;

(2) the early dominance of the polioviruses was followed by a quick decline—more rapid in the children over two years of age—which by the

TABLE 1. PROPORTION OF CHILDREN OF INDICATED AGE FED SINGLE DOSE OF TRIVALENT VACCINE IN TOLUCA, MEXICO—AUGUST 1959

AGE YEARS	TOTAL NO. IN INDICATED AGE	NO. FED VACCINE	PER CENT INDICATED AGE VACCINATED
<1	3,195	2,965	90
1	2,803	2,327	82
2	3,257	2,361	71
3	3,329	2,453	74
4	3,298	2,575	78
5	2,886	2,410	83
6—10	11,708	10,942	93
<1—10	30,476	26,033	86
Total Population All Ages	103,072	26,033	25

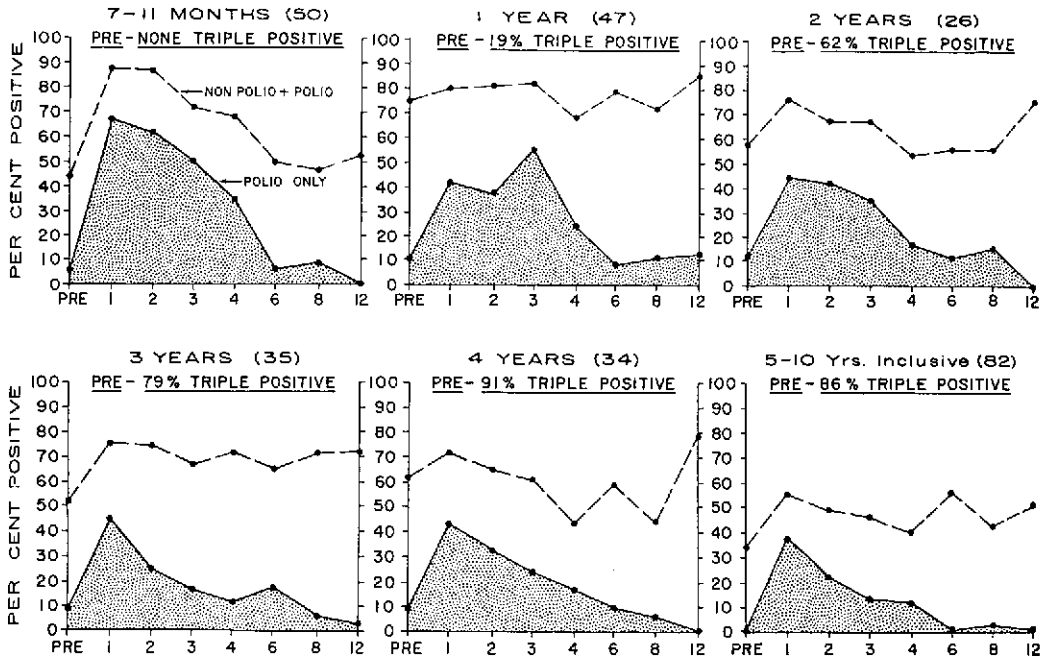
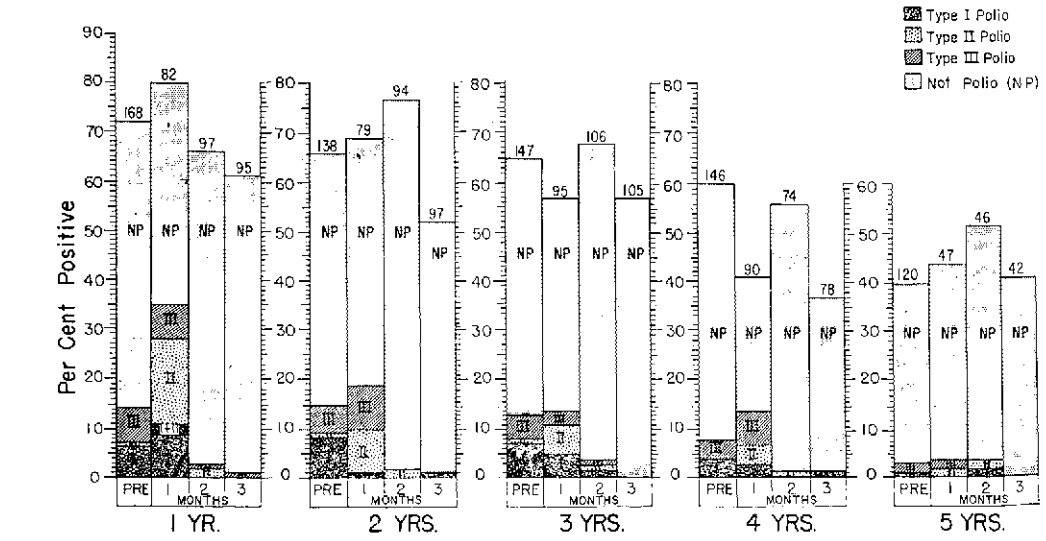


FIG. 3. Polioviruses and other enteric viruses in Group of 274 children tested just before and at weekly intervals after single dose of trivalent oral poliovirus vaccine.

Toluca, Mexico—Aug. to Nov. '59.



		1 YR.		2 YRS.		3 YRS.		4 YRS.		5 YRS.	
Antibody % Positive	I	57%	83%	75%	96%	94%	97%	97%	100%	91%	100%
	II	30%	83%	79%	100%	85%	97%	100%	100%	87%	100%
	III	43%	77%	79%	88%	88%	91%	94%	100%	96%	96%

FIG. 4. Incidence of various types of poliovirus and of other enteric viruses (NP) in 1 to 5 year old children randomly selected just before and at indicated monthly intervals after single dose of trivalent oral poliovirus vaccine.

Toluca, Mexico—Aug. to Nov. '59.

TABLE 2. SELF-LIMITED CHARACTER OF DISSEMINATION OF POLIOVIRUSES AFTER RAPID MASS ORAL ADMINISTRATION OF TRIVALENT VACCINE IN A TROPICAL COMMUNITY

Carrier Rate of Polioviruses and Other Enteric Viruses Among 1 to 5-Year-Old Children Selected at Random Before and 3 Months After *Single Dose ($10^{5.2}$—$10^{5.6}$ TCD_{50}) of Trivalent Oral Poliovirus Vaccine (Sabin)* Fed Within Few Days to 86 Per Cent of Children (Newborn to 10 Years) in Toluca, Mexico.

	BEFORE VACCINE AUG. 1959	THREE MONTHS AFTER VACCINE
Total No. Tested	719	417
Incidence of Polioviruses (P)	11.0%	0.7%
Incidence of Other Enteric Viruses (NP)	50.8%	50.6%
Ratio NP/P	4.6	72.3

Carrier Rate Based on Detection of Virus in Single Rectal Swabbing—Actual Carrier Rate is Somewhat Higher

end of three months reached a point that was markedly lower than before the mass feeding of the live poliovirus vaccine.

The self-limited character of dissemination of polioviruses after this rapid mass feeding of live poliovirus vaccine, under conditions which favor the continued extensive dissemination of enteric agents, is strikingly brought out in Table 2, which shows the results of tests on 1,135 randomly selected one to five-year-old children. The isolation rate of non-poliomyelitis enteric viruses was about 51 per cent before and also three months after the vaccination program, while the isolation of polioviruses dropped from 11 per cent to 0.7 per cent—a 16-fold diminution.

The serologic effect of this temporary dominance of the polioviruses is shown in Table 3, in which the antibody conversion rates during the first 10 weeks after the mass feeding of a single dose of trivalent vaccine in Toluca is compared with the natural antibody conversion rates in the city of Querétaro, which is similar in

TABLE 3. DEVELOPMENT OF POLIO NEUTRALIZING ANTIBODIES DURING COMPARABLE 10-WEEK PERIOD IN TWO MEXICAN CITIES

(a) Toluca—After one dose of trivalent vaccine (0.01 ml. of each—$10^{5.2}$ to $10^{5.6}$ TCD_{50}).
(b) Querétaro—No oral vaccine.

Conversion rates for indicated type based on simultaneous tests on paired sera from originally negative children.

TYPE	TOLUCA-VACCINE		QUERÉTARO—NO VACCINE	
	NO.*	%	NO.	%
1	36/53	68	2/33	6
2	64/77	82	3/33	10
3	33/76	43	5/38	13

*Children without antibody who were carriers of poliovirus at time first blood specimen was obtained are excluded from this calculation.

TABLE 4. POLIO ANTIBODY CONVERSION RATES IN TOLUCA, MEXICO AMONG "SINGLE-NEGATIVE", "DOUBLE-NEGATIVE" AND "TRIPLE-NEGATIVE" CHILDREN 10 WEEKS AFTER FEEDING SINGLE DOSE OF TRIVALENT VACCINE (0.01 ML. OF EACH—$10^{5.2}$ TO $10^{5.6}$ TCD_{50})

TYPE	SINGLE-NEGATIVE		DOUBLE-NEGATIVE		TRIPLE-NEGATIVE		TOTAL	
	NO. TESTED	% CONVERTED	NO. TESTED	% CONVERTED	NO. TESTED	% CONVERTED	NO. TESTED	% CONVERTED
1	4	100	16	88	33	55	53	68
2	17	82	25	80	35	83	77	82
3	17	59	24	42	35	37	76	43

TABLE 5. INCIDENCE OF VIRUS RECOVERY FROM RECTAL SWABS IN RELATION TO DEVELOPMENT OF ANTIBODY. ESTIMATE OF TOTAL INFECTION RATE DURING 10-WEEK PERIOD FOLLOWING SINGLE ORAL DOSE OF TRIVALENT VACCINE IN TOLUCA, MEXICO

CATEGORY	TYPE 1		TYPE 2		TYPE 3	
	NO.	%	NO.	%	NO.	%
VIRUS IN RECTAL SWABS AMONG THOSE WHO:						
a) DEVELOPED ANTIBODY	12/36	33	52/63	82	21/33	64
b) FAILED TO DEVELOP ANTIBODY	2/17	12	3/14	21	2/43	5
ESTIMATE OF TOTAL INFECTION RATE	42/53	80	67/77	87	36/76	47
DEVELOPMENT OF ANTIBODY AMONG TOTAL NO. INFECTED (DEMONSTRATED+ESTIMATED)	36/42	86	63/67	94	33/36	92

TABLE 6. CONVERSION RATES AT DIFFERENT TIMES AFTER FEEDING TWO DOSES OF TRIVALENT POLIOVIRUS VACCINE ($10^{5.2}$ TO $10^{5.6}$ TCD_{50}) IN TOLUCA, MEXICO

TYPE	NO. OF CHILDREN TESTED	PER CENT CONVERTED AT INDICATED TIME AFTER			
		FIRST DOSE — MASS FEEDING		SECOND DOSE-neg.only	BOTH DOSES
		First 10 weeks	Subsequent 11 weeks	6 weeks	
		○AUG'59 to +OCT'59	○OCT.'59 to +JAN'60	○JAN.'60 to +FEB.'60	○AUG'59 to +FEB'60
1	53	68	7	85	96
2	77	82	0	73	96
3	76	43	3	48	72

size, climate, hygienic conditions, and geography, but where no vaccine was given. The incidence of antibody conversion 10 weeks after the single dose of trivalent vaccine in single-negative, double-negative, and triple-negative children is shown in Table 4. The extent of interference resulting from the simultaneous administration of all three types, and the dominance of the Type 2 strain in this mixture are clearly evident. Thus, for Type 1, the conversion rate was 100 per cent in the small number of single negatives, 88 per cent in the double negatives, and 55 per cent in the triple negatives; for Type 3 it was 59 per cent, 42 per cent, and 37 per cent, respectively, while for Type 2 there was no difference in the three categories, the conversion rates being 82, 80, and 83 per cent.

An analysis of the data (Table 5) on the children from whom rectal swabbings were obtained at weekly intervals, indicated two significant facts: (1) many of the children who developed antibody did not excrete sufficient virus to be detected in the rectal swabs; and (2) some of the children who had no demonstrable antibody at 10 weeks excreted enough poliovirus to be detected in at least one of the rectal swabbings obtained during the first four weeks. When the children in the second category are included, one obtains an estimated total infection rate of 80 per cent for Type 1, 87 per cent for Type 2, and 47 per cent for Type 3 during the first 10 weeks after the single trivalent dose of vaccine. Further analysis of these data indicates that among the total number infected, antibody was demonstrable at 10 weeks in 86 per cent for Type 1, 94 per cent for Type 2, and 92 per cent for Type 3.

The virologic evidence of the marked drop in the dissemination of polioviruses that occurred eight to 12 weeks after vaccination is supported by the serologic data shown in Table 6, which indicate that among the children who failed to develop antibody during the first 10 weeks, very few converted during the subsequent 11 weeks.

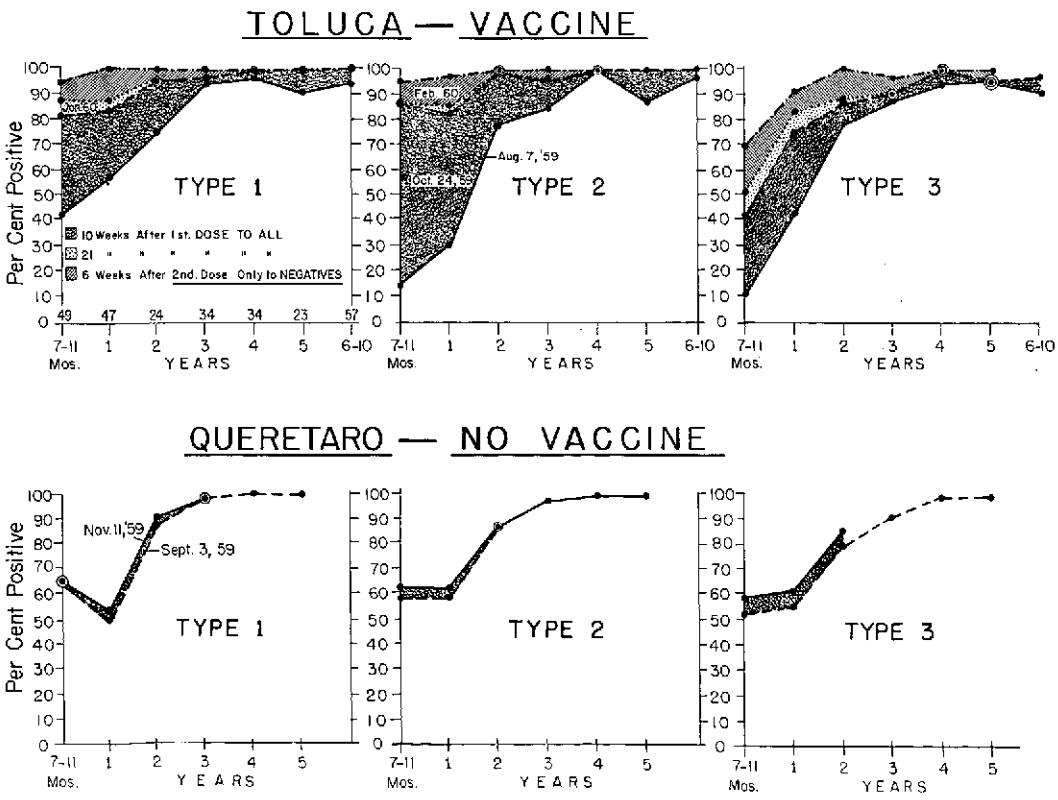


FIG. 5. Status of poliovirus antibody in two Mexican cities.

At this point, the remaining negative children in the group that were being followed serologically, were fed another dose of trivalent vaccine and they then exhibited a further marked immunogenic effect. The total conversion rate after the two doses of vaccine was 96 per cent for Types 1 and 2, and 72 per cent for Type 3—despite the interfering effects resulting from the massive infection with other enteric viruses (which was still 55 per cent in January 1960), and the simultaneous administration of all three types of poliovirus. The lower final conversion rate for Type 3 may also be related to the fact that we did not use the pH test for low-avidity antibody, which occurs more commonly after infection with the Type 3 virus. It should also be noted that the second feeding was not a mass feeding but involved only 44 children under four years of age, who were distributed among a large number of families throughout the city. The results might have been even better if nature had been given another chance at dissemination of the polioviruses in the community by feeding all the children under four years of age another dose of the vaccine.

Fig. 5 shows that with these two feedings of vaccine it was possible to achieve an immunogenic effect within a few months and without any cost in paralysis, comparable to that achieved by nature only after four years and at a price of about 56 cases of paralytic poliomyelitis.

The comparable serologic effectiveness of the same lots and doses of vaccine used in trivalent or monovalent form under different conditions in children in northern climates is shown in Table 7. It is evident that when the interval between the separate, sequential feedings of the three types was not less than four weeks, the antibody conversion rates were 100 per cent or close to it, while the mixture of all three types yielded conversion rates of only 82, 80, and 71 per cent, respectively, for the three types in a mixed group of single-negative, double-negative, and triple-negative children, living under conditions

TABLE 7. EFFECTIVENESS OF ALIQUOTS OF SAME LOTS AND SAME DOSES OF VACCINE USED IN TOLUCA, MEXICO WHEN GIVEN TO CHILDREN OF COMPARABLE AGE DURING WINTER AND SPRING MONTHS IN NORTHERN CLIMATES

Trivalent Versus Monovalent (1-3-2)

INVESTIGATOR PLACE AGE OF CHILDREN TIME OF YEAR	METHOD OF ADMINISTRATION	NO. OF CHILDREN WITHOUT ANTIBODY FOR INDICATED TYPE	PER CENT DEVELOPED ANTIBODY FOR INDICATED TYPE		
			1	2	3
Smorodintsev *Leningrad, USSR* Children's Homes *Up to 7 years* May, 1958	*Trivalent* Tested *4 months* later All together in camp during summer	29 neg. for 1 54 neg. for 2 38 neg. for 3	82	80	71
Smorodintsev *Leningrad, USSR* Children's Homes *Up to 3 years* Feb., March, Apr. 1958	*Monovalent* 4 week interval Tested one month after each type	147 neg. for 1 68 neg. for 2 67 neg. for 3	97	100	96
Verlinde *Leiden, Holland* Individual Families *Up to 14 years* May or Dec. 1957	*Monovalent* 3 week interval	20 neg. for 1 18 neg. for 2 28 neg. for 3	100	90	100

that were conducive to natural spread of the vaccine strains among them. These results, as well as other data collected by the Russian investigators in 1959, indicate that the separate sequential administration of the three types of vaccine at intervals of not less than four to six weeks, is optimum during the cold or cool months of the year in areas with good sanitation and hygiene.

CONCLUSION

The results obtained in Toluca, Mexico are of significance for the vast majority of the world population, among whom immunity to poliomyelitis is naturally acquired during the first few years of life at a varying and sometimes considerable price in paralysis. In such areas the feeding of trivalent vaccine on two brief occasions at an interval of six to eight weeks to all children under four or five years, depending on the age incidence of recorded cases of paralytic poliomyelitis or on the results of a serologic survey in the region, constitutes a rational, initial approach at eradication of poliomyelitis. The rapid disappearance of polioviruses from Toluca under the conditions of the present study, indicates that the oncoming generations of children will have to be similarly vaccinated at the optimum time during the first six months of life, because they will have little or no opportunity for natural acquisition of immunity to poliomyelitis.

REFERENCES

1. Sabin, A. B.: Status of field trials with an orally administered live attenuated poliovirus vaccine. *J. Am. M. Assn.* ***171:*** 863-868, 1959.
2. ————: Recent studies and field tests with a live attenuated poliovirus vaccine. In: *Live Poliovirus Vaccines, First International Conference.* Scient. Pub. no. 44 of the Pan American Health Organization, pp. 14-33, 1959.
3. Ramos Alvarez, M., Gómez Santos, F., Rangel Rivera, L., and Mayés, O.: Viral and serological studies in children immunized with live poliovirus vaccine—preliminary report of a large trial conducted in Mexico. In: *Live Poliovirus Vaccines, First International Conference.* Scient. Pub. no. 44 of the Pan American Health Organization, pp. 483-494, 1959.
4. Plotkin, S. A. and Koprowski, H.: Epidemiological studies of the safety and efficacy of vaccination with the CHAT strain of attenuated poliovirus in Leopoldville, Belgian Congo. In: *Live Poliovirus Vaccines, First International Conference.* Scient. Pub. no. 44 of the Pan American Health Organization, pp. 419-436, 1959.

15. USE OF SABIN'S LIVE POLIOVIRUS VACCINE IN MEXICO. RESULTS OF A LARGE-SCALE TRIAL

MANUEL RAMOS ALVAREZ, M.D., MIGUEL E. BUSTAMANTE, M.D., AND RAFAEL ALVAREZ ALBA, M.D.*

The Children's Hospital, Mexico City, Mexico; Ministry of Public Health and Welfare; and the Mexican Institute of Social Security

DR. RAMOS ALVAREZ *(presenting the paper)*: Studies with Sabin's live poliovirus vaccine have been conducted in Mexico during the last three years.

In 1957, carefully controlled laboratory and clinical observations were carried out on 181 children, mostly under three years of age, living in an orphanage in Mexico City to whom various doses of the attenuated strains separately or in mixtures were fed. In February 1958, investigations were extended to 2,800 children under six years of age living in 28 nurseries distributed in different sections of Mexico City, to whom the vaccine strains were fed separately at three to four-week intervals in the recommended order (Type 1, 3, and 2) in concentrations of approximately $10^{5.6}$ to $10^{5.9}$ TCD_{50} for each type. In addition, clinical observations were also made on 7,000 contacts of these 2,800 children. No untoward reactions were observed in any of the vaccinated children or in any of their contacts. Serologic studies on serum samples obtained from a group of these children before and after vaccination, showed the following antibody conversion rates: among 42 children negative for Type 1, 74 per cent converted to positive; among 52 children negative for Type 2, 77 per cent converted to positive; and among 37 children negative for Type 3, 67 per cent converted to positive.

The results of these preliminary studies, as well as the results obtained by other investigators working with the same strains, showed the innocuity of the vaccine and its immunogenic properties. Accordingly, the Ministry of Health of Mexico approved a large-scale vaccination program to be carried out at the end of 1958. A preliminary report of this trial was presented at the First Conference last year. The present report is intended to give a more detailed description of the conditions under which the campaign took place and the results obtained.

The administration of the vaccine was carried out on a voluntary basis in four different cities: Mexico City in the central part of the country; the City of Guadalajara, located approximately 400 miles northwest of Mexico City; the City of Monterrey, in the nothern part of the country; and the City of Puebla, approximately 100 miles south of Mexico City. It was limited to children under five years of age, who are the most susceptible population group according to previous laboratory and epidemiological observations.

A serological survey of the population in three of the cities under study just before the beginning of the vaccination program (Fig. 1), indicated that immunity to poliomyelitis is naturally acquired during the first four years of life. Eighty to 90 per cent of the children of four years of age showed antibodies to all three types of poliovirus.

The available data in these cities for the past five years indicate the following:

(1) Major outbreaks of poliomyelitis occurred every two years, with summer rises varying somewhat according to the year, sometimes as early as March, and sometimes as late as June or July.

(2) Mexico City, with a total population of 4,500,000 people, had an average of 349 paralytic cases per year; the City of Guadalajara, with a population of approximately 700,000, had an average of 132 cases per year; the City of Monterrey, with a population of 600,000, had an average of 72 cases; and the City of Puebla, with a popu-

* The following persons also cooperated in different parts of this work: Dr. Luis Rangel Rivera, Dr. Arturo González Durán, Otila Mayés, Q.F., and Lucía Bustamante, Q.F., of the Children's Hospital, Mexico City.

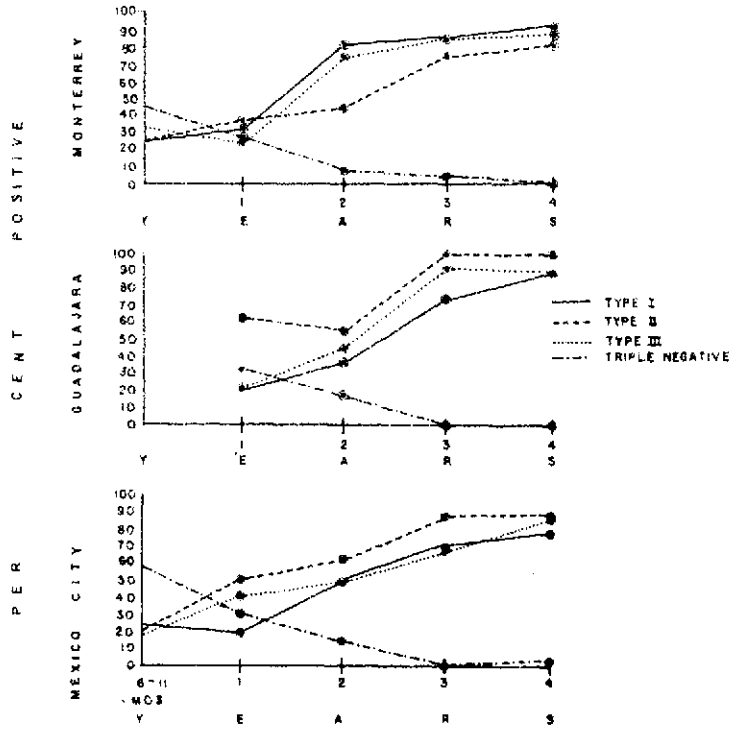


FIG. 1. Polio antibodies in children in 3 Mexican cities before feeding vaccine.

lation of 300,000, had an average of 31 cases of paralytic poliomyelitis per year.

(3) Approximately 90 per cent of these affected were children under five years of age.

Although the trial was scheduled for November and December 1958, for reasons beyond our control, the administration of the vaccine did not start until 23 February 1959 in Mexico City, and 27 April in the other three cities. It is important to emphasize that this delay was very unfortunate because, based on the experience of previous years, we were expecting an epidemic in both Mexico City and the City of Guadalajara. The epidemic in Mexico City had indeed started early in January, and in Guadalajara, it was picking up late in April. By the time the vaccination program was started, 21 cases each of paralytic poliomyelitis had occurred in Mexico City and Guadalajara, three cases in the City of Monterrey, and one case in the City of Puebla. It was in this kind of setting that the oral vaccine was administered.

All the vaccine used in these studies was kindly supplied by Dr. Sabin, from the large lots prepared by him in 1956, and fed by different investigators to several million people.

The strains were administered individually four to five weeks apart in the following order: Type I, 3, and 2 in amount of 0.1 ml., containing approximately $10^{5.6}$ to $10^{5.9}$ TCD_{50} for each type, in a teaspoon with cherry syrup. Table 1 shows the total number of children vaccinated and the period of time during which the different types of viruses were fed. In Mexico City, only 17.1 per cent of the estimated number of children between six months and less than five years of age were vaccinated; in Guadalajara and Puebla, about 30 per cent; and in the City of Monterrey, 52 per cent. The actual proportion of children of each age group who received the vaccine in the different cities is shown in Tables 2, 3, 4, and 5.

Tables 6, 7, and 8 show an estimation of the actual number of children without Types 1, 2, or 3 polio antibodies who received the different

TABLE 1. TOTAL NUMBER OF CHILDREN UNDER FIVE YEARS OF AGE IN FOUR DIFFERENT CITIES VACCINATED WITH SABIN'S LIVE POLIOVIRUS VACCINE

CITY	ADMINISTRATION OF VACCINE		ESTIMATED POPULATION 6 MOS. TO <5 YRS.	NUMBER OF CHILDREN FED INDICATED VIRUS PER CENT OF ESTIMATED POPULATION					
				TYPE 1		TYPE 3		TYPE 2	
	TYPE	DATE		NUMBER	PER CENT	NUMBER	PER CENT	NUMBER	PER CENT
Mexico City	1 3 2	Feb. 23 - April 4 April 6 May 14 May 15 June 20	630,000	107,919	17.1	93,988	14.9	89,012	14.1
Monterrey	1 3 2	April 27 June 6 June 8 July 18 July 20 Aug. 29	70,000	36,610	52.3	34,389	49.1	31,566	45
Guadalajara	1 3 2	Apr. 27 May 30 June 1 July 4 July 6 Aug. 8	75,555	29,904	30.3	19,480	25.7	17,594	23.2
Puebla	1 3 2	Apr. 27 May 30 June 8 July 18 July 20 Aug. 8	39,900	12,653	31.6	12,280	30.7	11,729	29.3
Total			815,455	180,086	22	160,137	19.6	149,901	18.3

TABLE 2. NUMBER OF CHILDREN OF DIFFERENT AGE GROUPS IN MEXICO CITY VACCINATED WITH SABIN'S LIVE POLIOVIRUS VACCINE

AGE GROUP YRS.	ESTIMATED POPULATION	NUMBER OF CHILDREN FED INDICATED VIRUS. PER CENT OF ESTIMATED POPULATION					
		TYPE 1		TYPE 3		TYPE 2	
		NUMBER	PER CENT	NUMBER	PER CENT	NUMBER	PER CENT
6—11 Mos.	81,000	16,463	20.3	13,930	17.1	13,013	16
1—<2	121,500	20,173	16.6	17,540	14.4	16,559	13.6
2—<3	144,000	20,108	13.9	17,680	12.2	16,753	11.6
3—<4	144,000	20,325	14.1	17,722	12.3	16,797	11.6
4—<5	139,500	30,850	22.1	27,116	19.4	25,890	18.5
All	630,000	107,919	17.1	93,988	14.9	89,012	14.1

TABLE 3. NUMBER OF CHILDREN OF DIFFERENT AGE GROUPS IN THE CITY OF GUADALAJARA VACCINATED WITH SABIN'S LIVE POLIOVIRUS VACCINE

AGE GROUP YRS.	ESTIMATED POPULATION	NUMBER OF CHILDREN FED INDICATED VIRUS. PER CENT OF ESTIMATED POPULATION					
		TYPE 1		TYPE 3		TYPE 2	
		NUMBER	PER CENT	NUMBER	PER CENT	NUMBER	PER CENT
6—11 Mos.	6,714	3,106	46.2	2,628	39.1	2,441	36.3
1—<2	15,107	4,341	28.7	3,690	24.4	3,170	20.9
2—<3	17,905	4,358	24.3	3,726	20.8	3,421	19.1
3—<4	17,345	4,468	25.7	3,751	21.6	3,427	19.7
4—<5	18,484	6,631	35.8	5,685	30.7	5,135	27.7
All	75,555	22,904	30.3	19,480	25.7	17,594	23.2

types of virus in Mexico City, Guadalajara, and Monterrey. These calculations are based on the serologic results obtained in a representative group of these children prior to feeding of the vaccine. Out of 167,433 children vaccinated with Type 1 virus in three of the cities, an estimated 75,000 did not have antibodies for this type of virus. Similar calculations for Types 2 and 3

TABLE 4. NUMBER OF CHILDREN OF DIFFERENT AGE GROUPS IN THE CITY OF MONTERREY VACCINATED WITH SABIN'S LIVE POLIOVIRUS VACCINE

AGE GROUP YRS.	ESTIMATED POPULATION	NUMBER OF CHILDREN FED INDICATED VIRUS. PER CENT OF ESTIMATED POPULATION					
		TYPE 1		TYPE 3		TYPE 2	
		NUMBER	PER CENT	NUMBER	PER CENT	NUMBER	PER CENT
6—11 MOS.	9,000	5,552	61.6	5,159	57.3	4,747	52.7
1—<2	13,500	8,227	60.9	7,681	56.8	7,021	52
2—<3	16,000	7,517	46.9	7,093	44.3	6,485	40.5
3—<4	16,000	7,255	45.3	6,825	42.6	6,323	39.5
4—<5	15,500	8,059	51.9	7,631	49.2	6,990	45
All	70,000	36,610	52.3	34,389	49.1	31,566	45

TABLE 5. NUMBER OF CHILDREN OF DIFFERENT AGE GROUPS IN THE CITY OF PUEBLA VACCINATED WITH SABIN'S LIVE POLIOVIRUS VACCINE

AGE GROUP YRS.	ESTIMATED POPULATION	NUMBER OF CHILDREN FED INDICATED VIRUS. PER CENT OF ESTIMATED POPULATION					
		TYPE 1		TYPE 3		TYPE 2	
		NUMBER	PER CENT	NUMBER	PER CENT	NUMBER	PER CENT
6—11 MOS.	5,130	1,538	29.9	1,472	28.6	1,378	26.8
1—<2	7,695	2,273	29.5	2,141	27.8	2,018	26.2
2—<3	9,120	2,259	24.7	2,194	24	2,110	23.1
3—<4	9,120	2,329	25.5	2,269	24.8	2,162	23.7
4—<5	8,835	4,254	48.1	4,204	47.5	4,061	45.9
All	39,900	12,653	31.6	12,280	30.7	11,729	29.3

virus gave the following results: out of 138,172 children fed Type 2 virus, 47,667 lacked type-specific antibodies, and out of 147,857 children fed Type 3 virus, an estimated number of 61,510 had no antibodies for this type of virus.

These calculations, showing the susceptibility of the vaccinated population and the epidemiological observations that indicate that no disease with central nervous system symptoms was associated with the feeding of the various types of virus, show clearly the safety of the vaccine.

TABLE 6. ESTIMATED NUMBER OF CHILDREN IN MEXICO CITY WITHOUT TYPES 1, 2, OR 3 POLIOVIRUS ANTIBODIES VACCINATED WITH SABIN'S TYPES 1, 2, AND 3 LIVE POLIOVIRUS VACCINE

AGE GROUP YRS.	SEROLOGIC STUDIES				TYPE 1		TYPE 3		TYPE 2	
	NUMBER TESTED	PER CENT WITHOUT INDICATED ANTIBODY			NUMBER VACCINATED	ESTIMATED NUMBER WITHOUT TYPE 1 ANTIBODY	NUMBER VACCINATED	ESTIMATED NUMBER WITHOUT TYPE 3 ANTIBODY	NUMBER VACCINATED	ESTIMATED NUMBER WITHOUT TYPE 2 ANTIBODY
		TYPE 1	TYPE 2	TYPE 3						
6—11 Mos.	42	76	79	81	16,463	12,511	13,930	11,283	13,013	10,280
1—<2	59	80	49	59	20,173	16,138	17,540	10,348	16,559	8,113
2—<3	59	51	37	51	20,108	10,255	17,680	9,017	16,753	6,198
3—<4	43	30	12	33	20,325	6,097	17,722	5,848	16,797	2,015
4—<5	46	22	11	15	30,850	6,787	27,116	4,067	25,890	2,848
Total	249	53	38	48	107,919	51,788	93,988	40,563	89,012	29,454

TABLE 7. ESTIMATED NUMBER OF CHILDREN FROM THE CITY OF GUADALAJARA WITHOUT TYPES 1, 2, OR 3 POLIOVIRUS ANTIBODIES FED SABIN'S TYPES 1, 2, AND 3 LIVE POLIOVIRUS VACCINE

AGE GROUP YRS.	SEROLOGIC STUDIES				TYPE 1		TYPE 3		TYPE 2	
	NUMBER TESTED	PER CENT WITHOUT INDICATED ANTIBODY			NUMBER VACCINATED	ESTIMATED NUMBER WITHOUT TYPE 1 ANTIBODY	NUMBER VACCINATED	ESTIMATED NUMBER WITHOUT TYPE 3 ANTIBODY	NUMBER VACCINATED	ESTIMATED NUMBER WITHOUT TYPE 2 ANTIBODY
		TYPE 1	TYPE 2	TYPE 3						
<2	18	78	39	78	7,447	5,808	6,318	4,928	5,611	2,188
2—<3	11	64	46	55	4,358	2,789	3,726	2,049	3,421	1,573
3—<4	12	25	0	8	4,468	1,117	3,751	300	3,427	0
4—<5	21	10	0	10	6,631	663	5,685	568	5,135	0
Total	62	42	19	37	22,904	10,377	19,480	7,845	17,594	3,761

TABLE 8. ESTIMATED NUMBER OF CHILDREN FROM THE CITY OF MONTERREY WITHOUT TYPES 1, 2, OR 3 POLIOVIRUS ANTIBODIES FED SABIN'S TYPES 1, 2, AND 3 LIVE POLIOVIRUS VACCINE

AGE GROUP YRS.	SEROLOGIC STUDIES				TYPE 1		TYPE 3		TYPE 2	
	NUMBER TESTED	PER CENT WITHOUT INDICATED ANTIBODY			NUMBER VACCINATED	ESTIMATED NUMBER WITHOUT TYPE 1 ANTIBODY	NUMBER VACCINATED	ESTIMATED NUMBER WITHOUT TYPE 3 ANTIBODY	NUMBER VACCINATED	ESTIMATED NUMBER WITHOUT TYPE 2 ANTIBODY
		TYPE 1	TYPE 2	TYPE 3						
6—11 MOS.	16	75	75	69	5,552	4,164	5,159	3,559	4,747	3,560
1—<2	21	67	62	76	8,227	5,512	7,681	5,837	7,021	4,353
2—<3	27	19	56	26	7,517	1,428	7,093	1,844	6,485	3,631
3—<4	20	15	25	15	7,255	1,088	6,825	1,023	6,323	1,580
4—<5	27	8	19	11	8,059	644	7,631	839	6,990	1,328
All	111	33	45	36	36,610	12,836	34,389	13,102	31,566	14,452

TABLE 9. SUMMARY OF TESTS OF ANTIBODY RESPONSE OF CHILDREN WITH NO DETECTABLE ANTIBODY FOR THE RESPECTIVE TYPE OF POLIOVIRUS PRIOR TO VACCINATION IN THREE OF THE CITIES IN WHICH SABIN'S ORAL VACCINE WAS USED

City	Serologic response					
	Type 1		Type 2		Type 3	
	Number tested	Number and percent positive	Number tested	Number and percent positive	Number tested	Number and percent positive
Mexico City	61	54 88%	46	40 87%	64	51 80%
Guadalajara	9	8 89%	6	5 83%	8	7 87%
Monterrey	29	21 72%	39	29 74%	27	14 52%
All	99	83 84%	91	74 81%	99	72 73%

The immunogenic activity of the vaccine is shown in Table 9, which shows the antibody conversion rate in a randomly selected group of children from three of the cities under study. These data are based on simultaneous tests using the cytopathogenic effect method in roller-tube cultures of human kidney cells on paired sera collected before feeding Type 1 and three to four weeks after feeding Type 2.

The conversion rate for all three types was very similar in Mexico City and in the City of Guadalajara, although the number of children tested in the latter is very small. Thus, for Type 1 the conversion rate was 88 per cent and 89 per cent, for Type 2, 87 per cent and 83 per cent, and for Type 3, 80 per cent and 87 per cent respectively. In the City of Monterrey, the antibody conversion rate was lower; for Type 1, 72 per cent, for Type 2, 74 per cent, and for Type 3, 52 per cent. The over-all response in the three cities was 84 per cent for Type 1, 81 per cent for Type 2, and 73 per cent for Type 3. The actual titers observed after vaccination are shown in Table 10. Although the antibody rise is somewhat variable with the individual, it can be noted that among those who responded, the majority of them (72 per cent for Type 1, 53 per cent for Type 2, and 74 per cent for Type 3) showed titers of 1:100 or more.

The relationship of age to development of antibodies after feeding of the vaccine is shown in Table 11. It is clearly evident that antibody response increases with age. This observation probably could be explained on the basis of the interference upon the vaccine strains of a great variety of non-polio enteroviruses, which are known to be present in a high percentage of children under one year of age in Mexico.

During the course of the vaccination program, some minor complaints, such as diarrhea, and occasionally vomiting and fever, were reported in some of the vaccinated children; however, these minor symptoms were not more frequent than would normally be expected in a population that is known to be highly infected with all kinds of enteropathogens.

The number of paralytic cases reported in the vaccinated areas is presented in the following five tables. Table 12 shows the data for the City of Monterrey, in which 52.3 per cent of the estimated population of children under five years of age were fed the vaccine. This city had experienced epidemics in the last two years and an outbreak was expected in 1959. Only 14 cases of paralytic poliomyelitis were reported throughout the year and all of them among non-vaccinated children. The data for the City of Puebla are presented in Table 13. In this city, about

TABLE 10. POLIO ANTIBODY TITERS AFTER FEEDING $10^{5.6}$ TO $10^{5.9}$ TCD_{50} OF SABIN'S TYPE 1, 2, AND 3 ORAL POLIOVIRUS VACCINE IN CHILDREN WITH NO DETECTABLE ANTIBODY FOR THE RESPECTIVE TYPE PRIOR TO VACCINATION

Antibody titer per 0.2 ml.	Number of children developing indicated titer after vaccination								
	Type 1			Type 2			Type 3		
	Mexico City	Guadalajara	Monterrey	Mexico City	Guadalajara	Monterrey	Mexico City	Guadalajara	Monterrey
320 or >	31	4	11	18	2	14	34	6	6
100	8	2	4	7	2	3	4		3
32	6	2	3	7	1	8	9		4
10	3			4			1		1
8	6		3	4		4	3	1	
0	7	1	8	6	1	10	13	1	13
All	61	9	29	46	6	39	64	8	27
Conversion rate	88%	89%	72%	87%	83%	74%	80%	87%	52%

TABLE 11. ANTIBODY RESPONSE OF CHILDREN OF DIFFERENT AGE GROUPS IN MEXICO CITY WITH NO DEMONSTRABLE HOMOTYPIC PRE-ANTIBODY FOLLOWING VACCINATION WITH $10^{5.6}$ TO $10^{5.9}$ TCD_{50} OF EACH OF THE THREE TYPES OF POLIOVIRUS 4-5 WEEKS APART

Age group Yrs.	Serologic response					
	Type 1		Type 2		Type 3	
	Number tested	Per cent positive	Number tested	Percent positive	Number tested	Per cent positive
6 – 11 Months	16	75	15	93	17	59
1 – <3	32	91	24	79	29	83
3 – <5	13	100	7	100	18	94
All	61	88	46	87	64	80

32 per cent of the susceptible population were vaccinated. It may be noted that 30 cases of paralytic poliomyelitis among non-vaccinees were reported, while no one case was observed among vaccinees.

The circumstances in the City of Guadalajara and in Mexico City were quite different. Vaccination programs in these two cities in only a small fraction of the susceptible population were carried out in the face of a Type 1 epidemic.

TABLE 12. INCIDENCE OF CASES OF PARALYTIC POLIOMYELITIS IN THE CITY OF MONTERREY DURING THE LAST FIVE YEARS. CASES AFTER INITIATION OF THE ORAL LIVE POLIOVIRUS VACCINE PROGRAM ON 27 APRIL 1959

Year	Number of reported paralytic cases mostly in age group 6 months to <5 years in indicated month												
	J.	F.	M.	A.	M.	J.	J.	A.	S.	O.	N.	D.	Total
1955	3	4	3	10	17	15	9	6	3	7	2	2	81
1956	0	0	2	2	2	0	1	2	2	5	2	5	23
1957	0	5	17	14	29	35	7	0	0	3	4	1	115
1958	1	2	6	1	12	28	46	16	10	4	2	0	128
Unvaccinated 1959	1	1	1	1	2	2	2	0	0	2	2	0	14
Vaccinated	-	-	-	0	0	0	0	0	0	0	0	0	0

TABLE 13. INCIDENCE OF CASES OF PARALYTIC POLIOMYELITIS IN THE CITY OF PUEBLA DURING THE LAST FIVE YEARS. CASES AFTER INITIATION OF THE ORAL LIVE POLIOVIRUS VACCINE PROGRAM ON 27 APRIL 1959

Year	Number of reported paralytic cases mostly in age group 6 months to <5 years in indicated month												
	J.	F.	M.	A.	M.	J.	J.	A.	S.	O.	N.	D.	Total
1955	0	0	0	0	3	23	20	8	5	3	0	3	65
1956	0	1	2	0	2	0	1	1	2	1	0	1	11
1957	0	1	0	1	2	2	7	1	4	5	6	3	32
1958	1	0	2	0	0	2	1	5	1	1	2	0	15
Unvaccinated 1959	0	1	0	0	2	3	7	6	3	6	2	0	30
Vaccinated	-	-	-	0	0	0	0	0	0	0	0	0	0

In Guadalajara, as is shown in Table 14, the epidemic started picking up at the beginning of May, just about the time the feeding of the vaccine had started. From the beginning of the vaccination program on 27 April, to the end of the year, 265 cases were reported; of these 265 cases, 248 occurred among non-vaccinated, and 17 among vaccinated children.

Table 15 shows the data for Mexico City. Although the expected epidemic did indeed appear, it should be noted that this epidemic began early in January before the initiation of the vaccination program. A total number of 382 cases were reported after initiation of the program. Of these 382 cases, 364 occurred among non-vaccinated and 18 among vaccinated children.

TABLE 14. INCIDENCE OF CASES OF PARALYTIC POLIOMYELITIS IN THE CITY OF GUADALAJARA DURING THE LAST FIVE YEARS. CASES AFTER INITIATION OF THE ORAL LIVE POLIOVIRUS VACCINE PROGRAM ON 27 APRIL 1959

Year	Number of reported paralytic cases mostly in age group 6 months to<5 Years in indicated month												
	J.	F.	M.	A.	M.	J.	J.	A.	S.	O.	N.	D.	Total
1955	0	0	1	0	2	6	22	20	26	4	4	3	88
1956	2	0	2	0	0	4	10	5	10	2	0	2	37
1957	2	5	4	6	11	28	49	67	25	3	6	1	207
1958	1	3	1	4	3	8	4	4	6	4	3	2	43
Unvaccinated 1959	2	8	3	8	47	67	75	44	10	4	1	0	269
Vaccinated	-	-	-	-	1	7	3	6	0	0	0	0	17

TABLE 15. INCIDENCE OF CASES OF PARALYTIC POLIOMYELITIS IN MEXICO CITY DURING THE LAST FIVE YEARS. CASES AFTER INITIATION OF THE ORAL LIVE POLIOVIRUS VACCINE PROGRAM ON 23 FEBRUARY 1959

Year	Number of reported paralytic cases mostly in age group 6 Months to <5 years in indicated month												
	J.	F.	M.	A.	M.	J.	J.	A.	S.	O.	N.	D.	Total
1955	22	24	28	92	115	109	159	77	36	28	15	9	714
1956	3	5	4	8	15	26	29	29	51	14	9	6	199
1957	1	8	8	11	24	58	78	37	36	24	13	10	308
1958	3	6	6	7	8	16	21	22	10	13	9	2	123
Unvaccinated 1959	11	11	19	37	61	86	65	34	22	23	8	5	382
Vaccinated	-	-	1	1	9	3	1	3	0	0	0	0	18

Table 16 presents in summary form the number of paralytic cases observed in all four cities in which the oral vaccine was used. It may be noted that, based on the population at risk, there was a definite difference betwen the number of expected cases and the number of observed cases. This is particularly striking in the City of Monterrey and in the City of Puebla, where not a single case of paralytic poliomyelitis was reported among the vaccinated children.

Detailed clinical and laboratory studies in most of the vaccinated children reported with the clinical diagnosis of paralytic poliomyelitis in Mexico City and in the City of Guadalajara, are given in the text of this paper; a summary is presented in Table 17. Out of the 18 cases reported

TABLE 16. TOTAL NUMBER OF CASES OF PARALYTIC POLIOMYELITIS IN FOUR DIFFERENT CITIES AFTER INITIATION OF THE ORAL LIVE POLIOVIRUS VACCINE PROGRAM IN 1959

City	Population in age group 6 Mos. to <5 years of age		Per cent vaccinated	Total number of cases mostly under 5 years of age			
				Vaccinated		Unvaccinated	Total
	Vaccinated	Unvaccinated		No. of expected cases	No. of observed cases		
Monterrey	36610	33390	52.3	12	0	11	11
Puebla	12653	27247	31.7	13	0	29	29
Guadalajara	22904	52651	30.3	111	17	256	273
Mexico City	107919	520081	17.1	75	18	364	382
All	180086	633369	22.1	187	35	660	695

in Mexico City, one was definitely not poliomyelitis. This was a child one year and five months old who on 31 March received Type 1 vaccine; 17 days later, on 17 April 1959, he developed fever and paralysis of both legs. A spinal-fluid examination 10 days after the beginning of fever showed a slight increase in proteins and a number of cells within normal limits. Virus isolations in stool samples collected 10 days after onset of the disease were negative. Tests on blood samples collected 35 and 92 days after onset of disease failed to detect antibodies for any of the three types of poliovirus.

Out of the other 17 cases, Type 1 poliovirus

TABLE 17. TIME INTERVAL BETWEEN ADMINISTRATION OF TYPE 1 VACCINE AND ONSET OF CLINICAL SYMPTOMS OF POLIOMYELITIS IN CHILDREN FROM MEXICO CITY AND GUADALAJARA

City	Number of cases	Type of virus recovered from stools	Number in group	Number of cases in which clinical symptoms started on indicated days after feeding type 1 vaccine				
				<17	35 - 40	45 - 50	60 - 80	>80
Mexico	17	Polio 1	16	1	1	2	6	7
		Non polio	0					
Guadalajara	16	Polio 1	6		1	4	1	
		Non polio	10	1	1	1	2	5

TABLE 18. INCIDENCE OF EACH OF THE 3 TYPES OF POLIOVIRUS AMONG CASES OF PARALYTIC POLIOMYELITIS IN NON-VACCINATED CHILDREN IN MEXICO CITY AT DIFFERENT TIMES AFTER INITIATION OF VACCINATION PROGRAM

Administration of vaccine in this city was carried out on the following dates:
Type 1 from February 23 to April 4
Type 3 from April 6 to May 14
Type 2 from May 15 to June 20

Month	Number of cases Tested	Number of isolations of indicated type of virus				
		Type 1	Type 2	Type 3	Non polio	Negative
March	9	6	3	0	0	0
April	14	10	0	0	3	1
May	23	9	1	2	11	0
June	13	7	0	0	6	0
July	6	4	0	0	2	0
August	3	3	0	0	0	0
September	5	3	0	0	1	1
October	3	3	0	0	0	0
November	3	3	0	0	0	0
December	1	1	0	0	0	0
Total	80	49	4	2	23	2

was recovered from the stool of all 16 patients from whom specimens could be obtained; one case developed the disease 16 days after vaccination. Unfortunately, studies in this case are incomplete and do not permit any conclusion as to whether or not the child was actually infected with a wild Type 1 poliovirus at the time of vaccination or a few days thereafter. Studies on the isolated strain, using the various genetic markers, could help to clarify this point. The interval between vaccination and onset of illness in the other 16 children was 35 to 80 days in nine cases and more than 80 days in seven cases. On the basis of available data, it is difficult to explain these failures of immunization. Nevertheless, the high incidence of infection with different enteroviruses, which has been demonstrated in this type of a population in Mexico (25 per cent as shown by the tests in a small group of 12 children studied two weeks before vaccination in Mexico City), strongly suggests that the phenomenon of interference played an important part in the unsucessful implantation of the Type 1 poliovirus vaccine.

Studies on 16 of the 17 cases reported in the City of Guadalajara showed the following results: Type 1 poliovirus was recovered from the stool of six cases, in all of which, the interval between administration of Type 1 vaccine and onset of illness was more than 30 days; in 10 patients, a non-poliovirus was recovered from the stools; in seven of these 10 patients, the non-poliovirus was isolated within the first or second week after onset of disease, in one case three

TABLE 19. INCIDENCE OF EACH OF THE 3 TYPES OF POLIOVIRUS AMONG CASES OF PARALYTIC POLIOMYELITIS IN NON-VACCINATED CHILDREN IN THE CITY OF GUADALAJARA AT DIFFERENT TIMES AFTER INITIATION OF VACCINATION PROGRAM

Administration of vaccine in this city was carried out on the following dates:
Type 1 from April 27 to May 30
Type 3 from June 1 to July 4
Type 2 from July 6 to August 8

Month	Number of cases tested	Number of isolations of indicated type of virus				
		Type 1	Type 2	Type 3	Non polio	Negative
May	2	2	0	0	0	0
June	9	6	0	0	3	0
July	15	8	0	0	4	3
August	3	2	0	1	0	0
Total	29	18	0	1	7	3

weeks after onset, and in two of them six weeks after onset of disease. Unfortunately, proper serologic evidence is not available to discard in a definite way the diagnosis of poliomyelitis.

The results of virus isolations from the stools of non-vaccinated patients with the clinical diagnosis of paralytic poliomyelitis in Mexico City and in the city of Guadalajara are presented in Tables 18 and 19. Table 18 shows the data for Mexico City. It can be seen that Type 1 poliovirus was responsible for the majority of the non-vaccinated cases throughout the year; there were only four cases due to Type 2 virus, but these cases were reported before the feeding of Type 2 vaccine. It is most interesting to note the high incidence of isolation of non-polioviruses (23 out of 80 cases tested). Although no serologic evidence and detailed clinical observations are available, these results may suggest that a certain proportion of the non-vaccinated children and also of the vaccinated children, may actually have not been poliomyelitis cases, but rather cases of transitory paralysis due to other enteroviruses.

Table 19 shows the data for the City of Guadalajara. As in Mexico City, Type 1 poliovirus was responsible for the vast majority of the cases among non-vaccinated children. It may be noted that in the City of Guadalajara a high incidence (seven out of 29 cases tested) of non-polio enteroviruses was found in the stool of these patients diagnosed clinically as paralytic poliomyelitis.

SUMMARY AND CONCLUSIONS

The results of the present studies indicate, first of all, the safety of Sabin's live poliovirus vaccine. No untoward reactions related to the feeding of the various strains were noted among the vaccinated children of four different Mexican cities and no evidence of association with vaccinated children was found among non-vaccinated cases.

The results of serological tests on paired sera collected from a randomly selected group of children before and after vaccination, show that Sabin's attenuated strains were highly immunogenic. The over-all antibody conversion rates were as follows: for Type 1, 84 per cent, for Type 2, 81 per cent, and for Type 3, 73 per cent.

The results obtained in Mexico City and in the City of Guadalajara point out the special problems created by the interference phenomenon and indicate that future vaccination programs should include at least 80 per cent of the susceptible population in the shortest possible period of

time, in a manner comparable to that achieved in Toluca and previously described by Dr. Sabin.

The administration of the vaccine under the conditions described in Mexico City and Guadalajara over a period of several weeks, including only a fraction of the susceptible population (17 per cent in Mexico City and 30 per cent in the City of Guadalajara), failed to immunize a certain proportion of the vaccinated children and did not influence the dissemination of the naturally occurring paralytogenic strains, as shown by the subsequent course of the Type 1 epidemic encountered in these two cities at the time of vaccination.

Although it would be difficult to calculate the real effectiveness of the vaccine under the conditions of the present studies, it should be emphasized, nevertheless, that on the basis of the population at risk, there was a striking difference between the number of expected cases and the number of observed cases in the vaccinated children, which was particularly evident in the cities of Monterrey and Puebla. Thus, it may be assumed that the oral vaccine was effective in preventing a large number of paralytic cases.

ACKNOWLEDGMENTS

The authors wish to acknowledge the cooperation of the staff of the Ministry of Public Health and Welfare of Mexico and of the staff of the Mexican Institute of Social Security, particularly Drs. Juan Assael and Samuel Inclán.

SUMMARY OF DATA ON VACCINATED CHILDREN IN MEXICO CITY WHO DEVELOPED PARALYTIC POLIOMYELITIS

CASE NUMBER NAME—AGE—YRS.	VACCINE FED		DATE OF ONSET	CLINICAL OBSERVATIONS	VIRUS ISOLATIONS		SERUM ANTIBODY TITERS PER 0.2ML. VS. INDICATED VIRUS				REMARKS
	TYPE	DATE			DATE OF STOOL	VIRUS RECOVERED	DATE OF SERUM	TYPE 1	TYPE 2	TYPE 3	
1.—P.A.G. 10 Mos.	1	3–13–59	3–29–59	Fever, constipation, paralysis of left leg on 4–5–59. Spinal fluid examination on 4–8–59. Proteins 67 mg.—Pandy pos.—Chlorides and glucose normal. Cells/cc:0.	4–7–59	Polio 1	4–7–59	320	0	10	10–16–59 great improvement of paralysis. Expect full recovery.
2.—J.L.M.V. 1 7/12	1 3	3–5–59 4–15–59	4–22–59	Paralysis of right leg and weakness of left leg on 4–27–59. Spinal fluid examination 4-28-59. Proteins 20 mg. Pandy neg. Cells/cc:48 (92% monocytes)	4–28–59	Polio 1	4–29–59 7–13–59	3200 3200	0 0	10 32	Time interval between feeding of Type 1 vaccine and onset of illness: 47 days. Failure of immunization. 5-24-59 fully recovered.
3.—S.H.A. 1	1 3	2–25–59 4–8–59	5–1–59	Fever, vomiting nucal rigidity, paralysis of right leg on 5–4–59. Spinal fluid examination on 5–7–59. Proteins 30 mg. Pandy neg. Cells/cc:18 (88% monocytes)	5–6–59	Polio 1	5–5–59 7–14–59 7–22–59	0 320 320	0 0 0	0 0 0	Time interval between feeding of Type 1 vaccine and onset of illness: 65 days. Failure of immunization. 1–14–60 paralysis improving.
4.—J.M.G. 1 6/12	1	3–20–59	5–18–59	Paralysis of left leg on 5–22–59. Spinal fluid examination on 5–23–59. Proteins 33 mg. Pandy	5–23–59	Polio 1	—	—	—	—	Time interval between feeding of Type 1 vaccine and onset of illness: 60 days. Failure of immuni-

				neg. Cells/cc:18 (88% monocytes).							zation.
5.—A.V. 1 6/12	1 3	4–1–59 5–10–59	5–19–59	Paresis of left leg	7–3–59	Polio 1	7–3–59	320	320	8	Time interval between feeding of Type 1 vaccine and onset of illness: 48 days. Failure of immunization.
6.—A.T.A. 1	1 3	4–12–59 5–14–59	5–21–59	Fever, pharyngitis, paralysis of right leg on 5–24–59. Spinal fluid examination on 5–26–59. Proteins 35 mg. Pandy neg. Cells./cc:66 (90% monocytes).	5–25–59	Polio 1	5–25–59 6–26–59 7–14–59	0 320 320	0 320 320	0 0 0	Time interval between feeding of Type 1 vaccine and onset of illness: 39 days. Failure of immunization 1–14–60. Great improvement of paralysis. Expect full recovery.
7.—J.M.H. 1 1/12	1 3	3–4–59 4–22–59	5–22–59	Paralysis of both legs.	6–10–59	Polio 1	6–10–59 7–7–59	320 320	320 320	100 32	Time interval between feeding of Type 1 vaccine and onset of illness: 78 days. Failure of immunization.
8.—M.V. 8 Mos.	1 3	3–2–59 4–22–59	5–25–59	Paralysis of right leg.	6–4–59	Polio 1	6–4–59 7–15–59	3200 3200	0 0	0 0	Time interval between feeding of Type 1 vaccine and onset of illness: 83 days. Failure of immunization.
9.—J.H. 1 1/12	1 3	3–4–59 4–22–59	5–31–59	Paralysis of right leg.	5–31–59	Polio 1	—	—	—	—	Time interval between feeding of Type 1 vaccine and onset of illness: 86 days. Failure of immunization.

SUMMARY OF DATA ON VACCINATED CHILDREN IN MEXICO CITY WHO DEVELOPED PARALYTIC POLIOMYELITIS

CASE NUMBER NAME—AGE—YRS.	VACCINE FED		DATE OF ONSET	CLINICAL OBSERVATIONS	VIRUS ISOLATIONS		SERUM ANTIBODY TITERS PER 0.2ML. VS. INDICATED VIRUS				REMARKS
	TYPE	DATE			DATE OF STOOL	VIRUS RECOVERED	DATE OF SERUM	TYPE 1	TYPE 2	TYPE 3	
10.—L.M.A. 3	1 3 2	3–2–59 4–15–59 5–28–59	6–22–59	Fever and headache on 6–22–59. Paralysis of right leg on 7–1–59.	7–8–59	Polio 1	7–7–59 7–12–59	3200 3200	100 100	320 320	Time interval between feeding of Type 1 vaccine and onset of illness: 80 days. Failure of immunization. 1–7–60. Great improvement.
11.—R.G.G. 1	1 3 2	4–5–59 5–10–59 6–18–59	6–25–59	Paralysis of left leg	7–8–59	Polio 1	7–8–59 7–14–59	0 0	0 0	0 0	Time interval between feeding of Type 1 vaccine and onset of illness: 80 days. Failure of immunization.
12.—V.M.M. 1 6/12	1 3 2	2–25–59 4–8–59 5–20–59	6–28–59	Paralysis of left leg	7–6–59	Polio 1	7–6–59 7–14–59	100 320	320 320	10 10	Time interval between feeding of Type 1 vaccine and onset of illness: 120 days. Failure of immunization.
13.—Ma.J.V. 8 Mos.	1 3 2	3–12–59 4–27–59 6–10–59	7–1–59	Fever, vomiting on 7–1–59, Quadriplegia on 7–6–59. Spinal fluid examination on 7–14–59. Proteins 93 mg. Pandy pos. Cells/cc:14	7–8–59	Polio 1	7–10–59	1000	0	0	Time interval between feeding of Type 1 vaccine and onset of illness: 110 days. Failure of immunization. 1–17–60. Great improvement of paralysis.
14.—L.H.H. 1 2/12	1 3 2	3–5–59 4–21–59 6–5–59	8–1–59	Fever, diarrhea, paralysis of right leg 8-6-59. Spinal fluid examination 8—8—	8–10–59 8–17–59	Polio 1 Polio 1	8–10–59 9–2–59 9–17–59	32 3200 3200	<10 <10 <10	320 320 320	Time interval between feeding of Type 1 vaccine and onset of illness: 140

				59. Proteins 40 mg. Pandy neg. Cells/cc:8							days. Failure of immunization. 1–17–60. Great improvement, expect full recovery.
15.—M.C. 11 Mos.	1 3 2	4–16–59 5–25–59 6–30–59	8–2–59	Fever, diarrhea, paralysis of right leg 8–6–59. Spinal fluid examination on 8–11–59. Proteins 72 mg. Pandy pos. Cells/cc:12 (80% monocytes)	8–13–59	Polio 1	8–10–59 1–19–60	10 100	10 10	320 10	Time interval between feeding of Type 1 vaccine and onset of illness: 106 days. Failure of immunization. 1–19–60. Great improvement.
16.—G.L. 1 8/12	1 3	3–10–59 4–22–59	8–20–59	Fever, vomiting, paralysis of right leg on 8–20–59. Spinal fluid examination on 8–29–59. Proteins 15 mg. Pandy neg. Cells/cc:12 (70% monocytes)	8–27–59	Polio 1	8–27–59	3200	3200	320	Time interval between feeding of Type 1 vaccine and onset of illness: 160 days. Failure of immunization.
17.—R.G.R. 1 5/12	1	3–31–59	4–17–59	Fever, paralysis of both legs. Spinal fluid examination on 4–27–59. Proteins 58 mg. Pandy pos. Cells/cc:10	4–25–59	Neg.	5–25–59 7–13–59 7–22–59	0 0 0	0 0 0	0 0 0	1–14–60 Fully recovered. Final diagnosis: Infectious polyneuritis.
18.—A.A.E. 6 Mos.	1 3	4–4–59 5–14–59	5–20–59	Paralysis of right leg. Weakness of left leg.							Time interval between feeding of Type 1 vaccine and onset of illness: 46 days. Failure of immunization.

SUMMARY OF DATA ON VACCINATED CHILDREN IN THE CITY OF GUADALAJARA WHO DEVELOPED PARALYTIC POLIOMYELITIS

CASE NUMBER NAME—AGE—YRS.	VACCINE FED		DATE OF ONSET	CLINICAL OBSERVATIONS	VIRUS ISOLATIONS		SERUM ANTIBODY TITERS PER 0.2 ML. VS. INDICATED VIRUS				REMARKS
	TYPE	DATE			DATE OF STOOL	VIRUS RECOVERED	DATE OF SERUM	TYPE 1	TYPE 2	TYPE 3	
1.—F.B.L. 14 Mos.	1	5–6–59	6–8–59	Fever, diarrhea, and paralysis of left arm	6–19–59	Polio 1					Time interval between feeding of Type 1 vaccine and onset of illness: 32 days. Failure of immunization.
2.—J.A.P. 1	1 3	4–27–59 6–8–59	6–18–59	Fever, vomiting, diarrhea, flaccid paralysis of left arm.	6–25–59	Polio 1					Time interval between feeding of Type 1 vaccine and onset of illness: 51 days. Failure of immunization.

3.—S.V.P. 19 Mos.	1 3	5–6–59 6–10–59	6–25–59	Fever, vomiting, paralysis of right leg.	7–9–59	Polio 1					Time interval between feeding of Type 1 vaccine and onset of illness: 49 days. Failure of immunization.
4.—CP.C.L. 13 Mos.	1 3	6–4–59 6–30–59	7–16–59	Fever, vomiting, partial paralysis of left leg.	7–29–59	Polio 1	7–29–59	320	1000	<10	Time interval between feeding of Type 1 vaccine and onset of illness: 42 days. Failure of immunization.
5.—M.L.B. 2	1 3	5–26–59 6–30–59	7–30–59	Fever, vomiting, partial paralysis of right leg.	8–5–59	Polio 1	8–5–59	320	10	100	Time interval between feeding of Type 1 vaccine and onset of illness: 52 days. Failure of immunization.
6.—Ma.L.Z.P. 1 5/12	1 3 2	5–28–59 6–30–59 8–4–59	8–31–59	Fever, vomiting, diarrhea, paralysis of both legs.	10–7–59	Polio 1	10–7–59	320	10	10	Time interval between feeding of Type 1 vaccine and onset of illness: 63 days. Failure of immunization.

SUMMARY OF DATA ON VACCINATED CHILDREN IN THE CITY OF GUADALAJARA IN WHOM CLINICAL DIAGNOSIS OF POLIOMYELITIS COULD NOT BE CONFIRMED BY VIRUS ISOLATIONS

CASE NUMBER NAME—AGE—YRS.	VACCINE FED		DATE OF ONSET	CLINICAL OBSERVATIONS	VIRUS ISOLATIONS		SERUM ANTIBODY TITERS PER 0.2 ML. VS. INDICATED VIRUS				REMARKS
	TYPE	DATE			DATE OF STOOL	VIRUS RECOVERED	DATE OF SERUM	TYPE 1	TYPE 2	TYPE 3	
7.—P.O.C. 7 Mos.	1	4–29–59	5–5–59	Fever, vomiting, paralysis of left leg.	5–6–59	Untyped non-polio-virus					On the basis of virus isolations this may not be poliomyelitis.
8.—O.M.G. 9 Mos.	1 3	5–26–59 7–3–59	7–15–59	Fever, questionable weakness of right arm	7–20–59	Untyped non-polio-virus					10–15–59 Fully recovered probably non-poliomelitis.
9.—V.M.P. 1	1 3	5–16–59 6–20–59	6–19–59	Fever, paralysis of both legs on 6–23–59	8–8–59	Untyped non-polio mouse pathogenic virus	8–8–59	3200	<10	<10	Improper specimens available for test. No conclusions could be drawn.
10.—J.J.G. 1	1 3	4–28–59 6–2–59	6–22–59	Fever, paralysis of left leg.	7–14–59	Untyped non-polio-virus					Improper specimens available for test. Without conclusions.
11.—Y.L.A. 2 6/12	1 3	4–28–59 6–13–59	6–26–59	Fever, weakness of right leg.	7–9–59	Untyped non-polio-virus					10–9–59 Fully recovered. This case may not have been poliomyelitis.

12.—F.T.L. 1	1 3 2	5–7–59 6–9–59 7–14–59	8–3–59	Fever, nausea, vomiting, paralysis of both legs.	8–12–59	Untyped non-polio-virus	8–11–59	1000	10	10	Although the available data on virus isolations is not conclusive, one should expect to recover a polio-virus nine days after onset of illness. Consequently, this may not be a case of poliomyelitis.
13.—B.A.A. 1	1 3 2	5–22–59 6–22–59 7–31–59	8–6–59	Fever, paralysis of left leg on 8–9–59	8–21–59	Untyped non-polio mouse patho-genic virus	8–20–59	3200	10	320	Although no conclusive evidence is available for discarding the diagnosis of poliomyelitis, the fact that a non-polio mouse pathogenic virus was iso-lated from the stool 12 days after paralysis, may suggest that this is not a case of poliomyelitis.
14.—F.A.L. 11 Mos.	1 3	5–6–59 7–10–59	8–14–59	Fever, diarrhea, paralysis of right leg on 8–21–59	8–29–59	Untyped non-polio-virus	8–28–59	3200	10	0	The isolation of a non-poliovirus from the stool of this child eight days after paralysis, suggests that this may not be a case of poliomyelitis.
15.–Ma.C.S.G. 10 Mos.	1 3 2	5–2–59 7–1–59 8–5–59	8–17–59	Fever, diarrhea, paralysis of right leg on 8-20-59		Untyped non-polio-virus	8–25–59 10–7–59	320 1000	0 0	0 0	Similarly to above case, the isolation of a non-poliovirus six days after paralysis strongly suggests that this is not a case of poliomyelitis.
16.–M.I.M. 2 10/12	1 3 2	5–2–59 7–1–59 8–4–59	8–23–59	Fever, facial paralysis	10–8–59	Untyped non-polio-virus	10–8–59	320	32	320	Improper specimens for test. No conclusions could be drawn.

DISCUSSION

CHAIRMAN LÉPINE: The papers by Dr. Sabin and Dr. Ramos Alvarez are now open for discussion.

DR. MELNICK: Dr. Sabin showed that after the mass feeding in Toluca polio isolations dropped from 11 to 1 per cent, but not to zero.

I should like to hear whether he might predict how long it will remain at this low level. Even if it dropped to zero, how long might it be before live viruses came into the community and caused reinfection? If wild viruses do come in, I believe Dr. Sabin's data showed that reinfections will occur. His paper indicated that immunity reached close to 100 per cent of the population by the age of four. Yet, even in this community, the immune mothers carried poliovirus to the extent of about 5 per cent. The viruses which they carried, as I recall, belonged to all three polio types and not just to one.

I should also like to raise the question of how we might prevent the reintroduction of polioviruses into mass vaccinated communities if we already know that immune persons can carry virus again, and perhaps again and again.

DR. SABIN: The first marked reduction occurred after a single feeding of trivalent vaccine. The second feeding was given to only a small number of children, and was not a mass feeding.

Whether or not the second trivalent feeding would have reduced the dissemination of polioviruses to a still lower level is something which may be expected but has not been demonstrated. But, as an indication of what nature itself does, we can look at the reported cases of poliomyelitis in the city of Toluca, which I think throws some light on the question. The pattern in Toluca, which is a city on trade routes with many people passing through it, is almost comparable to the pattern of measles. That is, you have one year with a high incidence and the next year with a very low incidence, followed by another year with a very high incidence.

That has been the pattern of recent years. This indicates that even after a period of high natural dissemination, which is associated with a high incidence of paralytic cases, there develop enough resistant intestinal tracts to stop the dissemination of the Type 1 poliovirus responsible for most of the clinical cases to such an extent that the following year there are very few cases —when I say very few, two to three has been the pattern—until additional children who are not immune come into the community.

The point about the mothers: I myself remarked that it seemed to me somewhat peculiar that, in a population in which presumably they would be exposed repeatedly, 5 per cent of the mothers should still be found to be carriers of poliovirus. But to be a carrier of polio is not an all-or-nothing affair as regards capacity to transmit virus. A person may become infected and yet may not be able to transmit.

It is quite possible, on the basis of many previous studies, that the young children who are primarily infected and excrete large amounts of virus are chiefly responsible for continuing dissemination. They can disseminate it to a number of others, including mothers, who may then have only low levels of multiplication. This is also comparable to experiences which many bacteriologists and epidemiologists have recorded for dysentery. It is not enough to be a carrier of dysentery. A person who has a few dysentery bacilli is not a disseminator, a person who has a million per gram is a disseminator.

The very fact that such marked reduction in dissemination could be achieved by a single feeding of trivalent vaccine indicates that we have to repeat it, as I said, in order to produce not only more immunization but also more intestinal resistance. Whether or not, and to what extent, such massive immunization will achieve, by continuing programs, the blockade to natural dissemination of polioviruses is something the virus laboratories will have to continue working on for the next few years and give us the answer. That available evidence warrants the attempt to eliminate naturally occurring polioviruses by this technique is to me beyond doubt.

DR. ANDERSON: My question is along the same line as Dr. Melnick's. Dr. Sabin has referred to a comparison with measles, pointing out that there is a high incidence one year and a low incidence the next. We know that, in a given year, the period of spread of measles is concentrated in a relatively short portion of that year.

It may be that Dr. Sabin explained this and I missed it, but I did not understand on what basis he assumed that this reduction from 11 per cent of shedding of poliovirus down to .7 per cent is something that was achieved by the immunization. Has he any control data to show that there may not have been seasonal variations and that a decline of this sort could have occurred independent of the immunization?

DR. SABIN: The only control data are that the factors conducive to the dissemination of enteric viruses continued, as is indicated by the fact that the other enteric viruses were spreading to the same extent.

The only thing that changed was the ratio of polio to non-polio. The ratio of change is due to the large number of resistant intestinal tracts that are created by massive spread of polioviruses, whether it be by nature or even more extensively by man, as was done in Toluca.

Nature can do the same thing—if it spreads Type 1 poliovirus extensively over a period of six months in one year, the result is a natural drop in Type 1 relative to other viruses the next year. We were merely trying to do what nature did, except in a much heavier and more extensive manner.

DR. BODIAN: I think this is a very interesting point and I wonder whether dissemination of polioviruses in Querétaro is somewhat indicative, although it can hardly serve as a completely adequate control in this regard. Is there any evidence that the wild polioviruses in the second city were diminishing seasonally?

DR. SABIN: We had our hands full with Toluca. Carrying out the serologic studies in Querétaro is about as much as we were able to do.

DR. BODIAN: I should like to ask a question of Dr. Ramos Alvarez, whose study I found most interesting, and that is whether he has some measure of the comparability of the two groups with respect to the incidence of poliomyelitis, that is, the unvaccinated and the vaccinated.

I do not believe I heard him mention any measure of comparability or any factors which were used to select those who received immunization.

DR. RAMOS ALVAREZ: The administration of the vaccine, as I mentioned, was made on a voluntary basis, and that was our method of selection. I think that both groups are comparable, since they belong to the same socioeconomic level. Does this answer your question?

DR. BODIAN: What I really was getting at was an independent measure of whether these two groups are comparable with respect to their probability of acquiring paralytic poliomyelitis. For example, age and antibody status are factors which would help in a comparison.

DR. RAMOS ALVAREZ: I think they are. I do not have with me the information you requested on the number of children in the different age groups. However, I shall be glad to make the data available later.

DR. MELNICK: Looking at Table 16*, of Dr. Ramos Alvarez's paper, which indicates the maximum effectiveness of the vaccine (from the number of expected cases and the number of observed cases), am I to understand that an effectiveness of 80 per cent is what he feels he achieved in this study?

DR. RAMOS ALVAREZ: I did not say it was an effectiveness of 80 per cent, merely that there was a striking difference between the groups.

DR. MELNICK: I should like an interpretation of Dr. Ramos Alvarez's data, where he shows 187 expected cases and 35 observed. At face value this comes out to about 80 per cent effectiveness. Do I interpret the table correctly?

DR. RAMOS ALVAREZ: Yes, but we must take one thing into consideration, that is, in Mexico City the numbers are not comparable. We vaccinated only 17 per cent of the estimated popula-

*see p. 398.

tion. I do not see how effectiveness can be measured on that basis.

DR. MELNICK: From your data, Dr. Ramos Alvarez, you indicated that in Mexico City you expected 75 cases in the 17 per cent vaccinated. Instead of findings 75 did you observe 18?

DR. RAMOS ALVAREZ: This is right.

DR. MELNICK: Calculated on this basis, do we not arrive at an effectiveness rate of about 80 per cent?

DR. RAMOS ALVAREZ: If you wish to take it that way.

CHAIRMAN LÉPINE: We have the figures. Are there any other remarks? If not, we shall now change continents, leaving America and going to Russia. The next paper is by Professor Chumakov and his associates entitled "On the Course of Mass Immunization of the Population in the Soviet Union with the Live Poliovirus Vaccine from Albert B. Sabin's Strains." The presentation of this paper will be made by Dr. Voroshilova.

TOPIC III. EFFICACY (B) FIELD EVIDENCE

16. ON THE COURSE OF MASS IMMUNIZATION OF THE POPULATION IN THE SOVIET UNION WITH THE LIVE POLIOVIRUS VACCINE FROM ALBERT B. SABIN'S STRAINS. REPORT NO. 3 AS OF 1 JUNE 1960

M. P. CHUMAKOV, M. K. VOROSHILOVA, S. G. DROZDOV, S. G. DZAGUROV, V. A. LASHKEVICH, L. L. MIRONOVA, N. M. RALPH, I. S. SOKOLOVA, I. N. DOBROVA, E. E. ASHMARINA, G. A. SHIRMAN, G. P. FLEER, V. I. ZHEVANDROVA, G. A. KOROLEVA, E. A. TOLSKAYA, O. D. YANKEVICH (MOSCOW); K. A. VASILIEVA, T. R. KUSLAP (TALLINN); T. S. PODSEDLOVSKY, Y. S. USPENSKY (VILNIUS); V. M. BOIKI (TASHKENT); AND K. M. SINYAK (LVOV)

DR. VOROSHILOVA *(presenting the paper):* The principal results of the work carried out in 1959, under the guidance of workers of the AMS USSR Institute for Poliomyelitis Research, on mass immunization of the population in the Soviet Union with live poliovirus vaccine from A. B. Sabin's strains, were published in Report No. 2 in Russian and English.*

These results were discussed in detail on two occasions and approved by the Presidium of the USSR Academy of Medical Sciences, the supreme scientific center of medical research in the USSR, which is authorized to approve new suggestions for the prevention or treatment of diseases. Based on the recommendations of the Presidium of the USSR Academy of Medical Sciences, and resolutions passed by the Board of the USSR Ministry of Public Health, an order was issued by the Minister of Health on 16 December 1959, providing for mass immunization of the population of two months to 20 years of age with live poliovirus vaccine during 1960, and mainly up to July 1960. This will involve about 35 per cent of the total population, or about 77 million persons.

For the first time in our country, and in the world, an immense task is before us to carry out immunization of many millions of people during one year, with the objective of eradicating epidemic manifestations of poliomyelitis on a scale of a large country. Further developments of specific poliomyelitis prophylaxis in our country and, possibly, in other countries, will be influenced to a considerable extent by the successful fulfillment of this task.

In this report, we are able to supplement some data on the results of mass immunization of the USSR population in 1959 and to give brief information on preliminary data of the large-scale poliomyelitis immunization program in the USSR in 1960.

Poliomyelitis is a severe but relatively rare disease. Nevertheless, it proved necessary to develop extremely broad measures for creating artificial immunity against this rare disease in the total population of the country by means of mass immunization within a short period of time. This is explained by the fundamental characteristics of poliomyelitis as an infection having a large number of completely asymptomatic forms which are difficult to diagnose.

Latent immunization with different poliovirus types circulating among the population occurs very irregularly in various areas and in different socio-economic groups. Of great importance for the occurrence of poliomyelitis epidemics is the peculiar "immunologic dystonia," due to inter-

* *On Mass Oral Immunization of Population in the Soviet Union against Poliomyelitis with Live Vaccine from A. B. Sabin's Attenuated Strains. Moscow 1960.*

ference between three types of poliovirus. For instance, prevalent dissemination of Type 2 poliovirus is frequently observed in inter-epidemic periods to the detriment of latent immunization with Types 1 and 3, which leads to the accumulation of menacingly large numbers of susceptibles to poliovirus Types 1 and 3; and, on the contrary, subsequent decrease of Type 2 virus circulation is possible in epidemic years when more active Type 1 or Type 3 strains are prevalent. Irregular alternations of poliovirus types prevalent in different periods, with constant separation of more pathogenic mutants, increase the chaotic state of latent immunization. The history of poliomyelitis for the past 10 years shows that poliomyelitis epidemics can occur in any country regardless of hygienic standards of living and of climatic conditions. Consequently, nobody can any longer depend on "favors from nature" in the form of very unreliable (because of its irregularity) latent immunizations with "wild" poliovirus strains.

Therefore, it is only with the aid of most extensive measures for rapid creation of artificial immunity in the entire susceptible population that the menace of poliomyelitis epidemics can be averted.

For this purpose it is necessary to have an effective, readily available, completely safe areactogenic vaccine presenting no difficulties in its most wide application.

Trials of live poliovirus vaccine from Sabin's strains carried out in a number of countries, particularly during 1959 in our country on a large scale, furnished convincing proof that it is the live vaccine that is most suitable for mass immunization.

A great advantage of the live vaccine, compared to the killed Salk vaccine, lies in the possibility to create in the vaccinated more or less complete immunologic resistance of tissue cells in the "portals of entry" of infection, that is, in the walls of the alimentary tract including the pharyngeal circle. This will probably cause a sharp reduction in the circulation of wild epidemic strains of poliovirus among the population as a result of repeated immunization with attenuated viruses. In this way an important basis for eradication of poliomyelitis epidemics is being created.

One of the most frequent objections raised against the use of live poliovirus vaccine is the fear of possible reversion of attenuated poliovirus strains to pathogenic properties which may occur as a result of selection in the human intestinal tract of virulent virus particles and of repeated passages through the susceptible population.

There are many facts available at present testifying to a sufficient stability of the genetic properties of Sabin's attenuated strains, which persists during several passages through the human body. There are no theoretical and experimental grounds, however, which would make it possible to exclude entirely the possibility of degeneration occurring in the vaccine strains under the effect of their propagation under inappropriate conditions, or due to a long-term selection of virulent virus particles and their multiplication on passages through susceptible human beings.

In connection with this, we put forward in 1958-1959 the principle of mass and simultaneous immunization with live poliovirus vaccine covering the territory of a whole district, city, or region, so as to ensure maximum involvement by immunization in the shortest possible time, and to minimize the possibility of a long-term circulation of any poliovirus strains among susceptible population and of increase in their neurovirulence during serial passages in children.

In carrying out mass vaccinations during 1959-1960 we closely adhered to this principle.

The necessity to use live vaccine for immunization of many millions of persons required searching for the most convenient form of vaccine preparation so as to minimize labor spent on vaccine distribution, and to simplify maximally the procedure of oral immunization of people.

Under our conditions, considering the unusually large scope and narrow time limits set for oral immunization in 1960, it would be extremely difficult to secure the dilution of thawed liquid vaccine and its distribution dropwise throughout the country by the local medical staff in the field (as it was done in 1959).

In this connection the Institute for Poliomyelitis Research, in cooperation with the Research Institute of confectionery industry, has elaborated a new presentation of the live vaccine incorporated into pasty candies and later into dragée-candy. This was based on the data (G. P. Fleer,

1959) proving satisfactory preservation of vaccine strains in strong solutions of inverted sugars and in the sweetmeat mass at sub-zero temperatures for at least three months, at 4° C. for about a month and at room temperature for three to five days.

Tests for the virus content in dragée-candy by the plaque method (data by S. G. Dzagurov and co-workers, V. A. Lashkevich and co-workers, and also by Dr. A. B. Sabin, 1960) confirmed the good preservation of the vaccine in candy and even distribution of the virus in individual pieces

Table 1. Frequency of Vaccine Virus Excretion in Children Immunized with Trivalent Attenuated Vaccine in Pomadka-Candy
(Town of Klin, 1959. G. P. Fleer)

Age-Group	Under Survey			Virus Isolation			
	Number of Children	Average Number of Stools from Each Child	Total No. of Stools Per Age-Group	Number of Children	% to the Number Tested	From Stool Specimens	
						No.	%
4 mos.—3 yrs.	30	4	118	28	93	78	66
3—7 yrs.	12	3	38	10	85	25	66
7—15 yrs.	48	3	158	43	89	97	61
Total	90		314	81	90	200	63.7

Table 1a. Establishment of Type 1 Vaccine in Dragée-Candy in Children from Children's Home No. 15

No. of Children Tested (Without Prevaccination Virus Carriage)	Number of Children Excreting Virus After Vaccination at Indicated Days								
	3	7	10	14	17	21	25	28	Total number of children excreting virus
27	11	12	19	14	16	9	3	2	27 100.0%

Table 2. Excretion of Type 1 Vaccine Poliovirus in Children Immunized with Live Type 1 Monovaccine in Dragée-Candy
(Moscow Children's homes, 1960. E. E. Ashmarina)

Number of Children Without Virus Carriage Before Vaccination	Number of Stool Specimens Tested (For 4 Weeks)	% of Finding of Vaccine Virus to the Number of Children
Children's home No. 11 37	331	94
Children's home No. 15 45	402	93
Total 82	733	93.9

TABLE 3. COMPARATIVE DATA OF SEROLOGIC SURVEY OF THE VACCINATED WITH LIQUID TRIVALENT VACCINE AND TRIVALENT VACCINE INCORPORATED INTO POMADKA-CANDY (Antibody Patterns)

TRIVALENT LIQUID LIVE VACCINE (ESTONIA, 1959, DOBROVA, I.N.)				TRIVALENT LIVE VACCINE INCORPORATED INTO POMADKA-CANDY (1959, FLEER, G.P.)			
BEFORE VACCINATION	TOTAL WITH ANTIBODY AFTER VACCINATION			BEFORE VACCINATION	TOTAL WITH ANTIBODY AFTER VACCINATION		
	1+	2+	3+		1+	2+	3+
000				000			
13 pers.	8	10	10	19 pers.	13	16	8
26.5%	61.5	78.9	76.9	43.1%	68.4	84.2	42.1
00+				00+			
4 pers.	3	3	4	3 pers.	3	2	3
8.2%	75.0	75.0	100.0	6.8%	100.0	66.6	100.0
0+0				0+0			
10 pers.	8	10	7	4 pers.	4	4	3
20.4%	80.0	100.0	70.0	9.0%	100.0	100.0	75.0
0++				0++			
12 pers.	9	12	12	6 pers.	6	6	6
24.5%	75.0	100.0	100.0	13.6%	100.0	100.0	100.0
+00				+00			
4 pers.	4	4	3	3 pers.	3	3	1
8.2%	100.0	100.0	75.0	6.8%	100.0	100.0	33.3
+0+				+0+			
3 pers.	3	3	3	4 pers.	4	4	4
6.1%	100.0	100.0	100.0	9.0%	100.0	100.0	100.0
++0				++0			
3 pers.	3	3	3	5 pers.	5	5	5
6.1%	100.0	100.0	100.0	11.3%	100.0	100.0	100.0
49	38	45	41	44	38	40	29
100.0%	77.5	91.8	83.7	100.0%	86.4	90.9	66.0
	Increase of antibody				*Increase of antibody*		
	1+ +57.1%				1+ +59.2%		
	2+ +40.8%				2+ +50.0%		
	3+ +44.9%				3+ +36.5%		

of dragée-candy (Tables 1, 2, 3, 4). Tests for the establishment and immunogenicity of the live vaccine in dragée-candy, carried out in 1959 by G. P. Fleer in the town of Klin in the Moscow region, showed quite satisfactory results, which could be favorably compared with those obtained in immunization with liquid live vaccine (see Table 5 and Fig. 1).

E. E. Ashmarina and S. G. Drozdov (1960) confirmed a high degree of establishment in children of monovaccines fed in the form of dragée-candy (Table 5).

TABLE 4. ANTIBODY RESPONSE TO TYPE 1 (ONE AND FOUR MONTHS) AND TO TYPE 3 (ONE MONTH) AFTER IMMUNIZATION OF CHILDREN UNDER TWO YEARS OF AGE WITH LIVE VACCINE INCORPORATED INTO DRAGÉE-CANDY STORED FOR THREE DAYS AT ROOM TEMPERATURE (+22° C.)
(M. K. Voroshilova, G. P. Taranova, 1959)

ONE MONTH AFTER INGESTION OF TYPE 1 VACCINE				FOUR MONTHS AFTER INGESTION OF TYPE 1 AND ONE MONTH AFTER INGESTION OF TYPE 3 VACCINE			
NUMBER OF CHILDREN WITHOUT ANTIBODY TO TYPES		DEVELOPED ANTIBODY No.	%	NUMBER OF CHILDREN WITHOUT ANTIBODY TO TYPES		DEVELOPED ANTIBODY No.	%
1°	19	14	73.7	1°	15	12	80.0
2°	15	0	0	2°	14	0	0
3°	19	0	0	3°	15	9	60.0

TABLE 5. ANTIBODY RESPONSE 1.5 MONTHS AFTER IMMUNIZATION WITH TYPE 1 MONOVACCINE IN DRAGÉE-CANDY STORED UNDER DIFFERENT CONDITIONS (1959)

CONDITIONS OF STORAGE	PERIOD OF STORAGE (DAYS)	ANTIBODY 1.5 MONTHS AFTER IMMUNIZATION					
		IN CHILDREN WITHOUT TYPE 1 ANTIBODY			IN TRIPLE-NEGATIVE CHILDREN		
		NO. OF CHILDREN	DEVELOPED ANTIBODY	%	NO. OF CHILDREN	DEVELOPED ANTIBODY	%
−20°C	3–30	15	13	86.7	10	8	80.0
+22°C	3–7	23	18	78.3	17	13	76.5
Total		38	31	81.6	27	21	77.8

TABLE 6. DEVELOPMENT AND RISE OF ANTIBODY IN CHILDREN UNDER THREE YEARS, IMMUNIZED WITH LIVE VACCINE IN DRAGÉE-CANDY (VACCINE STORED FOR 1-3 DAYS AT +20° C.)
(I. N. Dobrova, 1960, pH color test)

NUMBER OF CHILDREN WITHOUT PRE-VACCINATION ANTIBODY	DEVELOPED ANTIBODY		NUMBER OF CHILDREN WITH PRE-VACCINATION ANTIBODY	DEVELOPED RISE IN TITER	PER CENT
	No.	%			
Type 1—22	17*	77.3	21	11	52.4
Type 2—16	16	100.0	28	19	67.8
Type 3—23	23	100.0	21	13	61.9

* Including two of five triple-negative children.

Polio Type	Control (Frozen)	Days of Storage		
		3	7	15
1	5.6*	5.3	5.3	5.0
	100%	50%	50%	24%
2	5.4	5.2	5.1	4.6
	100%	64%	50%	16%
3	5.65	5.6	5.5	5.0
	100%	89%	71%	22%

* Average of plaque counts (pfu $\times 10^5$) of 10 individual pieces of candy.

Age of culture 8-9 days.
Adsorption—2½ hours at 22° C.
Overlay with 660 mg.% $NaHCO_3$
Incubated at 34° C. for 5 days.

Fig. 1. Stability on storage of polio vaccine in candy at 20° C.

In the fall of 1959-winter of 1960 M. K. Voroshilova, G. P. Taranova, I. N. Dobrova, and others again tested the immunogenicity of the live vaccine in dragée-candy and also regularly obtained good results. (Tables 6 and 7).

The technique of immunization with the vaccine in candy turned out to be extremely convenient and attractive in every respect. All this contributed to the widest use of dragée-candy with live vaccine for the purpose of poliomyelitis immunization. Large-scale production of live vaccine incorporated in dragée-candy has been set up at one of the confectionery factories of Moscow.

In 1960 the vast majority of poliomyelitis vaccinations (about 90 per cent) are being carried out with live vaccine incorporated into dragée-candy, and only 10 per cent with liquid vaccine dispensed dropwise, chiefly for immunization of infants unable to suck candies.

All production of live poliovirus vaccine from Sabin's strains in 1960 was concentrated at the Institute for Poliomyelitis Research, which was entrusted with the task of supplying live vaccine and exercising methodical guidance over vaccinations throughout the entire country.

The preliminary results showing fulfillment during 1960 of the program for mass immunization of the USSR population aged up to 20 years, are as follows.

A total of 197,810,200 inoculation doses of live vaccine prepared in Moscow from Sabin's strains were distributed throughout the country during the first five months of 1960 (data as of 1 June 1960). This includes monovaccines of Type 1, 67,819,900 doses, Type 2, 48,709,400 doses, Type 3, 51,825,000 doses, and trivalent mixture (Types 1, 2, and 3), 29,411,900 doses, used for revaccination of those who had been immunized previously with individual types of live vaccine or for pri-

Table 7. Antibody Response in Children Immunized on the Schedule Types 1-2-3 with Monovaccines in Dragée-Candy Stored at —20° C. and at Room Temperature (1.5 Months After Each Feeding, in Per Cent)
(Observations of 1959)

Conditions of Storage	Antibody Response in Seronegative Children		
	1	2	3
−20°C, 3 to 30 days	86.7	100.0	71.4
+22°C, 3 to 7 days	78.3	100.0	64.7

mary immunization. For primary immunization (Type 1 or trivalent mixture), 78,343,300 inoculation doses have been distributed.

Since a great deal of work connected with immunization of the population with live poliovirus vaccine had already been done in many republics during 1959, it was decided in these republics (Kazakh, Uzbek, Georgia, Azerbaijan, Moldavia, Lithuania, Kirghiz, Zajik, Turkmenistan, and Estonia) to give only two vaccinations in 1960: first, with Type 1 monovaccine, and second, with trivalent mixture (Types 1, 2, and 3) of live vaccine, which will cover the entire population from two months to 20 years of age.

In RSFSR, Ukrainian, Byelorussian, Latvian, and Armenian Republics, immunization was carried out in 1960 with monovaccines given three times. In some regions of the Ukraine, trivalent mixture was administered twice. All large-scale vaccination programs have been realized by 1 June 1960 in 13 out of 15 republics, and in RSFSR and Ukrainian SSR, they will be completed by 15-20 June.

After the termination of mass vaccination programs all over the country, immunization is scheduled for growing-up infants aged two to seven months who had not been involved by vaccinations during winter-spring immunization campaign.

There are no precise figures available at present showing the actual numbers of those immunized with the live vaccine in 1960. But taking into account the usual losses incident to the distribution of the vaccine during immunization (about 15-20 per cent), one may expect that before 15 June 1960, over 65 million persons in the USSR (or about 84 per cent of the population aged up to 20 years), will have completed vaccinations with three types of live poliovirus vaccine from Sabin's strains. Consequently, in less than six months we were able to immunize the vast majority of susceptible population and to fulfill over 84 per cent of the annual poliomyelitis vaccination program. As is well known, it took more than four years in the U.S.A. to give two or three shots of Salk vaccine to about 50 million persons (according to data of The National Foundation as of 31 December 1958). Undoubtedly, speed and broadness of involvement with live virus vaccinations greatly exceed those in vaccination with Salk vaccine. These factors, in turn, are important in reducing circulation of wild poliovirus strains. In order to completely deprive wild poliovirus strains of the possibility to circulate among the population regularly, and at the same time to provide uninterrupted immunization, we are planning in 1961 to carry out the following schedule of live virus vaccinations which are to involve all children of 0 to 3 years of age:

First vaccination of newborn children during the first seven days of life with Type 1 Sabin vaccine;

Second vaccination between the second and the third month of life with a mixture of Types 2 and 3 vaccine;

Third vaccination between the fourth and the sixth month of life with a trivalent mixture of Types 1, 2, and 3.

Accordingly, the cycle of immunizations will, as a rule, have been completed by six months of age. Besides, there will be annual revaccination of children aged one, two, and three years with one feeding of trivalent mixture (during certain times of the year). So there will be annual vaccination of four groups of children under three years of age. This will enable the population to be provided uninterruptedly with immunizing vaccine strains of poliovirus and to counteract the dissemination of wild poliovirus strains.

In addition to supplies necessary to ensure the fulfillment of internal programs of mass immunization with live poliovirus vaccine from Sabin's strains, the Soviet Union furnished, by way of friendly assistance, vaccines of Types 1, 2, and 3, separately for 2.5 million children in Hungary, Types 2 and 3 vaccines for 1.5-2 millions in Czechoslovakia, for 1.5 million children in the People's Republic of Viet-Nam, and for 2.2 million children in Bulgaria. Small amounts were given to Albania and China.

Because of the complete support on the part of medical workers and the entire population, we have no doubts that the large program envisaged by us for immunization with live vaccine in 1960 will be realized. Oral immunization with live poliovirus vaccine in dragée-candy makes it possible to accomplish mass vaccination of susceptible population within short periods of time. And this guarantees radical prevention of poliomyelitis epidemics.

Poliomyelitis vaccinations in 1960 were carried out through the local medical establishment on strictly a voluntary basis, with maximum public cooperation, this being due to broadly organized sanitary propaganda (through the press, radio, TV, newsreels, lectures, leaflets, etc.).

In 1959-1960 in vaccination areas, advisory teams were organized consisting of experienced physicians, charged with detailed investigation of all cases of CNS involvement suspected of polio. Over 5,000 persons were examined serologically and virologically in vaccination areas. The practice of laboratory diagnosis in vaccination areas was considerably broadened and substantiated in the Baltic republics, in Tashkent, Karaganda, Alma-Ata, Moscow, and other regions. All this enabled us to considerably improve the organization of mass vaccinations and to set up thorough survey of the vaccinated, to broaden diagnostic findings with regard to poliomyelitis and to secure adequate and complete recording of all polio cases during and after vaccination.

Based upon the research work and field trials carried out by our Institute in cooperation with other scientific institutions, several conclusions can be drawn with regard to the major problems concerning live poliovirus vaccine.

1. *Safety of live vaccine.* It can be taken for granted now that the live vaccine from Sabin's strains prepared with observation of certain conditions is a completely safe and areactogenic preparation. Large-scale field trials of the vaccine have confirmed this. Safety tests during live vaccine production possibly still require some simplification and improvement, but even such as they are, they ensure highly consistent and reproducible results.

The problem of reversion of pathogenic properties of live poliovirus vaccine proved to be practically non-existent under conditions of simultaneous mass immunization of the entire population in a given region. In the Soviet Union there were no poliomyelitis cases on record which could be attributed to the immunization with live vaccine from Sabin's strains.

Reactogenicity of live poliovirus vaccine was studied by many physicians on the basis of registered complaints put forward by the vaccinated. Specialists hold that under conditions of mass immunization there may be instances when vaccinations coincide with all kinds of symptoms produced by other affections. It was impossible to establish unequivocally any relationship between various complaints and symptoms and the effect of the vaccine. The total number of the so-called reactions to the vaccine itself is very insignificant (no more than three per 100,000). The question of the reactogenicity of the live vaccine, apparently, requires some further detailed study, but is now of no great practical importance.

2. *Immunologic activity of live poliovirus vaccine.* Workers of our Institute, as well as from laboratories in Tashkent, Riga, and some other places, have carried out very extensive serological investigations with several thousand paired blood specimens collected from those immunized with live vaccine under different epidemiological conditions or in connection with vaccination according to different schedules.

These investigations demonstrated that, as a rule, immunologic activity of Sabin's attenuated strains compared quite favorably with the best standards of killed Salk vaccine and was even superior to them with regard to the time of seroconversion and its duration.

During 1960 in the Soviet Union the most frequently used immunization schedule was that with individual monovaccines (Types 1-3-2) followed by subsequent revaccination with a trivalent mixture. Groups immunized in 1959 with trivalent mixture were revaccinated in 1960 twice: with Type 1 and then with trivalent mixture. The experience of 1959 demonstrated the schedule Types 1-3-2 to have yielded optimal serological results. In 1959 and 1960 vaccination schedules involving one or two feedings with trivalent mixture have been subject to large-scale trials. Judging by serological data and epidemiological observations of 1959, these schedules also gave fairly good results. It would evidently be more advisable to have a choice of several immunization schedules depending upon relevant epidemiological indications and local conditions. The results of serological investigations point to the influence of time factors upon gradual increase of immunologic response of the vaccinees to immunization, viz., the results of antibody survey three months after immunization with Type 1 were definitely better than one month thereafter.

In the course of observations on vaccine virus excretion during revaccination it was possible to establish that in contrast to the killed Salk vaccine, the live poliovirus vaccine produced not only serological, but to a certain extent, also local immunity, i.e., immunological resistance of cells of the alimentary tract. This may result in progressive reduction of poliovirus circulation among the population.

The problems associated with the dynamics of the development of local immunological resistance to poliovirus demand, however, further study and more precise definition. It is necessary also to solve the problem of providing continuous immunization considering possible complete disappearance of latent poliovirus strains. We believe that for this purpose children of 0 to 3 years of age should be immunized with live vaccine annually.

3. *Virus carriage and contact transmission.* As evidenced by numerous observations, including those performed at our Institute, oral immunization with live poliovaccine is accompanied by more or less protracted excretion of vaccine strains. The duration of virus excretion in children without antibodies is approximately twice as long as in those possessing them. In the environment of the vaccinees (under conditions of family contact) all persons susceptible to poliomyelitis pick up vaccine within a very short time and become immunized latently. No untoward reactions could be noted in connection with virus carriage and transmission of vaccine viruses by contact. Quite the contrary, it can be regarded as established that virus carriage and contact transmission during mass vaccination represent an exceptionally favorable factor conducive to all-round and more rapid immunization within families and communities.

4. *Interference.* In oral immunization with live poliovirus vaccine the phenomenon of interference between wild and vaccine poliovirus strains, between non-poliomyelitis enteroviruses (ECHO and Coxsackie groups) and live vaccine strains, and also interference between individual types in live vaccine acquire special importance.

As a result of extensive investigations it has been found that interference between vaccine and other enteroviruses occurs under various epidemiological conditions, with frequency in different months of the year. Winter months are, apparently, most advantageous for vaccination. The data obtained enable us to draw a preliminary conclusion to the effect that most instances of oral immunization failures are due to the influence of interference upon the vaccination process. The interference may, probably, be overcome by particularly massive vaccinations (with involvement of no less than 50 per cent of the susceptible population) and by repeated vaccinations over a period of one to three years.

Furthermore, complete study of patterns governing the interference between enteroviruses and vaccine strains of poliovirus is urgent.

5. *Epidemiological effectiveness of mass immunization with live poliovirus vaccine.* This was established for the first time in the Soviet Union on the basis of information gathered in 1959, the latter discussed in detail in our Report No. 2 (Moscow, 1960). Particularly convincing was the reduction in poliomyelitis incidence in Estonia and Lithuania, where triple immunization with live vaccine was carried out before the summer poliomyelitis season, as well as in Karaganda and in the Moscow region where conditions favored comparisons between the incidence in the vaccinated and the unvaccinated groups. In the first three months of 1960, a satisfactory situation with regard to poliomyelitis continued to prevail in Estonia, Lithuania, and other areas which were subject to observations (see Tables 5 and 6).

In Estonia during the last 12 months there were only 12 poliomyelitis cases. These were recorded only during the first six months, and the last six months were completely free from poliomyelitis. An unusually low number of poliomyelitis cases is recorded after vaccination in Lithuania. In Moscow, where every fourth inhabitant has been vaccinated (or over 90 per cent of the population under 20 years of age) a sharp reduction in poliomyelitis incidence is being observed. In all areas where vaccinations with live vaccine have been completed, the number of poliomyelitis cases is rapidly decreasing. Observations in carefully controlled trials indicated very high epidemiologic effectiveness of mass immunizations with live poliovirus vaccine (9.5-20.5-fold reduction in paralytic poliomyelitis incidence).

Of particular interest are materials covering the 1959 vaccination program in Tashkent. Simul-

taneous mass immunization with trivalent live vaccine during a period of steep rise in poliomyelitis incidence in Tashkent in 1959, was definite proof of the possibility of considerably reducing poliomyelitis incidence within five weeks, this being due to the effect of interference by the vaccine virus with the epidemic process and to progressive immunization of susceptible population groups.

In conclusion, I should like to call attention to the fruitful international cooperation in the matter of live poliovirus vaccine, which has existed between scientists of the U.S.A., the Soviet Union, Czechoslovakia, Hungary, German Democratic Republic, People's Republic of China, Bulgaria, and other countries on the basis of broad exchange of scientific information, personal contacts, mutual exchange of results of research work, and friendly support.

All that has been done heretofore is, undoubtedly, only a promising start of regular future international cooperation among medical scientists towards the speediest eradication of certain infectious diseases or, at least, sharp reduction of their number.

TABLE 8. DATA ON LIVE POLIOVIRUS VACCINE DISTRIBUTION IN 1960
(January-May, inclusive)

REPUBLICS	NUMBER OF DISTRIBUTED INOCULATION DOSES IN THOUSANDS					
	MONOVACCINES			TRIVACCINE	TOTAL	INCLUDING FOR PRIMARY VACCINATIONS
	TYPE 1	TYPE 2	TYPE 3			
RSFSR	39,407.3	34,657.5	37,610.4	4,409.8	116,085.0	40,907.3
Ukrain. SSR	12,080.6	9,206.3	9,466.6	6,454.6	37,208.1	14,504.0
Kazakhstan	2,294.0	—	—	3,502.7	5,796.7	3,600.0
Uzbekistan	3,176.0	200.0	200.0	4,406.0	7,982.0	4,400.0
Byelorussia	3,000.0	3,000.0	2,976.0	—	8,976.0	3,000.0
Georgia	1,500.0	—	—	1,699.2	3,199.2	1,500.0
Azerbaijan	1,404.0	30.0	—	1,557.0	2,991.0	1,434.0
Moldavia	600.0	—	—	900.0	1,500.0	1,100.0
Lithuania	750.0	—	—	2,000.0	2,750.0	2,000.0
Latvia	610.0	773.6	772.0	452.4	2,608.0	1,000.0
Kirghiz SSR	800.0	—	—	1,102.0	1,902.0	1,100.0
Tajikistan	750.0	—	—	1,102.2	1,852.2	1,100.0
Armenia	—	902.0	800.0	—	1,702.0	900.0
Turkmenia	550.0	—	—	928.0	1,478.0	900.0
Estonia	882.0	—	—	898.0	1,780.0	898.0
	67,819.9	48,769.4	51,825.0	29,411.9	197,810.2	78,343.3

TABLE 9. REDUCTION OF PARALYTIC POLIOMYELITIS INCIDENCE IN MOSCOW IN THE SECOND HALF OF 1959 AMONG CHILDREN VACCINATED WITH LIVE VACCINE

GROUPS OF CHILDREN UNDER 15 YEARS OF AGE	NUMBER OF CHILDREN IN THOUS.	NUMBER OF CASES	INDEX PER 100,000	RATIO OF INDICES
Vaccinated	1,058	59	5.6	1:9.25
Unvaccinated	392	203	51.8	

TABLE 10. PARALYTIC POLIOMYELITIS IN MOSCOW BY MONTHS

YEARS	J	F	M	A	M	J	J	A	S	O	N	D	TOTAL
1957												6	—
1958	18	14	23	21	22	37	58	57	42	20	13	11	336
1959	16	10	9	25	26	23	35	54	34	39	26	21	318
1960	7	11	13	12	5								

TABLE 11. SPECIFIED DATA ON PARALYTIC POLIOMYELITIS IN ESTONIAN SSR

YEARS	J	F	M	A	M	J	J	A	S	O	N	D	TOTAL FOR THE YEAR
1957	3	11	4	5	3	5	10	12	19	11	4	8	95
1958	7	2	4	7	3	5	12	74	138	89	25	19	385
1959	20	6	4	6	2	2	2	2	2	—	—	—	46
1960	—	2	—	—									

NUMBER OF PARALYTIC CASES

YEARS	HALF-YEAR PERIODS		PER CENT
	1	2	
1957	31	64	67.3
1958	28	357	92.7
1959	40	6	13.0

TABLE 12. PARALYTIC POLIOMYELITIS IN LITHUANIAN SSR (BY MONTHS)

YEARS	J	F	M	A	M	J	J	A	S	O	N	D	TOTAL
1955	2	2	8	3	9	15	48	78	75	49	26	16	331
1956	16	6	12	14	11	26	27	19	28	16	7	7	189
1957	7	10	8	9	5	7	26	25	15	6	6	7	131
1958	5	6	6	—	4	21	30	45	40	25	17	17	216
1959	10	12	6	4	3	4*	7	3†	1	2*	1	1	54
1960	—	3	1	—									

* Of these, two cases in June and one case in October 1959, in persons over 20 years of age (unvaccinated).
† Of these, one case in August with doubtful diagnosis.

TABLE 13. PARALYTIC POLIOMYELITIS IN LITHUANIAN SSR (BY HALF-YEAR PERIODS)

YEARS	FIRST HALF-YEAR	SECOND HALF-YEAR	PER CENT	RATIO (TO 1959)
1955	39	292	82.2	292:15=19.5
1956	85	104	55.0	104:15=6.9
1957	46	85	64.9	85:15=5.7
1958	42	174	80.5	174:15=10.2
1959	39	15	27.8	—

TABLE 14. SPECIFIED DATA ON POLIOMYELITIS CASES IN LITHUANIAN SSR, 1959 (BY MONTHS)

GROUP	J	F	M	A	M	J	J	A	S	O	N	D	TOTAL
I	10	12	6	4	3	4	8	5	1	2	1	1	57
II	—	—	—	—	—	—	1	2*	—	—	—	—	3
III	—	—	—	—	—	2	—	—	—	1	—	—	3
IV	10	12	6	4	3	2	7	3†	1	1	1	1	51
V	—	—	—	1	—	1	1	1	—	—	1	1	6

Composition of groups:

I. *All recorded cases,* including persons over 20 years of age (not vaccinated in Lithuania), and the so-called mild nonparalytic forms without laboratory confirmation.

II. Including only cases of the so-called nonparalytic forms without laboratory confirmation.
Note: * One case in this group in live virus vaccinec (case No. 50).

III. Only cases of paralytic polio in persons over 20 years of age (not vaccinated in 1959 in Lithuania).

IV. *Only paralytic cases in persons under 20 years of age.*
† One fatal case in this group in an unvaccinated person is questionable (insufficient investigation).

V. Including paralytic polio in persons vaccinated with live vaccine.

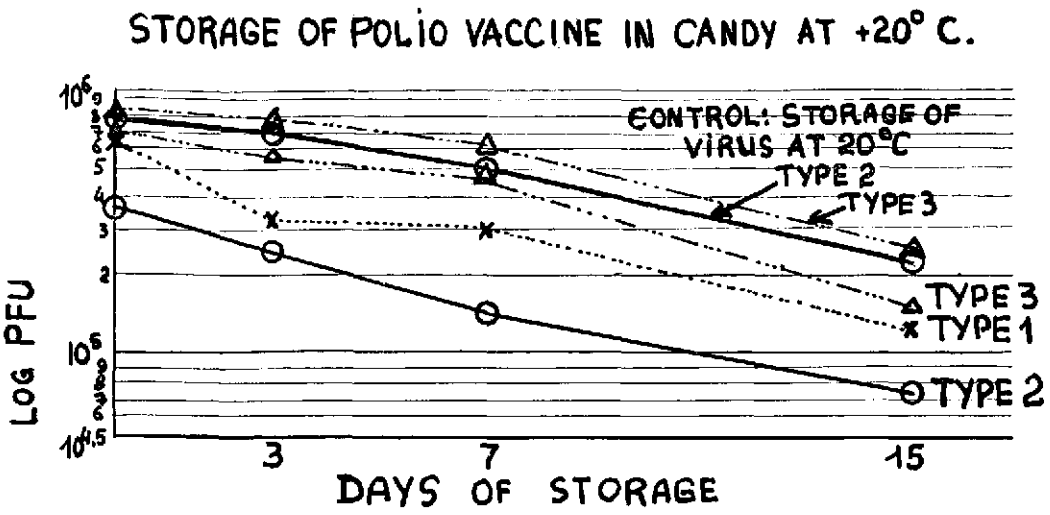


FIG. 2. Storage of polio vaccine in candy at +20° C.

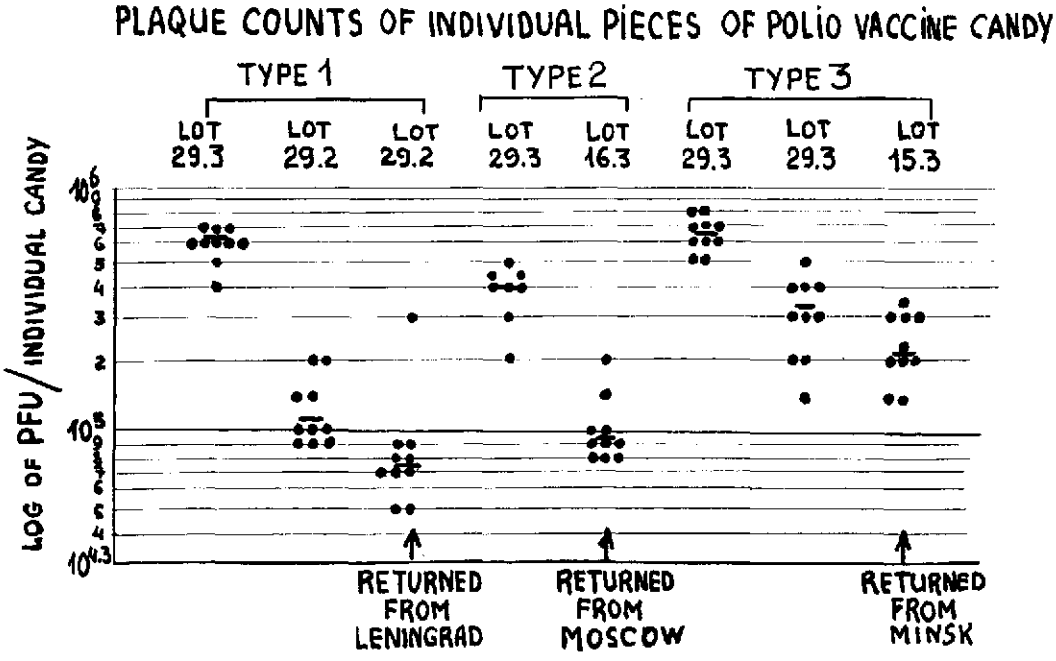


FIG. 3. Plaque counts of individual pieces of polio vaccine candy.

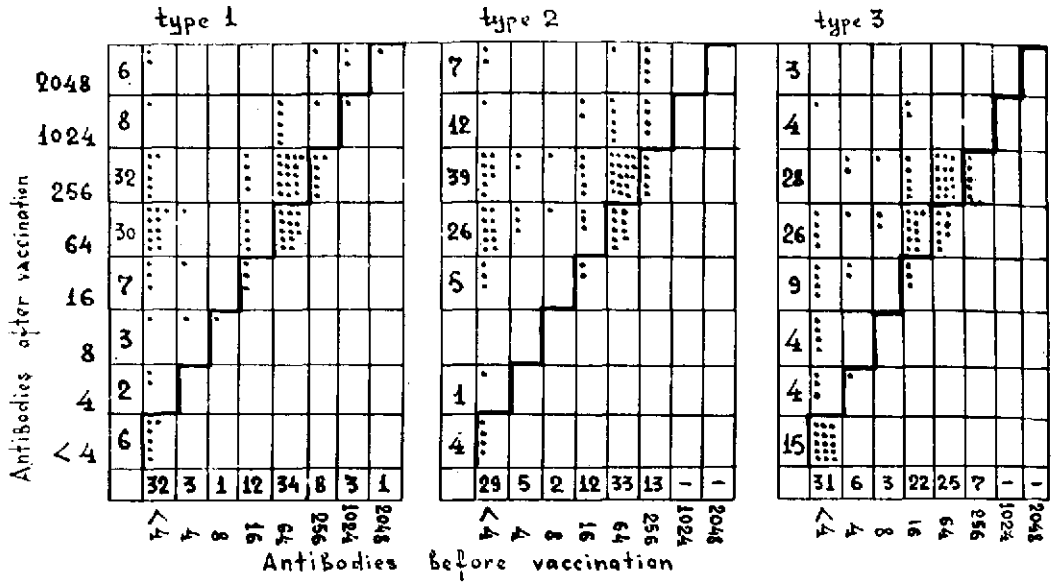


FIG. 4. Antibody response in children after vaccination with candy containing trivalent live poliovirus vaccine.
(Klin Mosc. reg. Fleer., 1959)

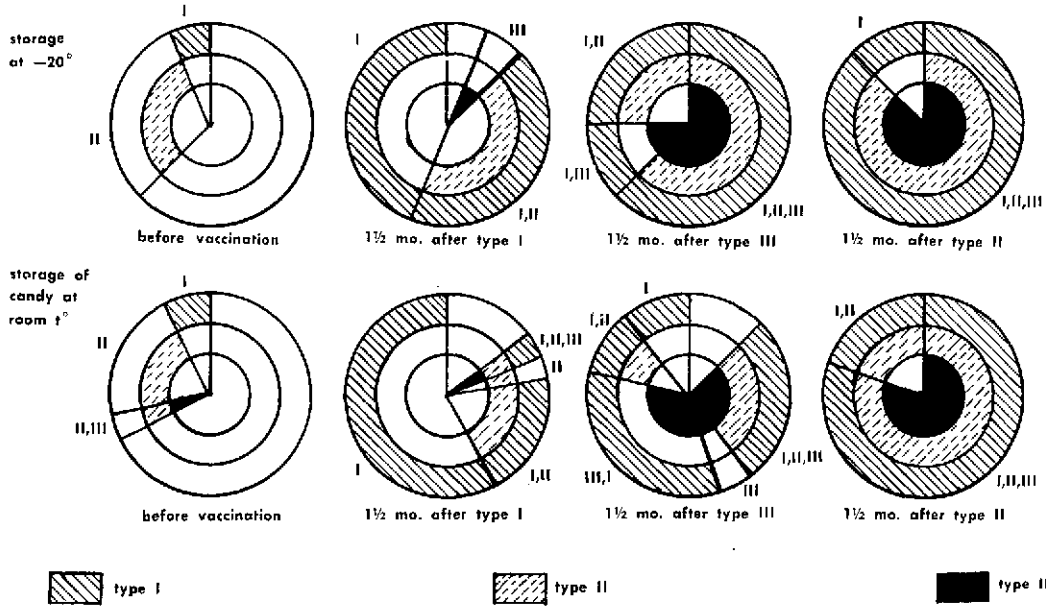


FIG. 5. Antibody patterns before and after vaccination with live vaccine in candy according to schedule I-III-II.

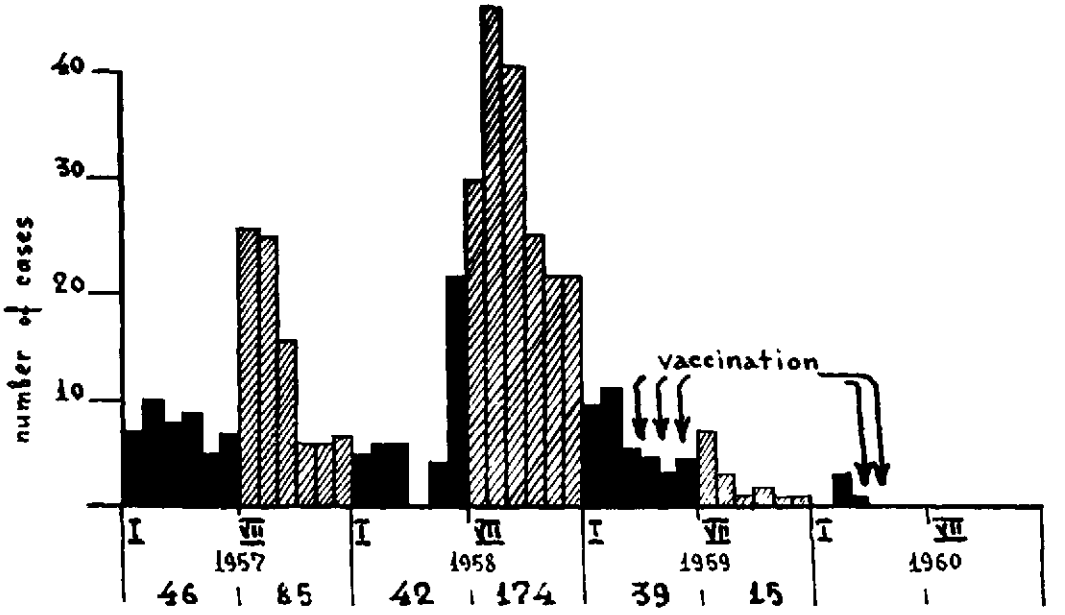


FIG. 6. Paralytic poliomyelitis in Lithuanian SSR.

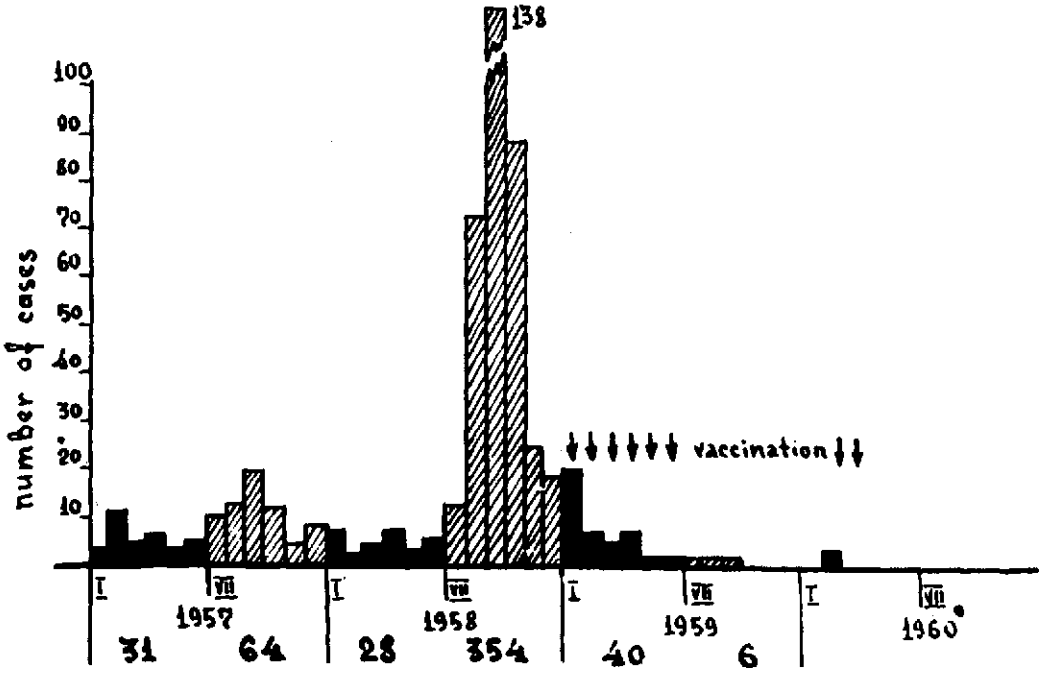


FIG. 7. Paralytic poliomyelitis in Estonian SSR.

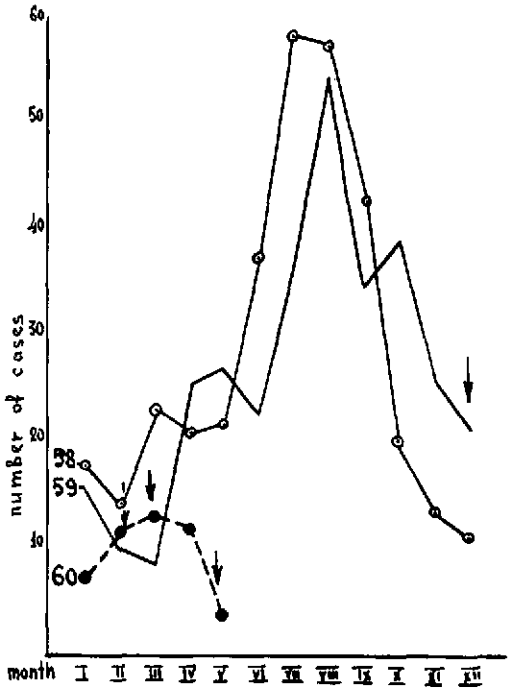


FIG. 8. Paralytic poliomyelitis in Moscow.

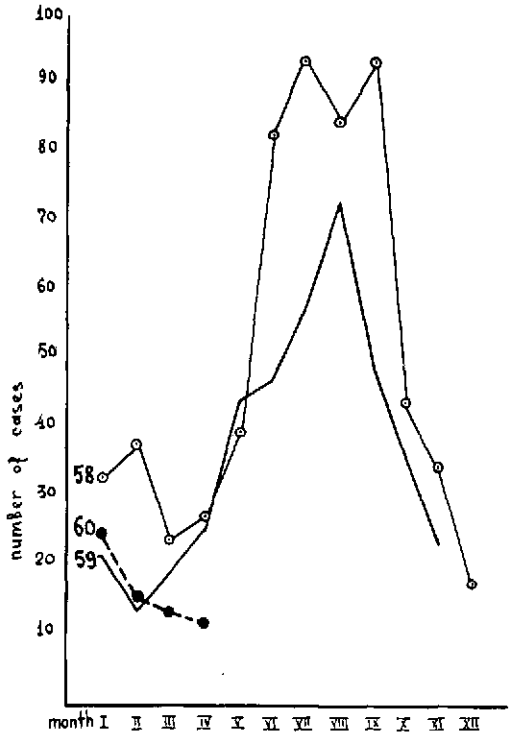


FIG. 9. Paralytic poliomyelitis in Moscow region.

DISCUSSION

CHAIRMAN LÉPINE: Thank you, Dr. Voroshilova. The paper is open for discussion.

DR. SMADEL: It goes without saying that our Russian colleagues deserve our congratulations on the many scientific accomplishments with live polio vaccine, certain of which they have reported here today. I should like to comment particularly on the matter of the Tashkent outbreak and to refer to Figure 11 of the booklet made available to us by our Russian colleagues and entitled "On Mass Oral Immunization of Population in the Soviet Union against Poliomyelitis with Live Vaccine from A. B. Sabin's Attenuated Strains," Moscow, 1960. This figure shows the difference in rate of paralytic disease among those who were vaccinated and among the unvaccinated portion of the population.

I am in agreement with the comments of Dr. Chumakov and his associates regarding the possibility that the prompt and appreciable drop in paralytic disease among the vaccinees during the period when the rate in the non-vaccinated continued to rise might represent interference by the attenuated virus with the wild virus.

I should hope, as a scientist and as a public health officer, that the opportunity may arise in the next year for similar studies to be undertaken not only by our colleagues in the USSR but by scientists in other parts of the world. It is important that we learn whether we can depend on the interference phenomenon to work for us instead of against us. This week we have heard more about wild enteroviruses in the intestine that interfere with implantation and growth of attenuated polio strains, thus hampering the development of immunity. Tashkent may have provided the first example of a "good" virus driving out of a "bad" virus in the population suffering an epidemic of polio.

CHAIRMAN LÉPINE: Dr. Voroshilova, do you wish to comment on this?

DR. VOROSHILOVA: I spoke only as the tongue of Dr. Chumakov. We may expect that such data as Dr. Smadel mentioned will be collected during future years.

DR. VERLINDE: I wish to comment very briefly on the slides that Dr. Voroshilova has shown with regard to the incidence curves of paralytic poliomyelitis before and after the administration of the live vaccine.

I should like to say that I too very much admire the studies that have been undertaken in the Soviet Union. Let me also state that I personally believe in the epidemiological effectiveness of the vaccine. But before drawing definite conclusions with regard to the epidemiological effectiveness, I think that we can make a judgment only after more years have passed. In this respect, may I remind you of one of the slides I showed yesterday in which one could see that in the Netherlands, after the epidemic year 1956 (in which a high incidence of poliomyelitis occurred), the incidence gradually decreased during the years 1957 and 1958 and that an extremely low incidence was reached in 1959.

If we had administered live poliovirus vaccine on a very large scale, this curve might perhaps be an excellent example of the epidemiological effectiveness of the vaccine. However, we have not administered the vaccine on such a scale.

DR. CHUMAKOV *(through an interpreter)*: Dr. Verlinde believes that several years will elapse before we can give a positive answer. I am not of that opinion. Our experiments in the Soviet Union show that the mass immunization—and I refer to the first part of this year—has already produced such a sharp decrease in the incidence of the disease that there is no doubt that this is connected with the vaccination.

I should add that we have shown only a few examples, but this is confirmed in all the areas where we have applied *mass immunization.* Everywhere we have achieved the sharpest decrease very successfully. Without exception, there has been no instance in any area where we have conducted mass immunization on a large scale that

it has not been accompanied by sharp reduction in the incidence.

It is possible, of course, that our conditions are different from those prevailing in small countries, such as the Netherlands, or in those where incidence can rise very sharply and then fall very sharply too. In our country, a very large country, this does not happen as a rule. A high incidence lasts for two or three years at least; it is not a uniform incidence. After the peak year, of course, there is a reduction, but the incidence does not last one year only.

I think that in our country it will be possible to give an answer within a short period of time on the question of effectiveness.

Furthermore, based on experience acquired over the last year and a half, together with some observations, I trust that Dr. Smorodintsev will present more facts today, indisputable proof that the epidemiological effectiveness of immunization with live vaccine is beyond doubt.

I do not think we should try to create any difficulties with virology or serology and epidemiology; they give full proof in this direction.

DR. SABIN: I should merely like to point out once more that nature can spread polioviruses and produce a certain effect, such as has been observed in the Netherlands, and man has recently been doing it on a very much larger scale than nature. We do not want to say here that man cannot achieve even better what nature has done, nor do we want to detract from the capacity of nature in achieving a similar effect.

Our friends who have worked a great deal with Salk vaccine are convinced, as I am, that when antibody is produced, one can obtain protection against paralysis.

I believe that the capacity of live virus vaccine to produce antibody has been demonstrated. Why anybody who is convinced that antibody can produce protection against paralysis should be surprised that live vaccine, producing such antibody, should be capable of providing the same protection against paralysis, is beyond me.

DR. BOZEMAN: I notice a sentence in Dr. Chumakov's paper, which reads as follows: "The total number of the so-called reactions to the vaccine itself is very insignificant (no more than 3 per 100,000)." Would Dr. Chumakov mind describing the type of reaction?

DR. CHUMAKOV *(through an interpreter)*: This section was not mentioned, not because it is insignificant but merely because we wanted to save time in the presentation of our report and avoid repetition of data, which had already been obtained and included in the second report.

The number of so-called reactions to the vaccine is indeed very insignificant. The character of these reactions differs widely and reflects the most varied symptoms that could be collected, if one wished to survey about 100,000 children. Of course, if such a survey is carried out, one will always find some kind of symptoms. However, among the symptoms which were observed and recorded, we have never had any serious reactions which could have implicated the vaccination work. More often than not, we found a relation to some digestive intestinal trouble for instance, vomiting and diarrhea. I myself am convinced that this was merely a concidence because there was no proof at all that this was connected with the vaccine. On the other hand, we found an increase in the temperature of the patients shortly after the feeding, but we believe this is merely a coincidence. It is true that in our country there are doctors who believe that this is perhaps connected with some immediate effect, but I think it is difficult to suppose that 30 minutes, an hour, or even 24 hours after feeding of the virus preparation, some gastric or intestinal symptoms, such as diarrhea and vomiting, or an increase in temperature could occur so soon.

So what we referred to as these extremely rare and insignificant reactions were, as I stated, merely coincidental.

DR. KITAOKA: Yesterday afternoon I mentioned that there is a possibility that the wild strains can be replaced by attenuated virus during mass vaccination in an endemic period. That is merely a possibility. As Dr. Sabin mentioned, even if the natural phenomenon is much more powerful than the artificial procedure, the new weapon should be used against it.

In my opinion, the attenuated virus should be recommended or used in such endemic periods where the wild virulent virus is known to be distributed, expecting that replacement by the attenuated virus may take place. The answer to the question of whether or not such a possibility is feasible, remains to be seen.

DR. STUART-HARRIS: I should like to ask Dr. Voroshilova whether she has any evidence concerning the properties of any strains of poliomyelitis viruses recovered during 1960, in such areas as Lithuanian and Esthonia, where mass campaigns with the attenuated virus vaccination have been carried out for the longest period of time.

I recall that when Dr. Sabin was writing about attenuated virus vaccines some years ago, he coined the phrase, I believe, of "causing a replacement of nature's viruses by the attenuated viruses from the laboratory." One of the interesting features in his own data, and also in the data from Czechoslovakia, is the lack of persistence of the attenuated viruses under field conditions. I wonder whether we could have any information on the status in any of the Baltic States or any other areas of the USSR.

DR. CHUMAKOV *(through an interpreter)*: In 1960, in Esthonia, at the beginning before revaccination, we carried out some investigations in a children's home and the results we have obtained are therefore of limited significance. We do not want to generalize these data and to apply them to the whole situation in Esthonia. The data, however, were interesting.

It so happened that out of 108 samples, about 58 had pathogenic agents, 25 strains being of Type 3 poliovirus. At any rate, we investigated eight of them for the *T* marker. All of them were *T*+, but neither in that children's institution, in the town, nor anywhere in the Republic, did we register a single case of poliomyelitis at the time. And since we had a mass circulation of strains of Type 3 in that children's institution, this fact led us to carry out the investigation on a larger scale, of the strains recovered in this Republic. But this investigation has not been completed.

We have preliminary data to the effect that this mass infection with strains of Type 3 poliovirus was connected with that particular institution only. So it has not been confirmed anywhere else and we have not discovered any other strain of poliovirus.

Furthermore, we had data last year in Esthonia when we carried out virological investigations on the various types of diseases—meningitis, for instance, and various incidents of intestinal diseases—at that time we discovered several strains of poliomyelitis virus. Several months after vaccination, poliomyelitis viruses were still circulating, if I may put it that way. But last year we did not have total vaccination. There were many gaps in that vaccination program, and we believe that regular vaccination from the very first days of life, mass immunization that will reach as high as 90 per cent of the susceptible population, will make it possible to exclude the circulation of these viruses more or less completely. I merely refer to the data which are now at my disposal.

DR. MELNICK: I should like to ask Dr. Chumakov if this institution in Esthonia, which he just mentioned to us, was an institution where vaccination had taken place the year before, and if so, whether the children had responded with antibodies?

The reason I ask the question is because, when I was in Moscow recently, Dr. Chumakov told me about a group of children who had been vaccinated one year before and in whom antibodies against Type 3 had been produced. But Type 3 wild viruses were found to have infected these children naturally a year later.

DR. CHUMAKOV *(through an interpreter)*: The information at my disposal refers to the children's institution where we had children from one and a half months to three years of age. It is obvious that there was a large turnover in the youngest group. This was not a stable group; during the year new children were admitted and others left—those who were over three years of age. So that before sampling, we also had unvaccinated children in this children's home who found themselves in an enviroment which was favorable to the circulation of various viruses.

The data obtained were as follows: Out of the 25 strains which belonged to Type 3, we had eight *T*+ strains which we succeeded in checking. Approximately half were found in children who had been vaccinated during the previous years and who had antibody.

It is possible that there is no direct correlation here with the presence of antibodies and virus of the Type 3. But this has been noted without the circulation of the virus. It had been men-

tioned already in the studies by Dr. Voroshilova and co-workers. It had been shown that the presence of antibodies does not always interfere with the subsequent circulation of the Type 3 virus. The question of intestinal resistance to Type 3 is a special question which deserves special examination.

DR. VAN ROOYEN: I cannot refrain from making a chronological reference to the subject of poliomyelitis, as it has been approximately 30 years since Dr. Sabin and I have been continuously involved in poliomyelitis research. Today, it gives me great pleasure to listen to this tremendous and fantastic accomplishment reported by the Russian workers in the field of public health. May I also extend to you, Dr. Sabin, my congratulations for your association in these developments.

DR. SABIN: Since this seems to be a time for flowers, I think that flowers are particularly due Professor Chumakov. Having been to the Soviet Union four times since 1956, and having observed all this work in the process of development, from Smorodintsev's early basic work to the present, I am personally convinced that if it had not been for Professor Chumakov's extraordinary energy, effort, and capacity for organization—capacity to get so many people from different republics to work with him, as well as his capacity to convince the Academy of Medical Sciences and the Ministry of Health—the Soviet Union would have been no further along in this effort than are some of the other countries. To Professor Chumakov, we owe this very special gratitude.

CHAIRMAN LÉPINE: Thank you. We certainly congratulate our Russian colleagues, and especially Dr. Chumakov and his team.

SEVENTH SESSION

THURSDAY, 9 JUNE 1960, 9:00 A.M.

Chairman

DR. ANDREW J. RHODES
Director, School of Hygiene
University of Toronto
Toronto, Canada

TOPIC III. EFFICACY. (B) FIELD EVIDENCE
(continuation)

Presentation of Papers by:

Dr. M. E. Flipse

Dr. G. M. Erickson

(DISCUSSION)

Dr. Stanley A. Plotkin

Dr. James H. S. Gear

(DISCUSSION)

Dr. A. A. Smorodintsev

TOPIC III. EFFICACY. (B) FIELD EVIDENCE
(continuation)

17. A PRELIMINARY REPORT ON A LARGE-SCALE FIELD TRIAL WITH THE ORAL COX-LEDERLE ATTENUATED POLIOMYELITIS VACCINE IN DADE COUNTY (MIAMI), FLORIDA

M. E. FLIPSE, G. M. ERICKSON, W. R. HOFFERT, M. M. SIGEL, N. J. SCHNEIDER, L. B. CLAYTON, A. W. MENZIN, R. E. MARKUSH, F. HOWELL, JR., M. I. CROSSLEY, T. E. CATO, A. V. HARDY, AND F. J. EVANS

Section of Preventive Medicine and Public Health, University of Miami School of Medicine; Dade County Department of Public Health; Dade County Medical Association; and Florida State Board of Health

DR. FLIPSE *(presenting the paper)*: During the late fall and early winter of 1959 the individuals and groups responsible for the control and prevention of poliomyelitis in Dade County, Florida, were forced to critically evaluate the past and possible future programs against this disease in this community. The factors which forced this evaluation and which influenced the decisions which were subsequently made, included:

1. The Salk-type polio vaccine did not provide a satisfactory degree of protection against paralytic and fatal poliomyelitis. In 1959 in Dade County 15 of 38, or 40 per cent and in Massachusetts 64 of 137, or 47 per cent, of the reported cases of paralytic poliomyelitis had previously received three or more of the Salk-type polio injections (Table 1). In Dade County the failure of the Salk vaccine occurred with nearly equal frequency to both Types 1 and 3. Likewise, the mortality data were disturbing (Table 2). In 1959 in Dade County, two of three, and in New Jersey four of seven deaths from poliomyelitis occurred in individuals who had previously received three Salk-type injections. One death from poliomyelitis in Florida was a school-age child who had received five Salk injections, the last only a few months before the onset of his fatal illness.

2. There was evidence of increasing apathy and decreasing participation in the serial and booster injections required by the killed vaccine programs. These trends were obvious in spite of repeated intensive health education and promotional campaigns by official and voluntary health agencies and professional groups, and in spite of the fact that polio vaccination was available without charge in Health Department clinics to all requesting it.

3. The presentations at the First International Conference on Live Poliovirus Vaccines and at the American Public Health Association Meeting gave evidence of the practicability of large-scale programs with apparently safe and antigenically potent oral polio vaccines.

4. All segments of the community seemed willing, even eager, to cooperate in a trial of a new method for the prevention of poliomyelitis, which appeared to have both theoretical and practical advantages over killed polio vaccine.

After extending invitations to interested in-

* Supported in part by a grant from the Lederle Laboratories Division of the American Cyanamid Company, Pearl River, New York.

TABLE 1. SALK VACCINATION STATUS OF CASES OF PARALYTIC POLIOMYELITIS—1959

AREA	TOTAL CASES	SALK STATUS							
		NONE		3 OR MORE		1–2		UNKNOWN	
		No.	%	No.	%	No.	%	No.	%
Dade County	38	13	34	15	40	10	26	0	0
Florida	144	82	57	34	23	24	17	4	3
Massachusetts	137	51	37	64	47	21	15	1	1
United States	5450	3418	60	928	17				

TABLE 2. SALK VACCINATION STATUS OF FATAL CASES OF POLIOMYELITIS—1959

AREA	TOTAL CASES	SALK STATUS	
		NONE	3 OR MORE
Dade County	3	1	2
Florida	14	9	5
New Jersey	7	3	4

dividuals and official and voluntary organizations to advise, participate, or observe, numerous informal and several formal conferences were held. After due consideration and on the recommendation of an expert advisory committee, it was decided to undertake a large-scale field trial of oral polio vaccine.

ORGANIZATION OF THE FIELD TRIAL

Sponsorship. The Dade County Community Polio Program, the name selected for the field trial, was sponsored by three organizations: the Dade County Department of Public Health, the University of Miami School of Medicine, and the Dade County Medical Association. The project was carried out with the official approval of the Florida State Board of Health, the Polio Advisory Committee and the Board of Governors of the Florida Medical Association, the Dade County Osteopathic Medical Association, the Miami Pediatric Society, and the Dade County School Board, with the knowledge and counsel of the U. S. Public Health Service. The field trial was truly an entire community effort supported by many individuals, professions, and organizations. Without the wholehearted support of the practicing physicians, public health nurses, school teachers, and the communications media (press, radio, television) the program would have been impossible; without the support of various governmental, health, fraternal and civic organizations, churches, pharmacists, and industry, the task would have been much more difficult.

Publicity. The extensive professional and public education and publicity program was conducted by the Dade County Medical Association. Major emphasis was placed on the fact that there was no cost to the individual and that no injections were required. Care was taken not to detract from the Salk-type vaccine, the only vaccine that would be available to most of the American population. The large volume of highly accurate reporting by press, radio, and television is a tribute to the public service programs of the communications media.

Certain features of the Dade County Community Polio Program require mention.

Age Selection. During recent years, approximately one half of the reported cases of paralytic poliomyelitis reported in Dade County have been in children less than five years of age. Except for a rare sporadic case in a person over 40 years of age, the remaining 50 per cent of cases were nearly equally divided between the

age groups from five through 19 and 20 through 39. On the basis of this information, it was decided to offer oral polio vaccine to all residents less than 40 years of age.

Season. Although paralytic poliomyelitis in Dade County occurs during all seasons of the year, the incidence is lowest during the months of December through April. By restricting the actual administration of oral vaccine to the months of February, March, and April, it was hoped that problems raised by coincidental "wild" poliovirus infections would be minimized. Certain epidemiological aspects of poliomyelitis in Dade County are considered separately in the companion paper by Erickson and associates.

Vaccine. The Lederle-Cox vaccine was used in the Dade County Community Polio Program. Detailed descriptions of the vaccine used by us, as well as in Minnesota, Latin America, and other areas of the world, were presented at the First International Conference on Live Poliovirus Vaccines and at other places by Drs. Cox, Cabasso, Ruegsegger, Barr, Martins da Silva, and others, and will not be reviewed at this time.

Although the published reports indicate a high degree of stability of the liquid poliomyelitis vaccine, adequate precautions were taken to keep it refrigerated but not frozen insofar as possible. Periodically, large shipments of the vaccine were flown into Miami and placed in the appropriately refrigerated storage. From this point it was distributed to immunization centers through Health Department channels. With the voluntary cooperation of the pharmaceutical profession the distribution to the 1,300 participating physicians was through routine trade channels. A central record system at the Health Department was maintained so that inventory and distribution control of the vaccine during and after the actual immunization program have been assured.

The dosage employed was a single administration of 2 ml. dose of the liquid trivalent attenuated poliovirus vaccine containing 1,200,000 tissue-culture doses ($10^{6.1}$ TCD_{50}) of strains Type 1-SM, Type 3-Fox, and 2,000,000 tissue-culture doses ($10^{6.3}$ TCD_{50}) of the Type 2 (MEF_1 strain). This dosage was used regardless of age, weight or other factors. The vaccine was usually administered in 30 ml. wax-lined paper cups diluted with approximately 6 ml. of distilled water, although it was also given undiluted by dropper or by spoon to infants.

The only contraindications specified were vomiting, diarrhea and unexplained fever. Vaccine was given during all trimesters of pregnancy and to the newborn. The use of the vaccine in relationship to oropharyngeal surgery, corticosteroid therapy and other surgical and medical conditions varied widely depending on the individual convictions of the responsible physician.

Records. A prerequisite for vaccination was submission of a completed and signed request form. All of the data are currently being transcribed to individual permanent business machine cards, which will be alphabetized and maintained as permanent records in the epidemiologist's office in the Dade County Health Department.

In addition to the name, address, birth date, age group, sex, race, and Salk vaccine history, the week and place of vaccination are recorded and by means of the "batch no." the original record on any individual can be located with minimal inconvenience or delay. Blank Mark-Sense columns are available for the addition of other information at a later date.

TABLE 3. RATE OF REPORTING OF ADMINISTRATION OF ORAL POLIO VACCINATION

	Totals	
Week	Weekly	Cumulative
1	7,760	7,760
2		7,760
3	57,942	65,702
4	59,450	125,152
5	59,442	184,594
6	37,532	222,126
7	35,218	257,344
8	26,192	283,536
9	17,041	300,577
10	20,278	320,855
11	17,744	338,599
12	20,359	358,958
13	52,321	411,279
Total	411,279	411,279

Administration of Vaccine. All vaccine was given under the supervision of physicians or registered nurses in schools, clinics, churches, places of employment, shopping centers, fairs, or other places where eligible residents were assembled or could be encouraged to congregate.

With good organization and prior completion of the request form, as is possible in schools, industry and other groups, very rapid administration of the vaccine was possible. In one senior high school a staff of six was able to complete 2500 vaccinations in thirty-five minutes. A preliminary idea of the rate of feeding can be obtained from the data contained in Table 3 which indicates the number of completed forms returned to the Health Department each week. Significantly, more accurate data will be available when all completed forms have been returned and the actual week of feeding has been tabulated. As of 1 June 1960, 411,279 completed forms have been received. During the first week of February, there was a "trial run" involving 7,760 university students. Beginning 15 February, and during the following five weeks, there was a rapid saturation of the school age population. During the last week there was some increase in the rate of administration of vaccine and during this period the practicing physicians responded to a request to return all completed forms still in their possession.

It is of interest to know that the practicing physicians supervised the administration of one third of the doses of vaccine given; approximately 75,000 in their offices, 58,900 in industrial, church, and other special groups, and 5,900 in private schools—a total of approximately 140,600 doses. The remaining doses, approximately 270,600, were given under Health Department supervision; 145,200 of these were administered in schools to pupils and staff, and 59,400 doses to the general public in regular and special Health Department clinics. Also included in the above total were 18,700 doses that were given by the mobile units of the local chapters of the tuberculosis, cancer, and rheumatism and arthritis groups. During the latter weeks of the program, the Health Department operated its own fleet of mobile units in areas where the response to other health education techniques had not reached expectations. The 47,300 doses dispensed from the tailgates of stationwagons among the "hard to reach" segments of our population, were particularly important, for they included 14,000 pre-school children.

TABLE 4. SALK STATUS OF POPULATION BASED UPON CRUDE INTERVIEW SURVEY DATA

AGE GROUP	PERCENTAGE WITH 3 OR MORE INJECTIONS
0—4 years	56%
5—14 years	81%
15—19 years	86%
20—39 years	45%
40 and over	16%

TABLE 5. SAMPLE OF POPULATION FOR POLIOVIRUS ANTIBODY DETERMINATION

AGE GROUP	NUMBER TO BE TESTED
1 year	3
1—4 years	82
5—14 years	565
15—19 years	86
20—39 years	245
40 and over	305
Total	1,286

Pre-Vaccination Survey. Immediately prior to the immunization program, the immune status of the entire population to poliovirus was determined by a quota sampling method by both interview and serologic techniques. The field work for this combined survey was carried out by the entire group of communicable disease investigators of the various county health departments throughout the state and with the consultative services of the Statistical Section of the Communicable Disease Center, U. S. Public Health Service, and the Bureau of Business and Economic Research of the University of Miami. The random sample included 4,630 individuals in 1,499 households for interviews and blood specimens before and after the immunization program from a 1,286 subsample. The data are being tabulated and analyzed in terms of age, sex, race, socio-economic status, prior polio

immunization, as well as other medical and social items. More reliable data than previously have been available concerning the immune status to poliomyelitis of a large metropolitan area will also be provided. The uncorrected interview survey data (Table 4) suggest that the percentage of the population that has received three or more injections of the Salk-type vaccine is about 83 per cent of the five-19-year-old-age group, 45 to 55 per cent in the 0-4 and 20-39-year-age group, and less than 20 per cent in the 40-and-over-age group. The serologic results required to complete Table 5 are not yet available.

Related Research. In addition to the actual field trial, we have undertaken or are cooperating in a series of related projects. These include the following:

1. A study of polioviruses and other enteroviruses from sewage samples collected from representative points at weekly intervals before, during, and after the oral immunization program.

2. An enterovirus surveillance program by Dr. A. Gelfand of the U. S. Public Health Service, based on rectal swabs submitted monthly from 100 Dade County infants and children.

3. Studies by Dr. R. Murray and associates at the National Institutes of Health on polioviruses excreted by humans during the field trial and of vaccine potency.

4. A prospective and retrospective multidisciplinary evaluation of the health education and other social factors, which contributed to the high degree of community acceptance and participation.

ANTIBODY RESPONSE AND EFFECTIVENESS

Although the final proof of the effectiveness of this and other polio vaccines depends on careful and prolonged epidemiologic surveillance for paralytic disease, it can be reasonably assumed that field effectiveness will parallel the antibody response to the vaccine.

In order to determine the antigenic potency of the vaccine used, blood specimens were collected immediately prior to, and three to four weeks after, oral vaccination from approximately 2,300 elementary school students and 850 infants and young adults. The elementary school students, aged six to 12 years, were volunteers, consisting of approximately 50 per cent of the student body of six representative elementary schools and a rather highly Salk-immunized group. The infants and young adults included university students, military personnel and their dependents, and Health Department clinic clientele, most of whom had received no prior Salk-type polio vaccine.

The poliovirus neutralizing antibody levels are being determined on paired sera in one of three laboratories: Variety Children's Research Foundation, directed by Dr. M. M. Sigel, Professor of Microbiology, University of Miami School of Medicine; Florida State Board of Health Laboratory, directed by Dr. N. J. Schneider; and by Microbiological Associates, Bethesda, a commercial laboratory with vast experience in this field. Microbiological Associates is using the metabolic inhibition (pH) test; the other laboratories are using the cytopathogenic effect (CPE) test. Specimens tested by the pH method which show absent or low titers by this technique and approximately 5 per cent of all other paired specimens, are being re-tested by one or more of the other laboratories by the different technique. All specimens are effectively coded, but by a system of unit packaging it is insured that all specimens from a given individual will be run simultaneously. Since this is not primarily a paper on laboratory technique and since the complete laboratory data are not yet available, the results here reported are those obtained from one laboratory using the pH test.

As of this date, we have received the reports on the results of tests from approximately 2,500 individuals. In this group there were 594 individuals who lacked measurable poliovirus neutralizing antibodies to one or more of the three types of poliovirus. The numbers of individuals, both by total and by broad age group, in each of the seven possible combinations of antibody gap to one or more types of poliovirus, are shown in Table 6. In this highly biased sample, the majority, 61 per cent, were in the age group of five through 14 years. Table 7 gives the Salk status of these 594 individuals. It will be noted that relatively few individuals lacking Type 2 antibodies had received three or more

TABLE 6. AGE GROUPING OF INDIVIDUALS LACKING POLIOVIRUS ANTIBODIES BEFORE ORAL VACCINATION

LACK OF ANTIBODY TO TYPE	AGE GROUPS			
	0–4 YEARS	5–14 YEARS	15 AND OVER	TOTAL
1	0	61	43	104
2	0	25	30	55
3	0	164	41	205
1 and 2	6	10	28	44
1 and 3	3	71	17	91
2 and 3	6	17	11	34
1, 2 and 3	18	13	30	61
Total	33	361	200	594
Per cent	6%	61%	33%	100%

TABLE 7. SALK STATUS OF INDIVIDUALS LACKING POLIOVIRUS ANTIBODIES BEFORE ORAL VACCINATION

LACK OF ANTIBODY TO TYPE	TOTAL NUMBER	SALK STATUS							
		NONE		1-2		3 OR MORE		UNKNOWN	
		No.	%	No.	%	No.	%	No.	%
1	104	45	43	12	12	44	42	3	3
2	55	43	78	6	11	2	4	4	7
3	205	46	22	19	9	138	67	2	1
1 and 2	44	37	84	6	14	1	2	0	0
1 and 3	91	26	29	10	11	51	56	4	4
2 and 3	34	24	71	6	18	3	9	1	3
1, 2, and 3	61	56	92	4	7	0	0	1	2
Total	594	277	47	63	11	239	40	15	3

Salk injections, but a high percentage of those lacking antibodies to either or both Types 1 and 3 had previously received three or more Salk injections. In view of the manner in which this sample was derived, the nature of the population from which it was drawn, and the unavailability of the as yet untabulated data on the individuals with antibodies to all three types of polioviruses, caution must be used in making generalizations from these data. However, it can be seen that, although the Type 2 component of the Salk vaccine in general use was locally antigenically potent, the Type 1, and especially the Type 3 components, left much to be desired.

We are in accord with the consensus of opinion that gross conversion from negative to positive antibody status is indicated by a change in titer from <1:4 to 1:4 or greater. However, the data to be reported will also indicate conversion rates based on titer changes of from <1:4 to 1:16 or greater. Since there are various ways of expressing vaccine efficacy by means of conversion data, several methods will be considered. Only summary data will be given here.

If one considers the individuals with the seven possible combinations of antibody gaps before vaccination (Table 8), it will be noted that the single feeding of the trivalent vaccine used resulted in 94 to 100 per cent conversions to Types 1 and 3, singly or together. Results were somewhat less effective where Type 2 antibody gaps were present, that is, 82 to 88 per cent except in the "triple negatives" which will be discussed in detail later. Even including the less responsive "triple negatives", 538 of the 594 persons, or 90 per cent, had antibodies to all three types of poliovirus following the single feeding. Forty-two of the 56 failures, or 75 per cent, involved Type 2, either singly or in combination.

If one considers the conversion rate by type (Table 9), the gross conversion rates for Type

TABLE 8. POLIOVIRUS ANTIBODY RESPONSE OF 594 INDIVIDUALS LACKING MEASURABLE ANTIBODIES TO ONE OR MORE TYPES OF POLIOVIRUS FED 2 ML. OF TRIVALENT ORAL POLIOMYELITIS VACCINE

PRE-VACCINATION STATUS		POST-VACCINATION STATUS							COMPLETE GROSS CONVERSION <1:4 TO 1:4 OR GREATER	
		NUMBER OF PERSONS NEGATIVE TO TYPE								
NEGATIVE TO TYPE	NUMBER	1	2	3	1 & 2	1 & 3	2 & 3	1, 2, & 3	NUMBER	PER CENT
1	104	2	–	–	–	–	–	–	102	98
2	55	–	8	–	–	–	–	–	47	86
3	205	–	–	0	–	–	–	–	205	100
1 and 2	44	0	5	–	3	–	–	–	36	82
1 and 3	91	2	–	2	–	1	–	–	86	95
2 and 3	34	–	2	1	–	–	1	–	30	88
1, 2, and 3	61	2	17	4	4	0	1	1	32	53
Totals	594	6	32	7	7	1	2	1	538	91
		42/56 = 75% of failures involved Type 2								

TABLE 9. POLIOVIRUS ANTIBODY RESPONSE OF 594 INDIVIDUALS LACKING MEASURABLE ANTIBODIES TO ONE OR MORE TYPES OF POLIOVIRUS FED 2 ML. OF TRIVALENT ORAL POLIOMYELITIS VACCINE

PRE-FEEDING NEGATIVES (TITERS <4)		POST-FEEDING TITERS					
		<4 (FAILURES)		4 AND OVER		16 AND OVER	
TYPE	NO.	NO.	%	NO.	%	NO.	%
1	300	15	5	285	95	266	89
2	194	42	22	152	78	119	61
3	391	11	3	380	97	341	87
Totals	885	68	8	817	92	726	82
		42/68=62% of failures were Type 2					

TABLE 10. POLIOVIRUS TYPE 1 ANTIBODY RESPONSE OF 300 PERSONS LACKING MEASURABLE HOMOLOGOUS ANTIBODIES FED 2 ML. OF TRIVALENT ORAL POLIOMYELITIS VACCINE

PRE-FEEDING TYPE 1 NEGATIVES (TITERS <4)		POST-FEEDING TYPE 1 TITERS					
		<4 (FAILURES)		4 AND OVER		16 AND OVER	
GROUP	NO.	NO.	%	NO.	%	NO.	%
Single	104	2	2	102	98	94	90
Double							
1 and 2	44	3	7	41	93	36	82
1 and 3	91	3	3	88	97	85	93
Triple	61	7	12	54	89	51	84
Total	300	15	5	285	95	266	89

TABLE 11. POLIOVIRUS TYPE 2 ANTIBODY RESPONSE OF 194 PERSONS LACKING MEASURABLE HOMOLOGOUS ANTIBODIES FED 2 ML. OF TRIVALENT ORAL POLIOMYELITIS VACCINE

PRE-FEEDING TYPE 2 NEGATIVES (TITERS <4)		POST-FEEDING TYPE 2 TITERS					
		<4 (FAILURES)		4 AND OVER		16 AND OVER	
GROUP	NO.	NO.	%	NO.	%	NO.	%
Single	55	8	15	47	84	40	73
Double							
1 and 2	44	8	18	36	82	29	66
2 and 3	34	3	9	31	91	27	79
Triple	61	23	38	38	62	23	38
Total	194	42	22	152	78	119	61

TABLE 12. POLIOVIRUS TYPE 3 ANTIBODY RESPONSE OF 391 PERSONS LACKING MEASURABLE HOMOLOGOUS ANTIBODIES FED 2 ML. OF TRIVALENT ORAL POLIOMYELITIS VACCINE

PRE-FEEDING TYPE 3 NEGATIVES (TITERS <4)		POST-FEEDING TYPE 3 TITERS					
		<4 (FAILURES)		4 AND OVER		16 AND OVER	
GROUP	NO.	NO.	%	NO.	%	NO.	%
Single	205	0	0	205	100	193	94
Double							
1 and 3	91	3	3	88	97	82	90
2 and 3	34	2	6	32	94	27	79
Triple	61	6	10	55	90	39	64
Total	391	11	3	380	97	341	87

1 were 285/300, or 95 per cent, Type 2, 152/194, or 78 per cent, Type 3, 380/391, or 97 per cent; and the conversion rates to titers of 1:16 or greater were 89, 61, and 87 per cent, respectively. In total, 817 of 885, or 92 per cent, of the antibody gaps were filled. Forty-two of the 68, or 62 per cent, of the failures were to Type 2.

Time does not permit discussion of the more detailed data tabulated in Tables 10-12, showing the gross and higher titer conversion rates by type for the various possible combinations of serologic gaps.

We have found only 61 "triple negatives" in approximately 2,500 individuals already tested. None were found among individuals who had received three or more Salk injections, and only four in individuals who had received one or two Salk injections. Although the highest percentage was in the youngest age group due to the method of sample selection, a significant number (30) was found in young adults (Table 13). Although only 32 of 61, or 52 per cent of "triple negatives" developed antibodies to all three types, the Types 1 and 3 components of the oral vaccine still showed approximately 90 per cent efficacy in producing serologic conversion. Thus, the gross conversion rates were: for Type 1, 54/61 (88 per cent); for Type 2, 38/61 (62 per cent), and for Type 3, 55/61 (90 per cent). The conversion rates to titers of 1:16 or greater were 84, 38, and 64 per cent, respectively. Twenty-three of the 36, or 64 per cent of the failures, involved the Type 2, either singly or in combination.

DISCUSSION

We believe that the preliminary data reported by us at this Conference strongly suggest that valid large-scale field trials of oral polio vaccine can and should be conducted in the social, geographic, and age groups in which its ultimate utilization is planned. Although it is more difficult to accumulate large numbers of "triple negatives" from the population of communities where widespread killed polio vaccine programs have been carried out, this problem is no different than that which develops in areas of limited Salk utilization when monovalent or bivalent oral vaccine is given, for this results in conversion of many "triple" to "double" or "single negatives", thus leaving varying but much smaller numbers of "triple negatives" to be challenged by the next type given. In either case, great reliance for vaccine testing will have to be placed on the results with less desirable but available "single" and "double negatives".

We deliberately selected the three-to-four-week interval in order to most accurately determine the primary antigenic potency of the vaccine, for longer intervals between feeding and repeated bleeding would have progressively increased the perhaps significant importance of unmeasurable variables, such as secondary spread of vaccine virus and spread of "wild"

TABLE 13. POLIOVIRUS ANTIBODY RESPONSE OF 61 INDIVIDUALS LACKING MEASURABLE ANTIBODIES TO ANY TYPE OF POLIOVIRUS FED 2 ML. OF TRIVALENT ORAL POLIOMYELITIS VACCINE

PRE-FEEDING TRIPLE NEGATIVES (ALL TITERS <4)		POST-FEEDING TITERS					
		<4 (FAILURES)		4 AND OVER		16 AND OVER	
TYPE	NO.	NO.	%	NO.	%	NO.	%
1	61	7	12	54	89	51	84
2	61	23	38	38	62	23	38
3	61	6	10	55	90	39	64
Total	183	36	20	147	80	113	62
		23/36 = 64% of failures were Type 2					

poliovirus throughout the community. On the basis of the results obtained in approximately 100 individuals who were bled before three to four weeks, and again six to seven weeks, after oral vaccination, we have no cause to regret this decision for the high conversion rates were not influenced by the increased time intervals, although they might well have been had we been using a less effective antigen.

The high conversion rates of over 90 per cent observed by us for both Types 1 and 3 in individuals lacking measurable antibodies to both these types would lead one to doubt that there was significant interference between these two vaccine poliovirus types or between the vaccine strains used, and the enterovirus usually presumed to be present in subtropical areas.

The reduced capacity of the Type 2 vaccine strain used to produce human infection, as evidenced by the lower conversion rates, is unexplained, but it raises important questions concerning the correlation of animal and human virulence.

The data on conversion rates obtained here and reported elsewhere by others, suggest that the administration of trivalent vaccine of this antigenic potency will be a public health tool that is superior to the sequential feeding of monovalent vaccine of comparable antigenicity. In the U.S.A., there is much experience to indicate that the percentage of persons satisfactorily completing an immunization or other nonemergency health procedure, which requires repeated revisits to the physician or clinic, varies inversely with the number of visits required to complete the immunization or treatment. Much evidence would be necessary to show that any theoretical superiority of sequential feeding of oral polio vaccine compensates for the increasing delinquency. It is probable that refeeding of the trivalent vaccine will be recommended to obtain maximal immunity for all individuals. Failure of an individual to receive this second dose, which is primarily used to insure Type 2 immunity, is of much less individual and public health significance than would be the failure to get either one or more of the monovalent strains given in sequential feedings. Also, to be considered in the trivalent versus monovalent question are such factors as the convenience of the public, economy of time of the professional staffs, and the length of time required to protect the maximal number of the population in the shortest period of time.

SUMMARY

1. A presentation of preliminary data of a field trial in Dade County, Florida, in which 412,000 persons were vaccinated with a single oral dose of Cox-Lederle trivalent attenuated poliovirus vaccine, is given.

2. Poliovirus neutralizing antibody studies have been reported on 594 individuals lacking antibodies to one or more types of poliovirus before oral vaccination. The efficacy of the vaccine, as measured by its ability to lead to antibody production in these individuals, was 95 per cent for Type 1, 97 per cent for Type 3, and 78 per cent for Type 2.

3. Our experiences lead us to believe that valid large-scale field trials of oral polio vaccine can and should be conducted in the social, geographic, and age groups in which its ultimate utilization is planned.

ACKNOWLEDGMENTS

Acknowledgment is made to the U. S. Public Health Service and to Dr. M. Martins da Silva of the Pan American Health Organization, for assistance given.

18. PRELIMINARY REPORT OF EPIDEMIOLOGICAL SURVEILLANCE IN A MASS FIELD TRIAL WITH ORAL POLIO VACCINE*

G. M. ERICKSON, M. E. FLIPSE, A. W. MENZIN, L. B. CLAYTON, R. E. MARKUSH, AND A. V. HARDY

Dade County Department of Public Health; Section of Preventive Medicine and Public Health, University of Miami School of Medicine; and the Florida State Board of Health with the cooperation of the Dade County Medical Association

DR. ERICKSON *(presenting the paper)*:

INTRODUCTION

In considering the possibility of studies of oral polio vaccine in Dade County, it was recognized that no one could predict how many residents of this American community would be willing to take a vaccine containing attenuated living poliovirus. Wide acceptance was essential to make it possible to conduct a mass field trial. The response far exceeded the most optimistic expectation. A total of 412,000 participated within a period of three months. The recommendation was that all residents of Dade County less than 40 years of age participate, and that older persons and non-county residents would be discouraged, but not refused. Pending final tabulations of data, the proportions of the different population groups taking the vaccine are not known, but it is apparent that at least two thirds of the resident population less than 40 years of age did so.

The major objectives of the study were to measure the individual's immediate response to the trivalent vaccine and to determine its long-range efficacy in the prevention of polio in the community. There was particular interest in examining the possibility that oral vaccination, through providing immunity to enteric poliovirus infection, would effectively prevent spread in the community and, through its wide use, virtually eradicate the clinical disease. It was believed essential to determine whether the response in this country would be in line with the favorable reports on mass field trials in other countries, as presented to the First Conference one year ago.

* Supported in part by a grant from the Lederle Laboratories Division of the American Cyanamid Company, Pearl River, New York.

The use of a placebo was neither considered indicated nor practicable. Community acceptance would be influenced unfavorably if the public realized that only half were receiving vaccine. Possible spread of live attenuated virus from vaccinated to unvaccinated individuals would make it difficult to delineate a control group. In addition, withholding a presumably effective vaccine from half of the eligible population implied that, at the rate of incidence in recent years, about 15 cases per year of paralytic poliomyelitis might needlessly occur. Indeed, the study was accepted with enthusiasm by the medical society and the county health department, since they were impressed by the published reports and by the recommendation of consultants, which indicated that oral vaccine probably would provide a particularly effective protection against polio. The interest was to test a promising vaccination program; this, rather than a placebo-controlled field study, was acceptable.

For the evaluation of oral polio vaccine it was concluded that a particularly exacting and prolonged epidemiological surveillance in a mass field trial was the urgent necessity. The multiple and extensive field trials in Latin America were done where available medical and public health resources were not always adequate to assure exacting surveillance. Published reports from the USSR and elsewhere have recorded progress in the administration of oral vaccine, but have provided less than a satisfying amount of detail on epidemiological surveillance. There have been detailed clinical, laboratory, and epidemiological observations in limited field trials, such as in Minnesota, but the small number of persons involved would not permit any defini-

tive epidemiological observations on a sporadically occurring infection.

This report describes plans for the continuing study necessary to evaluate efficacy and safety of the vaccine to the individual and to the community, with observations covering a period of from one to over three months following the administration of the vaccine to more than 400,000 people. Large numbers took the vaccine in the early weeks of the study, and over 200,000 have been observed for an average of three months following vaccination.

The Cox-Lederle trivalent vaccine was used exclusively throughout the study.

THE SETTING FOR THE STUDY

Dade County includes Miami, Miami Beach, 25 other urban municipalities, broad suburban areas, and a comparatively small rural region. It has a subtropical climate. The overall socio-economic level is relatively high. Its population has been increasing at approximately 7 per cent per year. Accurate population figures will be available only upon completion of the 1960 census. However, as of 1 February of this year, it was estimated that there were 920,000 permanent residents, of whom approximately 560,000 (60 percent) are less than 40 years of age. Thirteen per cent of the population is non-white.

Paralytic poliomyelitis in Dade County is an endemic disease occurring in all seasons. In most years, the highest incidence is in August, September, and October (Table 1). Annual paralytic attack rates have varied widely from a low of 0.8 cases per 100,000 in 1957, to a high for recent years of 16.4 in 1954. The case rate in 1959 was 4.2 per 100,000. In 1960, for the four months under consideration, the case rate was 2.3 per 100,000 person years. Deaths for the same years numbered one in 1957, eight in 1954, three in 1959, and one in 1960.

Immediately prior to the vaccination program, the immunization status of the entire population was estimated through both interview and serologic sampling technics. The data are being analyzed in terms of age, sex, race, education, socio-economic rating, and prior Salk vaccination history. The study population included a total of 1,499 households, with 4,630 people randomly selected from the community through use of a quota sampling technique. Blood specimens were obtained from a subsample of 1,286 persons. A second blood specimen is being collected one month following the termination of the vaccination program, or about four months after the initial specimen. Preliminary tabulations show that the approximate percentages of people who have had no Salk vaccine varied from about 15 per cent for those less than five years, 8 per cent for those five to 20 years, 30 per cent for those 20 to 40 years to 67 per cent for those over 40 years. In the same age groups, the percentages of those who have had three or more Salk inoculations were 56, 83, 45, and 16, respectively. Those most adequately immunized, therefore, were those of elementary through high school age. The record was more favorable in all ages for those in the higher socio-economic class and poorest in the lower socio-economic level.

Identifying data for each orally vaccinated person are being recorded permanently on IBM punch cards. These will serve as the check list for the verification of vaccination history for all subsequent central nervous system infections. These alphabetized records are not yet available and to date vaccination histories are based on the statement of patients and confirmation of clinic dates. Data drawn from these vaccination records, from the immunization survey, and from the 1960 population census, will clearly define the various segments of the population by Salk and by oral polio vaccination status.

SURVEILLANCE PROCEDURES

The staff of the Dade County Department of Public Health includes a full-time epidemiologist. For this study the epidemiology staff was augmented by an additional epidemiologist and a nurse. In recent years, the epidemiologist has personally investigated all known or suspect cases of central nervous system infection. Reporting of such diseases by practicing physicians has become relatively prompt and adequate. Moreover, in the hospital associated with the medical school and in the one community hospital to which recognized polio cases are admitted, infections of the central nervous system are receiving special study. The epidemiologist has worked closely with these and other clinical and research groups. There is an unusually favorable and close co-

TABLE 1. PARALYTIC POLIOMYELITIS IN DADE COUNTY. NUMBER OF CASES PER MONTH AND ANNUAL CASE RATES

	JAN.	FEB.	MAR.	APR.	MAY	JUNE	JULY	AUG.	SEPT.	OCT.	NOV.	DEC.	TOTAL	CASE RATE PER 100,000 POPULATION
1953	2	1	1	1	0	3	5	6	5	9	4	7	44	7.1
1954	6	4	4	8	8	10	20	14	20	8	3	3	108	16.4
1955	5	2	5	4	5	6	4	9	9	4	3	2	58	8.2
1956	1	4	4	1	5	3	4	2	3	2	1	1	31	4.2
1957	0	0	1	0	0	0	0	1	0	0	0	4	6	.75
1958	2	2	1	0	0	2	4	4	4	0	4	5	28	3.3
1959	2	2	2	6	6	3	3	9	2	3	0	0	38	4.2
1960	0	1	3	2	1									
Total in Vaccinees		1	2	1	1									
		Oral Program												

operative relationship among the health department, the practicing physicians, and the medical school, as exemplified by the joint sponsorship and participation in the present studies. In Dade County, therefore, there is a particularly favorable environment for exacting epidemiological surveillance.

The usually adequate epidemiological procedures were reinforced for this study. Discussion at the monthly meeting of the medical society and correspondence directed to all physicians, urged the necessity of reporting all known or suspect infections of central nervous system diseases or of alleged vaccine reactions. A large proportion of the physicians were voluntary participants in this program. They shared our scientific interest in assaying the merit of this new vaccine, still further assuring complete reporting. The Department of Public Health was the recognized focus for receiving reports of reactions to the vaccine. A specially trained interviewer received incoming calls and recorded all pertinent inquiries and observations.

The nature of the epidemiological field investigation depended on the characteristics of the case. The first level of surveillance comprised spontaneous reports from the public, usually attained by telephone. There were similar reports from physicians' offices. Few home visits were required to investigate these minor problems. Cases with any possible significance were scheduled for more detailed investigation. If a case were reported in which the physician had not done a lumbar puncture, an initial field visit was made by a public health nurse. When preliminary findings were significant, an investigation was made by an epidemiologist. Medical consultants were frequently called upon.

Whenever a lumbar puncture had been done, and in all cases of paralytic disease, the level of surveillance was maximum and carried out by the epidemiologists. Appropriate consultants were designated to review all cases where the diagnosis of poliomyelitis was under consideration.

Plans were effected to assure that appropriate specimens for desired laboratory tests would be collected and delivered properly to the virus laboratory.

Through special arrangements, the Florida State Board of Health and the Communicable Disease Center, U.S. Public Health Service, were to provide surveillance of any Dade County related poliomyelitis cases occurring elsewhere.

Despite all these precautions, a non-hospitalized fatal case occurred this year in Dade County and was reported as polio only after post-mortem study by the medical examiner.

OBSERVATIONS

There were relatively few telephone calls reporting possible minor reactions to the vaccine and in most instances persons were satisfied with appropriate information. All inquiries were reviewed by the epidemiologist. Nineteen reports of early reactions were of interest. Urticaria followed the taking of the vaccine in 18 individuals and there was one case of acute arthralgia. The various ingredients in the vaccine are to be offered separately to these persons to determine whether there is any sensitivity to some particular ingredient. Apart from these cases there was no reason to suspect that the disorders reported as following ingestion of vaccine were other than a sample of the general ailments occurring in the community.

During the first five months of this year, 29 cases of non-paralytic non-bacterial infections of the central nervous system were reported. Data on these are given in Table 2. Four occurred prior to the beginning of the vaccination program and in three recently reported cases the definitive diagnosis is pending. Among the remaining 22 cases, there were 12 cases of encephalitis, six due to mumps, two to varicella, one each to measles and herpes zoster, and two with etiology undetermined. There were 10 cases of aseptic meningitis, two with onset in February, five in March, one in April, and two in May. Twenty-one fecal specimens from seven patients have been examined for polio with the isolation of polio Type 3 virus from one, a case of aseptic meningitis. Of the 12 cases of encephalitis, seven took oral polio vaccine, one on the day of onset, the others with intervals of 14, 41, 41, 51, 72, and 96 days between administration of vaccine and onset of symptoms. Only four of the ten cases with aseptic meningitis had taken oral vaccine 15, 35, 53, and 75 days, respectively, prior to onset. In all, there were two cases only in which

TABLE 2. NON-BACTERIAL INFECTIOUS DISEASES OF THE CENTRAL NERVOUS SYSTEM (EXCLUDING PARALYTIC POLIOMYELITIS), DADE COUNTY, JANUARY 1-MAY 31, 1960

CASE	AGE, RACE AND SEX	SALK VACCINE		ORAL VACCINE AND DATE	ONSET OF ILLNESS	TIME INTERVAL ORAL VACCINE AND ILLNESS	VIRUS* ISOLATION FROM FECES BY M.K.T.	HOUSEHOLD† CONTACTS	FINAL DIAGNOSIS
		NO. OF DOSES	DATE OF LAST DOSE						
E.H.	5 Mos. C.M.	0	—	0	1/13/60	—	(2) Pending	None	Aseptic Meningitis
C.P.	29 Yrs. W.M.	0	—	0	1/17/60	—	(2) Negative	None	Aseptic Meningitis
J.B.	50 C.M.	0	—	0	1/22/60	—	No Specimen	None	Aseptic Meningitis
C.W.	22 C.F.	0	—	0	2/1/60	—	No Specimen	None	Aseptic Meningitis
G.S.	3 C.M.	2	10/23/58	0	2/19/60	—	No Specimen	None	Aseptic Meningitis
D.V.	16 W.M.	0	—	0	2/25/60	—	No Specimen	None	Aseptic Meningitis
C.M.	35 C.M.	0	—	0	3/2/60	—	No Specimen	(3) 3/14/60	Aseptic Meningitis
L.G.	17 W.M.	4	1957	0	3/4/60	—	(3) Negative	(3) 2/15/60	Aseptic Meningitis
P.R.	10 C.F.	3	1958	0	3/15/60	—	(1) Negative	None	Aseptic Meningitis
C.S.	3 W.F.	1	1/1959	0	3/22/60	—	Type 3 Polio (2)	None	Aseptic Meningitis Asso.—Polio
A.H.	8 W.M.	3	1957	3/14/60	4/18/60	35 Days	(1) Pending	(3) 3/11/60	Aseptic Meningitis
O.C.	6 W.M.	5	6/1959	2/22/60	5/7/60	75 Days	(1) Pending	(4) 2/22/60	Aseptic Meningitis
S.Y.	5 W.M.	4	Sum. 1959	3/31/60	5/23/60	53 Days	(1) Pending	(2) 2 mos. ago	Aseptic Meningitis
R.H.	11 C.F.	4	June 1959	2/18/60	3/4/60	15 Days	(2) Negative	(3) 2/18/60	Aseptic Meningitis

TABLE 2—Continued

CASE	AGE, RACE AND SEX	SALK VACCINE		ORAL VACCINE AND DATE	ONSET OF ILLNESS	TIME INTERVAL ORAL VACCINE AND ILLNESS	VIRUS* ISOLATION FROM FECES BY M.K.T.	HOUSEHOLD† CONTACTS	FINAL DIAGNOSIS
		NO. OF DOSES	DATE OF LAST DOSE						
H.P.	2 YRS. C.M.	0	—	0	3/8/60	—	(4) Negative	None	Mumps Encephalitis
B.H.	5 W.F.	2	11/4/59	0	3/16/60	—	(2) Negative	(2) 2/22/60	Mumps Encephalitis
A.B.	3 C.M.	1	5/16/57	0	3/24/60	—	No Specimen	None	Mumps Encephalitis
H.W.	19 C.M.	0	—	2x 4/14/60 4/21/60	4/29/60	14 Days	(2) Pending	None	Mumps Encephalitis
M.C.	4 W.M.	3	1958	0	5/1/60	—	No Specimen	None	Mumps Encephalitis
M.M.	6 W.F.	5	July 1959	3/30/60	5/11/60	41 Days	No Specimen	Date Unknown	Mumps Encephalitis
K.S.	10 C.F.	1	1956	3/11/60	4/21/60	41 Days	No Specimen	(3) 3/11/60	Varicella Enceph.

V.G.	7	W.F.	4	1959	2/29/60	4/20/60	51 Days	(3) Pending	(3) Date Unknown	Varicella Enceph.‡
R.D.	8	W.F.	4	1959	2/22/60	4/27/60	72 Days	(1) Pending	(1) 3/11/60	Measles Enceph.
J.A.	21	W.F.	4	1958	0	4/24/60	—	(1) Pending	None	Herpes Zoster Enceph.
M.F.	9	C.F.	2	1959	3/10/60	3/10/60	0	(7) Pending	(1) 3/10/60	Encephalitis
S.E.	8	W.M.	4	1959	2/18/60	5/14/60	96 Days	(1) Pending	(3) Date Unknown	Encephalitis‡
C.F.	16	C.M.	4	1959	2/24/60	5/26/60	92 Days	(1) Pending	(3) 2/24/60	Pending Prob. Aseptic Meningitis
P.C.	8	W.M.	3	1959	0	5/27/60	—	(1) Pending	None	Pending Prob. Mumps
D.G.	5	W.M.	4	1959	Late Feb.	5/27/60	3 Months	No Specimen	(2) 2/1960	Pending Prob. Mumps

* Number in parentheses indicates number of specimens.
† Number in parentheses indicates number household contacts who had taken oral vaccine. Date shown is earliest of such vaccinations.
‡ Fatal case.

TABLE 3. PARALYTIC POLIOMYELITIS CASES IN DADE COUNTY, JANUARY 1-MAY 31, 1960

CASE	AGE YRS.	SEX	RACE	SALK VACCINE		ORAL VACCINE AND DATE	ONSET OF ILLNESS	INTERVAL ORAL VACCINE AND ILLNESS	VIRUS* ISOLATION FROM FECES	HOUSEHOLD† CONTACTS	CLINICAL DIAGNOSIS AND COMMENT
				NO. OF DOSES	DATE OF LATE DOSE						
W.K.	23	M	W	0	—	2/4/60	2/13/60	9 Days	Polio (3) 1 and 3	None	Spinal
K.F.	2	M	W	2	July 1958	2/22/60	3/2/60	9 Days	Polio (3) 1 and 3	(6) 2/21/60	Spinal
D.S.	44	M	W	0	—	0	3/16/60	29 Days‡	No Specimen	(2) 2/16/60	Fatal Bulbar
R.M.	34	M	W	0	—	3/13/60	3/26/60	13 Days	Polio (3) 3	(3) 3/12/60	Spinal
R.P.	4	M	W	0	—	0	4/2/60	0 Days	Polio (2) 3	None	Spinal
M.G.	28	F	C	0	—	4/26/60	5/3/60	7 Days	Pending (2)	(3) 4/26/60	Spinal
J.A.	35	M	W	2	March 1955	3/22/60	4/5/60	14 Days	Pending	(4) 3/1/60	Spinal

* Number in parentheses indicates number of specimens.
† Number in parentheses indicates number of household contacts who had taken oral vaccine. Date shown is earliest of such vaccinations.
‡ Vaccine contact.

the interval between vaccination and onset was less than 28 days. One was a mumps encephalitis, with diagnosis established by more than a four-fold rise in titer and with known contact with mumps. The patient with an interval of 15 days had a titer of 1:256 for mumps, without a demonstrated change. Two fecal examinations in MKT were negative. The clinicians and the epidemiologist agree that findings this year concerning non-bacterial non-paralytic infections of the central nervous system, are in line with usual experience.

During the period under review (January-May 1960) seven cases of paralytic poliomyelitis were reported. (See Table 3.) (Individual case notes are included in the appendix.) In all cases, the clinical diagnosis was confirmed by consultants. One suspect "abortive polio," is being carried at present, classified as diagnosis undetermined. Two additional cases of central nervous system disease were carried as polio suspects, but were proved on study not to be poliomyelitis. Others were reported in which poliomyelitis was considered but in which different diagnoses were established.

It was presumed that if there should be any hitherto unreported clinical reaction to the oral vaccine, the clinical manifestation might well be mild and possibly atypical. However, the five cases observed in vaccinees were a sampling of the usual clinical picture of polio, varying from a minor paralysis to serious disability. Certainly, clinically these cases could not be differentiated from disease caused by wild polio viruses.

As previously indicated polio is not limited by season in Dade County. In the four months, February through May, the number of resident paralytic polio cases with onsets in these months were 14, 1, 3, and 16, respectively for the years 1956 through 1959. Thus in recent years, an average of 8.5 cases had onset during this interval. In 1960, during the same time, there were seven paralytic cases. On the basis of frequency alone, the occurrence of less than the average number of cases encourages the opinion that these may be coincidental infections, particularly since they had the usual range of clinical manifestations.

These paralytic cases, however, had unusual features demanding considerations. Of the seven cases, five (71 per cent) were more than 20 years of age. During the four years since Salk vaccine became generally available, there have been 103 cases in Dade County, of which only 24 (23 per cent) were in this adult age group.

The incidence of paralytic poliomyelitis in Florida in 1960 (February-May) has been low. Elsewhere in the State there were three cases in these four months. During the same period in the past four years, 37 per cent of all cases of paralytic polio occurred in Dade County. In 1960, in contrast, 70 per cent of the 10 cases in the State occurred in Dade County.

In the interval under study, the population of Dade County could be divided into three groups with respect to oral polio vaccination: (1) the unvaccinated; (2) the recently vaccinated (within 28 days); and (3) the vaccinated (28 days or more previously). Very preliminary figures pertaining to these are given in Table 4.

TABLE 4. PARALYTIC POLIO IN DADE COUNTY, FEBRUARY-MAY, 1960, IN RELATION TO VACCINATION STATUS

ORAL VACCINATION STATUS	PERSON YEARS	PARALYTIC POLIO CASES	CASES PER 100,000 PERSON YEARS
*Unvaccinated less than 40 years old	100,000	1	1
†Recently vaccinated less than 40	32,000	5	15.6
‡Vaccinated less than 40	55,000	0	0
Unvaccinated more than 40	119,000	1	0.8

* Did not take vaccine.

† Had taken vaccine within 28 days.

‡ Had taken vaccine more than 28 days previously.

It is noted that there were five cases in the recently vaccinated giving a case rate of 15.6 per 100,000, and none in the vaccinated after an interval adequate to permit the development of antibodies. In the unvaccinated less than 40 years of age, the case rate was 1.0 per 100,000, and in those 40 years and over, 0.8 per 100,000 person years. The case rates are to be compared with illustrative rates for Dade County of 4.2 per 100,000 total population in 1959, of 0.8 in 1957 (a year of low incidence), and of 16.4 in 1954 (a year of high incidence). Thus the case rate in the recently vaccinated compares with that for the population as a whole observed in a year of high incidence. The lack of cases in the 55,000 person years of experience of the orally vaccinated is encouraging.

Time relationships are of particular importance. The intervals between the taking of the vaccine and onset of symptoms were respectively 7, 9, 9, 13, and 14 days. Coincidental cases would be expected to have onsets distributed evenly at least for the first two weeks following vaccination. A chance grouping of onsets of all cases within the second week following vaccination would be improbable. In the experience with the live virus vaccine in the USSR, there are reports that onsets of illness in paralytic cases up to and including 10 days following oral vaccination were interpreted as cases probably related to pre-existing infection due to wild virus. Although these would be expected to occur in any large-scale use, cases of this type were not discussed in the official presentation from the USSR. We can therefore only emphasize the importance of records that show both the occurrence of paralytic polio in vaccinees from the day of vaccination to onset of illness, as well as complete reports of cases occurring in the comparable unvaccinated group. Data of this kind are obviously required.

There were two cases in 1960 in the orally unvaccinated. As noted in the case reports in the appendix, one occurred in a child of a rural family, no member of which had taken oral vaccine and in which the probability of contact with the vaccine virus was relatively remote. The other was the fulminating fatal case whose wife and child had taken oral vaccine one month earlier. The course of the disease spoke for infection with a highly virulent virus. If these are accepted as coincidental infections and disease, the evidence indicates the safety of an oral polio vaccination program to contacts and the community.

Of the seven cases, poliovirus was isolated from four, all yielding Type 3 virus and two of these Type 1 also. In two cases findings are pending. In the fatal case no feces were collected.

None of the seven cases had had a full course of Salk vaccination. Two had been inoculated twice. The child of two received the last dose 18 months prior to onset. The adult male had taken them "about five years ago."

The one suspect case in which diagnosis is still undetermined is a male 20 years of age. He noted malaise, headache, fever and difficulty in swallowing with onset 11 days after taking oral vaccine. His symptoms improved but the gag reflex is still absent. He had had no Salk vaccine. This patient has some neuromuscular congenital defects.

COMMENT

At this time we are able to present only very early and obviously incomplete observations. Final evaluation will need to include the results of marker studies and serological findings, neither of which is at hand. Thus our impressions must be based chiefly on epidemiological findings. The observations which impress us as being of greatest significance include:

1. There was a ready acceptance in Dade County of a living oral poliovirus vaccine by 412,000 of the population.

2. Significant immediate reactions to oral polio vaccination were not observed.

3. The usual incidence of aseptic meningitis and encephalitis noted in the previous year continued throughout the trial period without significant change.

4. There was no evidence of any significant association between attenuated vaccine virus and the 29 currently accepted cases of non-bacterial, non-paralytic infections of the central nervous system. Four of these 1960 cases occurred prior to the field trial.

5. There was substantial evidence of safety to contacts in the presence of an extensive use of oral polio vaccine.

6. The occurrence of seven cases of paralytic poliomyelitis in Dade County in this period of 1960 was less than the five-year median of 14 and the average of 10 cases for these same months.

7. Five of the seven currently accepted cases of paralytic polio had onset within two weeks of the ingestion of the vaccine.

8. There was a single case of paralytic disease in a vaccinee of pre-school age, none in those of school age, and four in adult vaccinees.

9. The clinical nature of diagnosed cases of paralytic poliomyelitis in vaccinees ranged in severity from mild to serious disability.

10. None of the seven cases of paralytic polio reported this year occurred in persons who had taken a full series of Salk vaccine.

SUMMARY

We feel that this field trial was timely and indicated. Our observations are all too preliminary to justify conclusions. More data will be available in the months ahead. Our current findings are only presented as a progress report.

APPENDIX
CASE REPORTS

W.K. W M 23. A student active in weight lifting. Took oral vaccine on 2-4-60. On 2-13-60 he developed a severe headache and malaise. He reported to the infirmary on 2-15-60 and was believed to have a URI. He was seen again on 2-17-60 complaining of continued headache and some nausea. Treatment was symptomatic. On 2-18 he was admitted to the infirmary because of dysphagia. On 2-20 he was seen in neurological consultation. On first examination left deltoid weakness and right palatal deviation were noted. There was mild dysphasia and hesitancy on voiding. Two months later the major residual was a winged right scapula. Weakness at the original site had greatly improved.

K.F. W M 2. Took vaccine on 2-22-60. Became ill on 3-2 with vomiting, malaise, and irritability. On 3-5-60 admitted to hospital because of inability to walk. The paralysis involved both lower extremities. This patient is still in the hospital and apparently will have extensive residual impairment.

D.S. W M 44. An advertising executive who did not participate in the vaccine program. His wife and five-month-old daughter took the vaccine on 2-16-60. On 3-16 he developed a severe backache and consulted his physician. Was diagnosed as "flu" and given antibiotics, codeine, and sedatives. On 3-18 he developed marked insomnia and fever of 102°. He remained at home. On 3-19 he aroused his wife during the night complaining of weakness of the legs and requested that she assist him to the bathroom. He died suddenly while at toilet. He became a medical examiner's case and the histopathological examination of cord and brain stem revealed classical findings of poliomyelitis.

R.M. W M 34. A salesman who took polio vaccine on 3-13-60. On 3-26 became ill with fever of 103° and pain in his back and legs. On 3-29 he developed paralysis in his right leg and was hospitalized on 3-30. He remained in the hospital until 5-7. It appears that he will have significant residual paralysis of the right lower extremity.

R.P. W M 4. This boy lives with his parents in a rather isolated rural home. Neither he nor any member of his family had taken vaccine. He developed a headache on 4-2-60. This cleared but recurred on 4-5. He became irritable and developed leg weakness. On 4-7 it was noted that he was dragging his right leg and was admitted to the hospital. He will apparently have significant impairment of his right lower extremity. He was discharged from the hospital on 5-25-60.

J.A. W M 35. A military reserve officer who took vaccine on 3-22-60. He became ill elsewhere on 4-5-60 with nausea, headache, and very mild diarrhea. He reported to a military hospital on 4-9 and was diagnosed as influenza. He developed muscle pain and found relief by walking. He was treated casually by a medical friend who furnished muscle relaxants and codeine. On 4-10 he felt much worse and developed nuchal rigidity accompanied by headache and fever. He was hospitalized at a military hospital on 4-10 and remained there until 5-12. Paralysis was noted on 4-18-60 involving both extremities and the abdominal muscles. It is likely that his residual paralysis will be mild to moderate.

M.G. C F 28. A vaccinee who participated on 4-26-60. She became ill on 5-3 with sore throat, fever, and headache. On 5-7 she experienced difficulty in walking and was hospitalized. She became psychotic. She had bilateral lower extremity flaccid paralysis. The residual paralysis probably will be marked. At present she is an in-patient on the neuropsychiatric service.

G.L. W M 20. A student with multiple congenital anomalies who took vaccine on 3-2-60. He became ill on 3-13. His presenting symptoms were dysphagia, fever, sore throat, and vomiting. The following day he developed a severe headache and his difficulty in swallowing increased. He was examined at a military hospital. It was found that he had hypersthesia of the posterior pharyngeal wall and absent gag reflexes bilaterally. A tissue biopsy from the right hypopharynx showed muscle atrophy. The diagnosis made retrospectively on 5-4 by the physicians at this hospital was "abortive poliomyelitis." Further investigation is pending and this case is being held as "diagnosis undetermined."

ACKNOWLEDGMENTS

Grateful acknowledgment is made to: Dr. M. Michael Sigel, Director, Variety Research Foundation; Miss Methyl I. Crossley, Director of Nursing Service, Dade County Department of Public Health, and to their staffs. Without the help they have given this program, completion of surveillance would have been impossible.

DISCUSSION

CHAIRMAN RHODES: These two papers are open for discussion.

DR. ALEXANDER: I was fortunate enough to be allowed to look over the shoulder, as it were, of the Dade County Health Department in connection with the surveillance of the cases just reported by Dr. Erickson. I should merely like to comment on the exactness, care, and thoroughness with which this surveillance was carried out.

The second point I wanted to mention was that, in reviewing these cases, I am reminded of a theoretical discussion during a recent meeting on polio in New Jersey, concerning the criteria necessary for designating a case as possibly having some vaccine association. Some members of that group are present here today.

There were a number of points that were put forth as possible criteria, the first being the presence of the laboratory evidence, particularly of genetic markers that would help to differentiate "wild" viruses from vaccine viruses. In this study, these are not yet available, have not yet been tested, but it is hard, actually, to postulate results that would be all-conclusive in allowing one to make a more definite decision on these cases.

The second criterion that was discussed was the total number of cases reported in relation to past experience. The study in Dade County shows how it is possible that the incidence may be well within "normal" experience, if one were to postulate that there might be a vaccine association of a low order of magnitude in a population that is relatively highly immunized, either naturally or artificially.

Finally, the discussion brought out that probably the best criterion would be one of the epidemiologic consistency. I should only like to suggest that the discussion is no longer theoretical, in that we have data before us on which such decisions must be made.

DR. BODIAN: I should like to commend the people involved in this study on their objectivity in presenting very carefully collected and assessed data concerning the cases which occurred in the vaccinated individuals.

I made a check list of points which might be used to determine the significance of these cases, and in almost every instance I find that there are two possible interpretations. It seems to me that we are confronted with a peculiarly difficult situation when we carry out a very careful casefinding and surveillance study and turn up with information which has two possible interpretations.

There are some points not covered by the speakers, which I should like to discuss. First of all, as Dr. Alexander mentioned, the unusual incidence could be interpreted as being an unusual result of the changed ecology of the disease in Dade County. It might be considered as evidence for the excellent protection of the formalinized vaccine in the younger age groups.

On the other hand, we also have to think about the possibility that we are looking at an unusual pathogenicity of the vaccine viruses for adults, perhaps unsuspected before.

One also wonders why there appear to be no non-paralytic cases to go with the paralytic cases, because this is a rather high ratio of paralytic to non-paralytic cases.

The case incidence in vaccines is, of course, unusually high. Five out of seven cases occurring during the time period in question occurred in vaccinees. I think this is subject to several interpretations, but until we have age specific attack rates in the vaccinated and unvaccinated, I do not see how we can conclude either that this is a natural occurrence at this time of year in Dade County or that it is causally associated with the vaccine.

The astonishing lumping of cases in the second week after feeding is a bit difficult to interpret as chance association. Nevertheless, one cannot completely eliminate the role of chance.

We must consider especially the pathogenic properties of the particular strains involved in this study.

First of all, we have heard from the Minnesota group that these strains grow well in the throat. Secondly, we have heard that the viruses associated with these cases, namely the Types 1 and 3, produce viremia readily. Third, we know from evidence which was presented last year and has been further elaborated on, that these strains have a greater neurovirulence than some of the other strains we have heard about, particularly as regards their ability to spread after gaining a foothold in the spinal cord, after intraspinal inoculation. Dr. Kirschstein submitted data on this in great detail, as you remember, in earlier meetings.

And, finally, we have to consider the possibility that, with strains having the ability to get into the bloodstream, which is essential for attaining a foothold in the nervous system, and which have the ability to spread, a small degree of reversion here would be just enough to produce paralytic disease.

DR. FLIPSE: Dr. Bodian, I should like to reply first to your comment by saying that we have revealed the complete picture as it now exists, and that I believe this group should be very cautious in trying to make interpretations, since we have tried not to muddy the waters by discussing some of the variances in opinions about these cases.

However, I think that this assembly would do well to realize that, in the case of patient J. A., our group questioned the non-polio diagnosis of the attending staff of a well-staffed military hospital, even though their diagnosis was based on five weeks of observation, in the absence of laboratory and serologic evidence of polio infection. We consider this a case that we should investigate further.

And so we could go through each one of these cases. I could likewise say that in four of the cases of vaccinees serologic evidence of recent polio infection is lacking.

To keep the record clear, I should point out that these are complement-fixation studies, and that our neutralization studies are not yet available.

This, I think, gives some indication of the lack of evidence so necessary even for the diagnosis of these cases, let alone the calculation of attack or other rates.

The cases, the temporal and other relationships, and other factors are being brought to your attention. We have weeks and months of laboratory and other work ahead of us before we or others obtain the data necessary for making interpretations.

Now, on the question of markers, I believe all of us using live virus vaccines are faced with the problem of proving or disproving paternity of isolated polioviruses. If we go back to the early days of blood typing, when there was only a single set of blood types on which to depend, one would have a very difficult time if brought in before a group like this to prove or disprove paternity. With the development of more and more different sets of blood types, the solution of paternity cases has become more certain. Similarly, as the field of markers is widened, it may well be possible that, by the statistical considerations of the results of 10, 12, or maybe more marker studies, the paternity of isolated polioviruses may be proved.

Unfortunately, the isolation and the proof of paternity of an agent from the feces of a patient in a field trial area may perhaps have little relationship to the etiology of the patient's illness. We have saturated our community with poliovirus and the finding of poliovirus in the stool of patients with all types of medical and surgical disorders is to be expected. Because of this, we can no longer diagnose or report non-paralytic polio. We have to report aseptic meningitis, associated with poliovirus isolation.

We are not trying to hedge by saying that the same should be applied to the paralytic cases, but we, and others, are faced with a difficult problem in diagnosis.

We have not had time to report fully on an epidemic of infectious central nervous system disease in St. Petersburg, Florida, another activity of neurotropic agents in the State. The picture is very complex and the evidence as yet incomplete. I think we have just presented the problems of coincidental and temporally related cases of illness faced by any group doing large-scale field trials. As you look into all the field trials on Salk vaccine and all the oral vaccines, you will find these cases. They are apparent in all vaccine trials of any type. We present them before this group, but more information is needed before interpretations can be made.

We did not have time to go into our own unfavorable Salk experience, but this is the main thing that led us to undertake an oral vaccine field trial.

Last year, 40 per cent of our paralytic cases were in people who had received three or more Salk shots. Two of our three deaths were triply Salk-vaccinated people, and there was a young child in the State of Florida who died from polio two months after his fifth Salk shot.

This, as I say, is the reason we went into an oral vaccine program. It may be that these cases of Salk failure in previous years were not all polio, but the laboratory and other studies in our State are good.

Not all of the material we have can be presented, since we have taken too much time already. We have merely presented the problem. There is an enormous amount of careful epidemiological and laboratory work yet to be done. Without these studies, and a very careful consideration of the whole problem, we cannot make any interpretation of our data, or we would have done so. We therefore question whether others should try it without the completed evidence.

DR. ANDERSON: I believe we are very much indebted to Dr. Flipse, Dr. Erickson, and their colleagues for two very forthright and carefully presented statements as to their experiences. Obviously, a great deal of attention is going to be paid to these five cases that occurred in vaccinated individuals. And certainly, as has been pointed out, one cannot overlook the temporal relationship. A point of major interest, certainly, is whether this temporal relationship was related to the vaccine or whether it was coincidental. Obviously, to draw any conclusions on five cases is extremely dangerous.

Reference has been made also to age distribution, i.e., that four of these five cases, occurring after the vaccine, were adults; this is somewhat different than the usual age distribution. Yet, as I recall some figures that Dr. Erickson showed me recently, the normal distribution in Dade County during the last few years has been about one third under five, one third five to 20, and one third 20 and over. Thus, the deviation from this distribution of one third, one third, and one third is not particularly striking when we have one case in the preschool age group, none in the age group of five to 19, and the four in the adult group.

I believe we should give some attention to the absence of cases in the younger age group. Table 4 in Dr. Flipse's paper shows that the Salk status of the children under five was essentially the same as the Salk status of persons 20 and over; in other words, about half of those under five had had Salk vaccine, and the same was true of about half of those over 20.

This means, then, that from the standpoint of prior immunization the two are essentially the same. But we should recognize also that the persons over 20 had, because of exposures to natural infection, undoubtedly acquired a certain level of resistance over and above what we might expect in a group under five. So we have to think of this group under five as a somewhat more susceptible group than the group 20 and over.

And yet it is striking, in my mind, that within the limits of the few cases available, this highly susceptible, most susceptible, group is the one in which we found only one case. When we get into the school-age group, where nearly 90 per cent of the children received the oral vaccine, we find no cases. In looking at these relationships, I believe we should balance off what might be considered equally suggestive data, namely, that we did not get cases in the age susceptibility group where we might have expected the most trouble.

Now, Dr. Flipse has made reference to the incidence of neurologic conditions that were in that area of Florida. He referred to St. Petersburg and to that general area.

I gathered from Dr. Bodian's comments that he had raised the possibility that with some other neurologic infection going through that area, these cases, causally related to the vaccine, were possibly the result of a synergistic effect of two different viruses in the same individual. Certainly we should not overlook the fact that there were these other infections that may or may not have had some relationship.

Before closing the discussion of this experience with the community-wide oral vaccine, I hope that you, Mr. Chairman, will call upon the Minnesota group, for whom I cannot speak. Dr. Barr and Dr. Eklund can present data as to the results that they have just obtained from the

feeding of oral vaccine to 100,000 persons (50,000 with placebo and 50,000 with vaccine) and, on the very careful surveillance that Dr. Eklund, here present, has been carrying on there. I think those data should be made available for comparison.

CHAIRMAN RHODES: I shall comply with your suggestion, Dr. Anderson; but first, Sir Macfarlane Burnet has asked to be recognized.

SIR MACFARLANE BURNET: I believe it is important to ask the question: If one expected paralytic infection from a very weak virus, in which groups should one look?

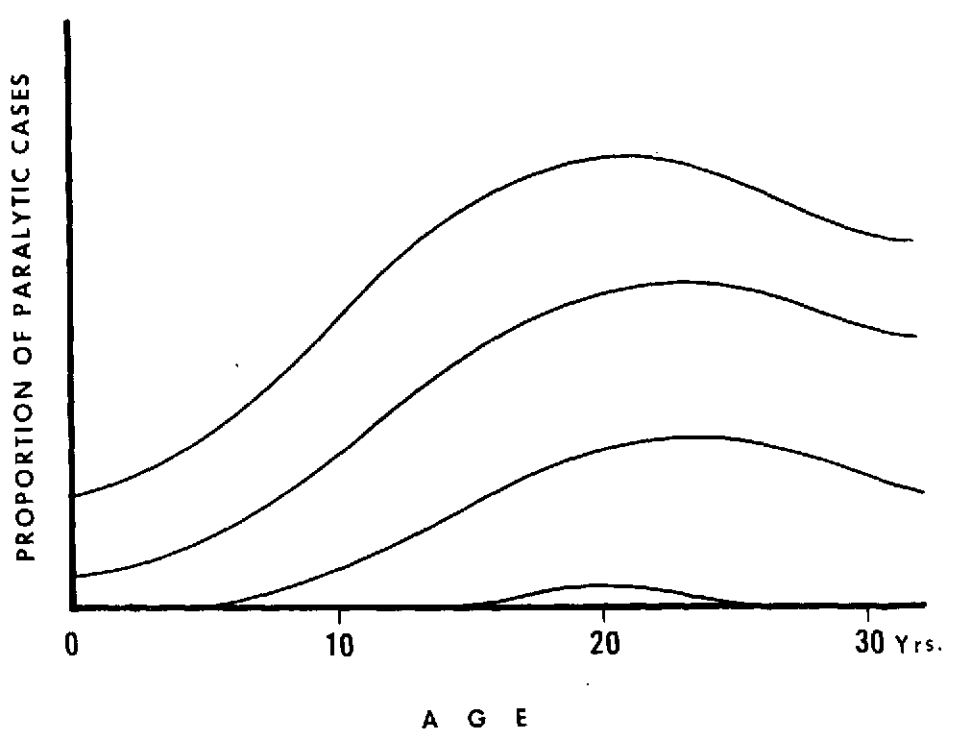
Ten years ago I published a theoretical graph in the *Bulletin of the Johns Hopkins Hospital*, indicating what experience of paralysis would be expected in the various age groups of a completely virgin, unimmunized population with different strains of virus, at different levels of virulence. Based upon experience in virgin soil epidemics the graph looked like this:

One can draw a family of curves with incidence of paralysis as ordinate and age as abscissa. In each curve the highest level of incidence is at the adolescent-young adult age level 15—30. With progressively decreasing virulence the curves become lower and at a certain level the only paralysis to be expected will be a small proportion in the young-adult age group.

I suggested at that time that if normally avirulent viruses were to produce paralysis in a wholly nonimmunized community, it would be in the young-adult age group. If we have reason to believe that a population undergoing immunization with a live vaccine contains a proportion of completely unimmunized individuals, and we are looking for possible cases of paralysis, then we should look for this among the young adults.

At this point, I should like to suggest that there is one other place where virus of very low virulence might produce paralysis, that is, in infants and very young children. It is in this group that one would expect to find immuno-

HYPOTHETICAL INCIDENCE IN VIRGIN-SOIL POPULATIONS



logical anomalies of the agamma-globulinemia type, or others not yet recognized. In children with poor defenses, either congenital or due to slow maturation of the defense mechanism, I believe you might again expect to find an occasional case of paralysis from virus which in a normal population is completely harmless.

I should also point out that if this high susceptibility of the young adult is a fact, then in any country with wide and early dissemination of wild polioviruses among children, there would be no adults left unimmunized by the natural process and their potential susceptibility would never become manifest.

In other words, in Central America, or other countries where natural immunization is at the very early age, you would certainly never see such adult cases. But if cases are going to occur with very low virulent virus in relatively non-immune communities, this is the age group to be watched.

DR. FLIPSE: Sir MacFarlane, I should appreciate your comment as to the probable risk or chance of seeing similar phenomenon in cortisonized individuals, patients whose reticulo-endothelial system has been damaged by X-ray and by drugs, such as those used to treat leukemia and Hodgkins disease, and also in patients with recent oropharyngeal surgery, because these groups have been challenged in significant numbers in our trial. I think your experience and judgment would be appreciated by this group.

SIR MACFARLANE BURNET: The only comment I could make would be in regard to the evidence that children being treated with cortico-steroid drugs have shown alarming reactions to chicken-pox, including some deaths. I should feel, again, that a nonimmune child under cortico-steroid therapy would be another possible candidate for abnormal susceptibility.

I am speaking only of the potential areas in which it would seem worthwhile to look for cases with a vaccine which we know from experience to be perfectly harmless to normal individuals.

CHAIRMAN RHODES: As for the question addressed to the Minnesota group, would Dr. Barr care to answer it?

DR. BARR: Minnesota planned a mass trial study in which 103,000 people were fed between 24 March and 20 May 1960. The studies were conducted in Minneapolis, St. Paul, Duluth, two suburbs of Minneapolis (Bloomington and St. Louis Park), and three rural counties located in the central part of the State.

The number of participants ranged from 31,000 in Minneapolis, 21,000 in Duluth, 17,000 in St. Paul, to 16,000 in Bloomington and St. Louis Park, and a like number in the three rural counties. They covered the entire gamut of people, from poor socio-economic groups in the three cities and the rural areas to the silk-stocking group in the metropolitan areas.

Feedings were tied in with the local school systems, with participation on a voluntary basis. As we have described before, the field trials were all done as double-blind studies in which the participants went to the school and did not know whether they received oral vaccine or placebo. Out of 103,000 fed, one half of the participants received placebos and one half received the Cox strains of the attenuated oral polio vaccine.

Interestingly enough, the vaccine we fed in Minnesota was from the same batch as that which was used in Miami. In fact, on 24 March (the day we started), we received information about the reported cases of polio in Miami. I called the health officer in Miami and asked for specific information, as we were starting our feeding studies in Minneapolis that night. On reviewing the information he gave us on the five cases, and consulting with the oral vaccine advisory committee to the State Board of Health (headed by Dr. Gaylord W. Anderson), we went ahead with the study.

I must admit that when we received this report at the very last minute we had some qualms. I might also add that Minnesota had five cases of Type 1 polio during the first three months of 1960. The last reported case occurred on 4 March; it was a non-paralytic case from which we isolated Type I virus.

To my knowledge, this case, which had first symptoms 20 days prior to the beginning of the study, is the last one that has occurred in the State of Minnesota to date.

All persons who participated did so on a volunteer basis. The communities of Bloomington and St. Louis Park asked to be included in the study;

one was sponsored by the local medical society and one by the Junior Chamber of Commerce. The tricounty medical society of the three rural counties also asked to be included. Studies in the three large cities were developed after we had received assurances of active cooperation of the health officer, the school authorities, and the medical societies concerned. Subsequently, we have had any number of requests for inclusion in the study.

The *Minneapolis Tribune*, which has a poll service for determining state-wide public opinion on subjects of public interest, reported on 26 May 1960 that 79 per cent of the people in Minnesota knew about the Minnesota feeding experiments, which included a placebo. Approximately 75 per cent of the people said that they would be willing to take part in such an experiment if given the opportunity. I believe this gives you some idea of the public confidence in the oral vaccine studies in Minnesota.

The Minnesota State Board of Health is an administrative board of health. It has had, throughout this whole polio experience described to you, an advisory committee on poliomyelitis, both for the Salk vaccine programs and for the oral vaccine studies. The Board specifically said that it felt it had a responsibility of doing everything it could in the evaluation of this oral vaccine.

This is one of the reasons why Minnesota has been involved in the study of the use of oral vaccine for such a long period of time. We are convinced, of course, that the vaccine is safe and that it is effective.

May I add that this study has been headed for the entire period by Dr. Bauer, whom you heard earlier.

I can only say that we feel, of course, very pleased. We have had no problems with the vaccine. We have maintained a very close surveillance. We approached the Surgeon General with the request that he assign observers to these feeding experiments, and we specifically requested that Dr. Carl Eklund be assigned as an observer to Minnesota. Since he spent a considerable amount of time interviewing families who participated in the study, I should like to have him recognized at this time.

DR. EKLUND: I feel very strongly that studies like this should be set up in a double-blind manner and planned in such a way as to produce answers.

In Minnesota there were nine areas, and no one outside of these areas could take any oral vaccine. We therefore had a chance to study spread from vaccinated groups to unvaccinated groups. Within the areas, the study was completely double blind, so it was possible to interview people without knowing whether they had taken vaccine or placebo.

My main concern has been to see whether any ill effects come from this vaccine. I went from family to family and interviewed people without knowing what they had taken. I interviewed physicians. I went to small rural schools. I could not tell a placebo lot from a vaccine group. All I knew was the lot numbers.

No illness accumulated in any lot number. Complaints would vary from one area to another, but there was no uniformity in complaints and no serious ones of any kind.

One saw miscellaneous diseases in the community—skin rashes, mumps, measles, chickenpox, broken legs, operations, tonsillectomies, etc. But to date, there has been no evidence of any ill effect.

I believe that future studies should be set up in this manner so as to avoid obtaining controversial data.

DR. BODIAN: Since Dr. Eklund has done a most remarkable single-handed job of case finding, I should like to inquire whether he also asked the people who had received placebo and vaccine, first, whether they had previously had Salk vaccine; second, how many of those interviewed might have been in the age group that Sir MacFarlane Burnet mentioned; and third, whether they were triple or double negatives.

DR. EKLUND: I think the points raised are very pertinent. First, you have to collect fecal specimens in every area to know what viruses are circulating before your study starts; second, you have to bleed part of the population; and third, you have to know the Salk status. All three of these steps were taken.

The majority of children, whether of preschool or school age, have had Salk type vaccine. I would estimate the number at 90 per cent. It varies somewhat from area to area, being less in the rural areas.

I would estimate that probably 40 per cent of the mothers have had Salk vaccine and approximately 10 per cent of the fathers. The fathers, for some reason, practically never get immunized.

In one area it is known that Type 1 and Type 3 were present before the vaccine was given.

DR. ERICKSON: I believe Dr. Bodian's question went unanswered. I should like to say that when our IBM data is tabulated, we shall have denominators to calculate the age adjusted rates.

From these data we shall also be able to estimate data of the population that is at risk, provided we believe the financial expenditure to be justified.

We have siblings in households who have been exposed to vaccinees for several weeks, and these data could be made into a very good retrospective study and, if financial resources were available, the information would be worth the cost.

My last comment is, I believe, in line with what Dr. Flipse has mentioned. The data are certainly too preliminary to draw any conclusions. Consequently, we have avoided trying to interpret our data, and we should hope that other groups would refrain from such an interpretation at this time.

DR. HARDY: Looking back on the planning of this study, we are amazed at how different our findings would have been had different decisions been taken. Had we elected to limit vaccine feeding to those under 20, our findings might have been very different. Again, looking back, I am inclined to feel that had we set this up as a placebo-control study, our observations would have less meaning than they do at present. We might well have been faced with a study group with possibly two or three cases among those fed the vaccine. But the numbers at that level would be so low that one really could not hope to form any reasonable opinion from them.

Looking to the future, we are going to be faced with precisely this difficulty. Should there be a risk here, it is apparently in the order of one in 100,000 and in the young, of much lower order than this. To seek to study a risk of that order by placebo study seems to me rather impractical.

I am impressed with the need for obtaining data in areas where the vaccine will be used. We went into this study with full confidence that the question of safety had been settled. We hope that there are no serious questions there now.

However, we did feel considerable reluctance to apply to our area, our population, with an entirely different immunological history, the results of findings in, for example, immunologically so strikingly different an area as Mexico. This, it seems to me, bespeaks the need for a continuation of necessarily large-scale field trials in this country.

I feel, too, that this is needed, not for one group of strains, but probably for all, before we can have assurance that, in the area where they will be used, the results can be predicted reliably.

DR. ARMSTRONG: We should, I believe, commend the workers of Dade County on their very frank and open statements. I also believe that the rest of us should lay aside our prejudices and give that group not discouragement but every encouragement. After all, there are only seven cases, two never having been vaccinated. To make a statistical analysis on this small number of cases is practically impossible. We are therefore confronted with a very difficult problem.

I should now like to direct a few questions to our Russian friends.

This is not said in a critical sense, because there are different standards of surveillance and what is proper and suitable for one country might not be for another.

I might ask Dr. Chumakov, along with my friend Dr. Murray from the National Institutes of Health, if they would sanction the use of vaccine put up in candy. One of the common fatal poisonings in the United States is due to aspirin. Aspirin is put up in candy capsules for children; if they get hold of them and swallow 15, 20, 30, or more, there is trouble.

What would happen in this country if the product were put up in candy might be disturbing. I do not know what would happen if children ate two, three, four, or more times as much

as they were supposed to have eaten. That is one of the things we would have to guard against.

Now, the number of questionable things we find in any field study depends directly on the surveillance. One thought on this: If we are confronted with an epidemic and put on a vaccine campaign, and if a few children become crippled, the vaccine will have to be taken because we are striving to save hundreds.

That is one theory, one view, and I must say that there is a good deal of hard common sense in that attitude. But it would never do in the United States, our people would not stand for it. Our people are very illogical; they do not react to 360 deaths over a weekend holiday, but they will be very perturbed if there is one death as a result of a product which was being used to save their lives.

I should like to ask Dr. Chumakov if they have seen any urticaria following their vaccinations, and I would like to ask him what their rule was following exposure? If an individual is vaccinated and develops polio the next day, it could hardly be due to the vaccination. But what is the upper limit, if you have one, for that period?

On the charts that were shown yesterday, we saw cases that had occurred after vaccination started, and presumably they were unvaccinated cases. They were not mentioned, but Dr. Flipse would have included them among the group that developed polio without being vaccinated, as possible vaccination contacts; we would have a record—otherwise such cases would not have appeared.

Perhaps some light could be shed on such matters if our friends would tell us what rules were followed in their surveys.

CHAIRMAN RHODES: I think this would be excessively difficult to answer, Dr. Armstrong. This is an almost philosophical question you asked, which could take up hours of our time, but perhaps Professor Chumakov or Dr. Voroshilova would care to answer it.

DR. VOROSHILOVA: I shall attempt to say something concerning this because during vaccination in the summer in our country, occurrences of poliomyelitis were observed among the vaccinees, as well as among the non-vaccinated.

First of all, it is necessary to say that by carrying out mass immunization during the winter in areas where there was no poliomyelitis, incidence of this disease among the vaccinated was non-existent. Therefore, when vaccination was begun during the summer we were, of course, aware of the possibility of the appearance of poliomyelitis among the vaccinated, and took all measures for an analysis of such cases.

Our Laboratory had a special experience in the City of Karaganda and in the Karaganda region, where virological investigations have been undertaken since 1952 and where the doctors are familiar with the requirements necessary for the collection of investigation material from the patients.

In the City of Karaganda and in the Karaganda region, 32 persons vaccinated with live vaccine were observed in 1959 and diagnosed by the clinicians as poliomyelitis cases. It should be noted that from each of these 32 patients, as well as from other patients, material for virological research was collected in the course of three successive days. Also, blood specimens from each individual were collected both upon their admittance to, and release from, the hospital.

Only four strains of poliomyelitis virus were found among the 32 patients. At the same time, as in the previous years, the virological and serological diagnosis did not present great difficulties. We found no viruses at all in 22 cases, and in six cases we found viruses that were not of a poliomyelitis nature. The viruses from the vaccinated patients were studied for temperature characteristics and three of them were of the *T*— type. The disease developed in one case one month after a single inoculation with trivalent live vaccine, but after serological investigation, no poliovirus antibodies were detected. This was a very mild case of poliomyelitis and there were no residual symptoms.

In another case, on the 19th day after a single inoculation with live trivalent vaccine, a Type 2 poliovirus strain was found with the characteristics of *T*—. At the same time, a Coxsackie A-4 virus was present in the same sample. An increase of antibodies to poliovirus Type 2 was not present in this patient. There was, however, an increase of antibodies to Coxsackie A-4 virus from 1:16 to 1:128. This was also a very mild

case of poliomyelitis, if it was poliomyelitis; again there were no residual symptoms.

Finally, poliomyelitis occurred on the sixth day after the second inoculation with live trivalent vaccine in the third case from whom a *T*—Type 1 poliovirus strain was isolated. The child already had a titer of 1:512 to poliovirus Type 2, and also a titer of 1:512 to a poliovirus Type 3. As for Type 1, there were no antibodies in the serum extracted on the third day after the onset of illness nor on the 33rd day of the illness.

It is very difficult to interpret these results. Is it possible to say that these cases were caused by the vaccine virus? I think that none of us would dare to draw such a conclusion. Taking into account all the favorable conditions that generally existed during such a mass immunization campaign, it is impossible to say whether they were usual cases of poliomyelitis or that they were mild cases of the disease because of the inoculation background.

Referring to the clinical picture among the vaccinees, we had only one case with residual symptoms among the vaccinated children. All other patients, who had been recorded as paralytic poliomyelitis cases, left the hospital without any residual symptoms. I believe that if the vaccination were to have a favorable result, all children, even those recorded as suffering from paralytic poliomyelitis, would leave the hospital without residual symptoms. This would be a great achievement.

At any rate, the statistics available at present on paralytic poliomyelitis among the vaccinated in 1959 include only one case with residual symptoms.

DR. ZHDANOV: The question is whether it is acceptable to prevent many illnesses by means of small amount of illnesses provoked by the vaccine. I believe such a philosophy to be a very bad one, not acceptable in any country.

That is why we started this work on poliomyelitis, and we carefully searched, step by step, for all possible complications, in all experiments. We investigated every possibility.

That is why our first field trial was undertaken not in the summer, when it was very difficult to understand what happens, but in the winter season in those regions where poliomyelitis was absent, or at least not so largely spread. Then, only after these small experiments with a few thousand who were vaccinated, did we go forward to other major regions, Esthonia and so on, where this disease was prevalent.

And do not forget, the first immunization was carried out during the winter season in places where all the cases could be searched and investigated. Of course, when we started with the last campaign, we were quite sure that complications would be avoided in every possible way.

CHAIRMAN RHODES: The next paper to be presented is by Dr. Plotkin on "Vaccination with the CHAT strain of Type 1 Attenuated Poliomyelitis Virus in Leopoldville, Belgian Congo." Following that, we shall have Dr. Gear's presentation on "Live Virus Vaccine Studies in Southern Africa."

VACCINATION WITH THE CHAT STRAIN OF TYPE 1 ATTENUATED POLIOMYELITIS VIRUS IN LEOPOLDVILLE, BELGIAN CONGO. III. SAFETY AND EFFICACY DURING THE FIRST TWENTY-ONE MONTHS OF STUDY*

STANLEY A. PLOTKIN, M.D., ANDRÉ LEBRUN, M.D., GHISLAIN COURTOIS, M.D., AND HILARY KOPROWSKI, M.D.†

DR. PLOTKIN *(presenting the paper):* First, let me say that this represents the work of Dr. Lebrun, Dr. Chyssels, Dr. Jourdain, and Dr. Courtois from Leopoldville.

Vaccination with the CHAT Type 1 strain of attenuated poliovirus has been progressing in Leopoldville, Belgian Congo since August 1958. By April 1960, approximately 21 months after the commencement of the campaign, approximately 75,000 African children under the age of five years had been vaccinated. This number represents an increase of approximately 30,000 over the total number of vaccinees on 30 April 1959, the date of the preliminary report presented at last year's Conference.[1, 2] This communication is concerned with a year's further studies.

Table 1 summarizes the vaccination and population statistics for the age group of African children in which almost all poliomyelitis cases occur, that is, six months through two years. As of 30 April 1960, 41,400 of approximately 46,200 children in the most susceptible age group had been fed CHAT virus. Vaccination has been widespread throughout all districts of Leopoldville.

Poliomyelitis Incidence—May 1959 through April 1960. The monthly incidence of paralytic poliomyelitis during 1958, 1959, and the first four months of 1960, is given in Table 2. A Type 1 epidemic occurred during the latter part of 1958 and early 1959, ending in March 1959. Beginning in May 1959 and continuing into 1960, there have been from three to 10 cases of paralytic poliomyelitis each month. No sharp epidemic peak has been observed.

The age distribution of the cases is shown in Table 3, in comparison to the age distribution of the 1958-59 epidemic, to which it is similar.

For the purposes of analysis, Leopoldville can be divided into three major geographic areas, the Ancienne Cité, the Nouvelle Cité, and the five smaller districts. Table 4 gives the number of cases in each area during the past year and also analyzes the patients according to those who had been previously given CHAT virus and those to whom no vaccine had been administered before onset of illness. Of 76 cases, 47 were in non-vaccinated infants and 29 in vaccinated infants. It is of interest to note that in the Ancienne Cité, the number of vaccinated cases exceeded the number of non-vaccinated cases, whereas the opposite is true in the other two districts. We shall return to this point later.

Virological studies have been done on poliomyelitis cases since the beginning of this vaccination campaign. Table 5 summarizes the monthly experience with isolation of viruses from cases of paralytic poliomyelitis. During the 1958-59 epidemic almost all isolated viruses were Type 1, with an occasional Type 2 or 3 polio or non-polio viruses. Beginning in July 1959, a change occurred in that Type 3 poliovirus was isolated more frequently. We have virological data only until the end of February 1960 and Type 3 virus was still being isolated in February. Table 6 summarizes the isolation data by type of

* These studies were supported by grants from the National Institute of Allergy and Infectious Disease.

† Dr. Plotkin (Epidemic Intelligence Officer, Communicable Disease Center, U.S. Public Health Service, on assignment at The Wistar Institute); Dr. Lebrun (Medical Director, Marcel Wanson Institute of Hygiene, Leopoldville, Belgian Congo); Dr. Courtois (Director, Princess Astrid Institute of Tropical Medicine); and Dr. Koprowski (Director, The Wistar Institute, Philadelphia, Pennsylvania). Participation of Dr. Plotkin in these studies in no way implies the endorsement of the U.S. Public Health Service; the opinions are those of the author.

TABLE 1. POPULATION AND VACCINATION STATISTICS, LEOPOLDVILLE AFRICAN CHILDREN 6 MONTHS TO 2 YEARS OLD (IN THOUSANDS)

AREA OF CITY	AVERAGE POPULATION		VACCINEES	
	1958	1959	30 APRIL 1959	30 APRIL 1960
Ancienne Cité	13.5	14.7	10.7	13.6
Nouvelle Cité	15.1	15.6	7.0	12.4
Five Districts	12.0	15.9	7.9	15.4
All	40.6	46.2	25.6	41.4

TABLE 2. REPORTED CASES OF PARALYTIC POLIOMYELITIS BY MONTH, JANUARY 1958 THROUGH APRIL 1960 AND BY VACCINATION STATUS

Reported Cases Each Month: Vaccinated Cases in Parentheses

YEAR	JAN.	FEB.	MAR.	APR.	MAY	JUNE	JULY	AUG.	SEPT.	OCT.	NOV.	DEC.
1958	12	5	6	1	2	0	1	0	1	3	9	37
								(−)*	(−)	(−)	(1)	(−)
1959	24	15	9	1	5	6	10	9	7	8	6	6
	(3)	(2)	(4)	(−)	(1)	(1)	(4)	(5)	(3)	(4)	(3)	(3)
1960	4	6	6	3								
	(1)	(3)	(1)	(−)								

* Vaccination began in August, 1958.

TABLE 3. AGE DISTRIBUTION OF PARALYTIC POLIOMYELITIS IN AFRICAN CHILDREN, LEOPOLDVILLE, SINCE BEGINNING OF VACCINATION

AGE IN YEARS	AUG. '58—APR. '59		MAY '59—APR. '60	
	NUMBER	PER CENT	NUMBER	PER CENT
<0.5	–	–	3	4
0.5–	25	25	14	18
1–	57	58	37	49
2–	15	15	16	21
3–	1	1	4	5
>3	1	1	2	3
Total	99	100	76	100

virus and also by the residential area and vaccination status of the individual from whom the virus was isolated. Seventeen Type 3 isolations were made during May to December 1959, as well as five Type 1's, five Type 2's, and six non-polio viruses. Fifteen viruses were isolated from vaccinated individuals, of which 10 were Type 3. Eighteen viruses were isolated from non-vaccinated individuals, of which seven were Type 3.

From these data it is clear that there has been

TABLE 4. PARALYTIC POLIOMYELITIS IN AFRICAN CHILDREN BY GEOGRAPHIC AREA OF LEOPOLDVILLE AND VACCINATION STATUS

AREA	CASES		
	VACCINATED	NON-VACC.	TOTAL
Ancienne Cité	14	7	21
Nouvelle Cité	10	21	31
Five Districts	5	19	24
Total	29	47	76

TABLE 5. VIRUS ISOLATIONS FROM STOOLS IN PARALYTIC CASES, AUGUST 1958-DECEMBER 1959

	POLIO 1	POLIO 2	POLIO 3	NON-POLIO
1958 Aug.	—	—	—	—
Sept.	—	—	—	—
Oct.	1	—	—	—
Nov.	1	—	—	—
Dec.	6	—	1	2
1959 Jan.	6	—	1	—
Feb.	5	1	—	—
Mar.	2	—	—	—
Apr.	—	1	—	—
May	—	—	—	—
June	2	1	1	1
July	2	1	2	1
Aug.	1	—	1	—
Sept.	—	—	6	—
Oct.	—	—	2	2
Nov.	—	—	—	1
Dec.	—	—	2	—
1960 Jan.	—	—	1	—
Feb.	—	3	1	—

TABLE 6. VIRUS ISOLATIONS FROM STOOLS IN PARALYTIC CASES ANALYZED BY LOCATION OF CASE AND VACCINATION STATUS

AREA	GROUP	VIRUS ISOLATION				
		POLIO 1	POLIO 2	POLIO 3	NON-POLIO	TOTAL
Ancienne Cité	Total	—	3	6	1	10
Nouvelle Cité	Total	2	2	3	3	10
Five Districts	Total	3	—	8	2	13
All	Vacc.	1	2	10	2	15
	Non-Vacc.	4	3	7	4	18
	Total	5	5	17	6	33

TABLE 7. INTERVAL BETWEEN VACCINATION AND ILLNESS IN VACCINATED CASES

INTERVAL IN MONTHS	NO. OF CASES
<1	5*
1–	—
2–	5
3–5	13
6–8	10
9–11	6

* Expected number = 7.8.

a shift in the causative agent of most cases of poliomyelitis in Leopoldville; from Type 1 to Type 3. It is interesting to speculate that this change might be due to the recent Type 1 epidemic or to the recent Type 1 vaccination. Future observations will be of considerable interest in clarifying the situation.

Safety—Study of Cases in Vaccinated Individuals. Since the beginning of the vaccination campaign, 39 cases of paralytic poliomyelitis have occurred in vaccinated individuals. The intervals between vaccination and illness are given in Table 7. Only five of the 37 had onsets within a period of two months from the time of vaccination. If one multiplies the number of vaccinations given each month by the expected incidence of poliomyelitis in that number of infants during that month, an expected number of 7.8 cases is obtained indicating that the five cases that did occur are not more than the chance occurrence. The five cases which occurred less than one month after vaccination are further analyzed in Table 8. The first two cases were thought not to be vaccine-caused because of the short incubation period which was available and also because the acid agar, *MS*, temperature, and serologic characteristics of the virus did not resemble those of CHAT. Because of similar laboratory observations, the virus isolated from the third case was thought not to be a descendant of the CHAT strain.

The fourth case did not develop complement-fixing antibodies to poliomyelitis after the onset of paralysis, although neutralizing antibodies to all three types of poliomyelitis were present. Consequently, it does not appear that the illness represented an acute poliovirus infection, although, unfortunately, a stool specimen was not obtained. From the last case a Type 3 virus was isolated. It should be stated that no attenuated Type 3 vaccine was used in Leopoldville until vaccination of the European population was begun in September 1959, well after the beginning and the peak of the natural Type 3 epidemic which, as shown in Table 5, was reported in September 1959, the cases therefore having been infected several weeks earlier.

All Type 1 polioviruses isolated in Leopoldville from paralytic cases both vaccinated and non-vaccinated, have been studied from the point of view of at least two laboratory tests. First, for the reproductive capacity at 40° C., and second, for the ability to form plaques under a specific anti-CHAT serum. The latter test consists of the production of an antiserum to a virus, in this case CHAT, by hyperimmunization

TABLE 8. SUMMARY OF CASES HAVING ONSET OF POLIOMYELITIS WITHIN A MONTH OF VACCINATION

PATIENT NO.	DATE OF VACCINATION (1959)	DATE OF ONSET OF ILLNESS (1959)	INTERVAL VACCINATION TO ONSET	FECAL VIRUS TYPE	C-F TEST POLIO TYPE 1	2	3
63	9 or 10 Jan.	11 Jan.	1–2 d.	1			
81	2 Feb.	8(?)Feb.	6 d. or less	1			
93	6 Feb.	26 Feb.	20 d.	1	+	0	0
201	15 April	30 April	15 d.	N.T.	0	0	0
46-59	20 Oct.	18 Nov.	29 d.	3			

N.T. = Not tested.

of guinea pigs, and then the use of this serum to neutralize the virus against which it was prepared and other viruses which one desires to compare with the prototype strain. For example, in a typical test, monkey-kidney monolayers in Petri dishes are separately inoculated with approximately 10 plaque-forming units of CHAT, Mahoney, and a virus to be tested for its similarity to CHAT. After time is allowed for adsorption, some of the monolayers are overlayed with agar containing several dilutions of CHAT antiserum. After incubation, the plates are read for the number and size of the plaques formed. The dilution which is used to read the test is that which just neutralizes the CHAT virus but allows Mahoney virus to form plaques. The Mahoney virus plaques will generally be approximately 30 per cent —40 per cent of the diameter of control Mahoney plaques. If the unknown virus is similar to CHAT, it will be neutralized to the same degree; if not, it will break through as does the Mahoney virus.

Table 9 shows the results of these tests when applied to known human intestinal passages of the CHAT virus. To this date, none of the viruses tested, which included up to three human intestinal passages, has differed from CHAT with respect to temperature, character or what we would call its antigenic character. The 10 virulent viruses referred to were the Type 1 viruses isolated from cases in Leopoldville up until April 1959.

Tests of Type 1 viruses isolated more recently are shown in Table 10. Nos. 211 and 212 were isolated from vaccinated infants. They are close to CHAT in their temperature and serologic characters. Viruses isolated from non-vaccinated children are represented by Nos. 233, 235, 224, and 229, whereas No. 220 was isolated from a vaccinated child. All of these viruses are able to grow at 40° C. and are capable of breaking through the CHAT antiserum.

Virus 525 is of particular interest. The patient from whom this was isolated was a 32-year-old European woman who developed poliomyelitis one month after coming to a village near Leopoldville. In this village and in neighboring villages during the latter part of 1959 there had been

TABLE 9. GENETIC AND SEROLOGIC MARKERS OF CHAT AND ITS HUMAN PASSAGE STRAINS

STRAIN	HUMAN INTESTINAL PASSAGE	MARKERS				
		TEMP.	MS	NEUT.*	IC PAR.	PATHOG. LESIONS
CHAT	—	Cold	MS	Yes	0/5	0/5
C–1	1	Cold	MS	Yes		
D—1		Cold	MS	Yes		
I–1		Cold	MS	N.T.		
L–1		Cold	MS	Yes		
R–1		Cold	MS	N.T.		
Q–1	↓	Cold	MS	Yes	0/4	0/4
O–5	2	Interm.	MS	Yes		
Q-5	2	Cold	MS	Yes	0/3	1/3
Q–2	3	Cold	MS	Yes	0/7	0/7
Mahoney	—	Hot	MS+	No		
Virulent (10)†	?	Hot(6) Interm. (1) N.T.(3)	MS+(10)	No(6) N.T.(4)		

* By anti-CHAT serum.
† Ten "wild" strains isolated from paralytic patients during an epidemic.

TABLE 10. VIRUSES (LEOPOLDVILLE)

VIRUS	GROUP	LOG $TCID_{50}$ AT 37°C − LOG $TCID_{50}$ AT 40°C	% CONTROL PLAQUE DIAMETER UNDER CHAT ANTISERUM
CHAT	Vaccine	> 6.0	0
Mahoney	Virulent Control	0.0	40
211	Vaccinated Children	> 2.7	10
212		> 4.5	0
233	Non-Vacc. Paralytic Cases	0.5	50
235		0.2	45
224		0.8	40
229		0.0	50
220	Vaccinated Paralytic Case	0.3	38
525	Non-Vacc. Case in Vaccinated Community	0.0	43

several cases of Type 1 paralytic poliomyelitis. Consequently, on 30 November 1959 CHAT was administered to most of the European and African population of the village. Thus 374 Europeans of all ages and 253 African children less than five years old were given the virus. It is not known how many remained unvaccinated in the village, but there is no question that there were many Africans who failed to appear for vaccination. On 10 December 1959, 20 days after the vaccination had been performed, the woman arrived in the Congo from Holland. On 10 January 1960, one month after her arrival in this village, she developed bulbar spinal poliomyelitis and Type 1 poliovirus was isolated from her stool. This is the virus shown in the table to be capable of breaking through the CHAT antiserum. Since this woman has two young children, it is conceivable that she was infected by a wild Type 1 poliovirus transmitted from African children to her own.

Efficacy of vaccination. In the first report of the vaccination campaign in Leopoldville, an attempt was made to calculate the efficacy of vaccination despite numerous problems in the evaluation of the results. It was determined, using two methods, that there was at least mathematical evidence of approximately 60 per cent protection afforded by the vaccine. It was also shown that the antibody response to vaccination, presumably because of viral interference, was only 60 per cent. Another year's data have been incorporated into calculations which are presented on the next two tables. Table 11 shows calculation of efficacy by the person-week method in which we have taken two periods of time, first the Type 1 epidemic (October 1958 through March 1959) and second, the recent experience (May 1959 through April 1960). For each period, those that are vaccinated at the beginning are multiplied by the number of weeks to the end of the period, and those that are vaccinated later are multiplied by the number of weeks remaining to the end of the period. Thus, cumulative experience of vaccinated and unvaccinated is built up. For the total experience, we find a

TABLE 11. ESTIMATED PROTECTION BY CHAT-VIRUS AGAINST PARALYTIC POLIOMYELITIS IN LEOPOLDVILLE AFRICAN CHILDREN 6 MONTHS THROUGH 2 YEARS OF AGE

DISTRICT	PERIOD*	GROUP	CASES	PERSON-WEEKS (P-W)	RATE PER 10^5 P-W	ESTIMATED PROTECTION
						(%)
Ancienne Cité	1	V	4	64,800	6.2	53
		NV	27	205,200	13.2	
	2	V	13	774,800	1.7	52
		NV	5	142,480	3.5	
Nouvelle Cité	1	V	2	47,800	4.2	71
		NV	41	284,200	14.4	
	2	V	8	677,560	1.2	80
		NV	18	295,880	6.1	
Five Districts	1	V	4	130,400	3.0	68
		NV	13	133,600	9.7	
	2	V	5	686,920	0.7	88
		NV	18	305,240	5.9	
All†		V	34		2.6	67
		NV	85		7.7	

* 1 = Period of Type 1 epidemic, October 1958 through March 1959.
2 = End of April 1959 through April 1960.
† Excluding period 1 in Nouvelle Cité.

rate of 7.7 cases per 100,000 person-weeks in the unvaccinated population, and 2.6 cases per 100,000 person-weeks in the vaccinated. The estimated protection from these figures is 67 per cent. Note that the Ancienne Cité has an estimated protection of somewhat less.

The second method of calculation (Table 12) employed the expected number of cases in vaccinated individuals, determined by multiplying the number of cases during a given week by the number vaccinated as of two weeks previously and dividing that by the total number of susceptible individuals. It was found that 69.55 cases would have been expected in vaccinated individuals whereas only 36 actually occurred. Chi-square was highly significant with a probability of less than .01.

It may be objected that the evidence of protection should not be quite so good considering that most of the recent cases apparently have been caused by Type 3 virus. In this regard, it is of interest that in the Ancienne Cité where no Type 1 viruses were isolated during the Type 3 epidemic, there was poorest evidence of protection, whereas in the other two areas where some Type 1 viruses were isolated during June, July, and August 1959, the apparent protection was higher. In other words, the areas which showed

TABLE 12. SIGNIFICANCE OF ESTIMATED PROTECTIVE EFFECT OF CHAT VACCINE AGAINST POLIOMYELITIS IN VACCINATED LEOPOLDVILLE AFRICAN CHILDREN 6 MONTHS THROUGH 2 YEARS

DISTRICT	CASES OBSERVED	CASES EXPECTED	CHI-SQUARE
Ancienne Cité	17	23.73	1.91
Nouvelle Cité	10	20.89	5.67
Five Districts	9	24.93	10.28
All	36	69.55	17.86*

* $n = 2$; $p < .01$.

the highest levels of significant protection were those in which Type 1 viruses were circulating at some time during this period in addition to Type 3. It is also possible, of course that Type 1 immunity partially protects against Type 3 poliomyelitis.

SUMMARY

Seventy-five thousand African children under the age of five years have been vaccinated in Leopoldville, Belgian Congo with CHAT Type 1 attenuated virus beginning in August 1958. Approximately 46,000 of these children are in the highly susceptible group of which 41,000 had been vaccinated as of 30 April 1960. During this period there has been an epidemic of Type 1 paralytic poliomyelitis, followed by a smaller Type 3 epidemic. A six-month period has passed without the isolation of Type 1 virus from patients with paralytic poliomyelitis. Cases in vaccinated individuals did not occur during the period immediately after vaccination more frequently than would be expected by chance. Virus strains isolated from vaccinated and non-vaccinated cases in Leopoldville were shown in the laboratory to differ from CHAT virus and its known human passages in at least two characteristics.

The efficacy of vaccination computed as closely as possible appeared to be on the order of 67 per cent. Where poliomyelitis was most exclusively caused by Type 3 virus, there was the least difference in incidence between expected and observed number of cases in vaccinees.

ACKNOWLEDGMENTS

We wish to express our thanks to Dr. Ghyssels and Dr. Jourdain of the Princess Astrid Institute of Tropical Medicine in Leopoldville, and to Suzanne Richardson of The Wistar Institute.

REFERENCES

1. Lebrun, A., Cerf, J., Gelfand, H. M., Courtois, G., Plotkin, Stanley A., and Koprowski, H.: Vaccination with the CHAT strain of Type 1 attenuated poliomyelitis virus in Leopoldville, Belgian Congo. 1. Description of the city, its history of poliomyelitis, and the plan of the vaccination campaign. *Bull. Wld. Health Org.* **22:** 203-213, 1960.
2. Plotkin, Stanley A., Lebrun, A., and Koprowski, H.: Vaccination with the CHAT strain of Type 1 attenuated poliomyelitis virus in Leopoldville, Belgian Congo. 2. Studies of the safety and efficacy of vaccination. *Bull. Wld. Health Org.* **22:** 215-234, 1960.

20. LIVE VIRUS VACCINE STUDIES IN SOUTHERN AFRICA

JAMES H. S. GEAR

Poliomyelitis Research Foundation
South African Institute for Medical Research
Johannesburg, Union of South Africa

DR. GEAR *(presenting the paper)*:

EARLY STUDIES

Work on the development of a live virus vaccine against poliomyelitis began at the South African Institute for Medical Research in 1946, when an attempt to attenuate the Lansing strain of the virus by passage in *Mystromys albicaudatus*, a veld rodent, was initiated. At the beginning, the virus paralyzed nine of 10 monkeys, *Cercopithecus aethiops pygerythrus*, inoculated intracerebrally, two of seven after 47 passages, and after 100 passages, and again after 150 passages, only one of the 10 monkeys inoculated intracerebrally developed paralysis, indicating that some attenuation of the virus for these monkeys had occurred. A feeding trial was contemplated at this stage and arrangements were actually made, but not carried out.

At about the 180th passage, the virus suddenly increased greatly in virulence for the rodents and most of them developed paralysis within 24 hours of inoculation. It was suspected that some other virus such as EMC or Columbia SK had been picked up in the course of passage, but identity tests showed that the virus was still a Type 2 poliovirus. This finding might be of some interest to those who, some years ago, were interested in low titer and high titer Lansing virus. It was found that its virulence for monkeys had also increased again, and six of the 10 vervet monkeys inoculated intracerebrally developed paralysis. It was also found to be relatively non-cytopathogenic in tissue cultures.

At this time, the whole energies of the staff of the institution had to be devoted to the preliminary studies and then to the production of a formalin-inactivated vaccine. Work on the attenuation of the virus was discontinued.

IMMUNITY SURVEYS

Immunity surveys, as part of a study of the epidemiology of poliomyelitis in this region and also to assess the need of various groups for protection, have been carried out on representative samples of the populations of the Territories of Southern Africa and of the neighboring islands in the Atlantic and Indian Oceans.

These have shown, in general, that most children of the indigenous African native population have developed antibodies against all three types of poliovirus by the time they are six years old. On the other hand, in those of European descent, most of whom live under good conditions of hygiene, there is a lower degree of immunity and some adults lack immunity to all three types of virus, a larger number to two, and a still larger number to one type of poliovirus.

The incidence of the paralytic disease in the past has been in keeping with these serological findings. In the African native population, nearly all cases occur in children under six years old, and most of the cases in infants under three years old. However, within the last five years, the condition, previously sporadic, has begun to occur in epidemics of increasing severity. The fact that a large proportion of the population in the large urban centers, for example about 70 per cent in Johannesburg, has been rehoused in model townships provided with waterborne sewerage, may be partly responsible for the increasing incidence. In the population of European descent most cases still occur in children under 10 years old, but there is a wider spread of incidence. Cases are frequent in older children and adolescents and not uncommon in adults, in whom the illness tends to be severe and often fatal.

TRIALS CONSIDERED

In the immunity survey of the population of the island of Tristan da Cunha, which is a lonely island halfway between Buenos Aires and Cape Town, in 1955, of the 70 individuals tested, varying in age from two to 45 years, most of them had antibodies to Type 3 virus, but only one had antibody to Type 1 virus. I believe that most of the antibodies to Type 2, about 23 per cent, are due to heterotypic antibody developed as a result of this wave of infection. As poliomyelitis due to Type 1 poliovirus was then prevalent in Cape Town, the port from which ships sailed to supply the island, it was considered advisable to protect the population against this infection and it was suggested that they should be immunized by feeding with Dr. Koprowski's SM and TN strains. Dr. Koprowski arrived in South Africa and was available for consultation shortly after this. The suggestion was approved by the medical authorities of the British Colonial Office and the British Medical Research Council, but for various reasons, it was not considered to be expedient by higher authorities and it was not carried out.

Early in 1956, the beginning of an epidemic in an African native township in East London was brought to our notice by Dr. Sinclair-Smith, the Medical Officer of Health of that city. The epidemic was proved to be due to a Type 1 virus, and the possibility of feeding an attenuated Type 1 strain in the face of this epidemic was discussed and a supply actually flown to East London. However, on further consideration, it was again decided not to administer the virus. The epidemic continued and developed into the worst and most severe experienced in such a township.

RECENT STUDIES

In 1957, the Advisory Committee on Virus Diseases of the Union Health Department agreed that trial feedings of attenuated virus could be carried out in the face of an impending epidemic, preferably when it occurred in a relatively isolated community. No such circumstances have since developed in the Union of South Africa, in which formalinized vaccine has been used on a relatively large scale.

Mauritius. In July 1959, the Director of Medical Services of the Island of Mauritius in the Indian Ocean, notified us of a sharp epidemic then developing in the island. It was suggested to him that an attempt should be made to bring the epidemic to an end by feeding attenuated virus of the same type as that causing the epidemic.

Specimens from 10 cases were flown to the Poliomyelitis Research Foundation and the virus identified as a Type 1 strain.

Dr. P. D. Winter, Head of the Vaccine Division of the Poliomyelitis Research Foundation, then flew to Mauritius, taking with him 250,000 doses of Sabin's LSc Type 1 strain, prepared in the laboratories of the Poliomyelitis Research Foundation, and tested for safety according to our own criteria.

An immunity survey of the population had been carried out in 1955. The results of the tests on 80 children under 10 years old are shown in Table 1.

In September 1957 Salk type vaccine was offered to all children between one and five years old, in September 1958 to all between the ages of one to two years, and in April 1959 to all between one and two years. By the end of June 1959, the vaccination status of the children between one to seven years old was:

3 doses vaccine	43,395
2 doses vaccine	35,603
1 dose vaccine	46,120
0 dose vaccine	25,790

TABLE 1. POLIOMYELITIS IMMUNITY—MAURITIUS—1955

AGE GROUP	TOTAL	ANTIBODIES					
		TYPE 1		TYPE 2		TYPE 3	
		+	−	+	−	+	−
1—5 years	37	21	16	23	14	27	10
6—10 years	43	35	8	38	5	29	14

TABLE 2. REPORTED CASES OF POLIOMYELITIS—MAURITIUS

YEAR	NO. OF CASES	PERIOD	CASE RATE PER 100,000	MORTALITY RATE
1945	1018	Feb—April	240	6
1948/49	552	Nov—Feb	128	5.2
1952	284	March—June	56	1.0
1959	97	June—Sept	15	0

The epidemic started while the vaccination campaign was still on, and occurred in midwinter. Three previous epidemics had occurred in summer or autumn. This is the first one in midwinter. The details are given in Table 2.

The attack rate in children of one to seven years who had received two to three doses of Salk vaccine, and amongst the unvaccinated was:

	Attack rate/100,000
Vaccinated, 3 doses	2.30
Vaccinated, 2 doses	11.23
Unvaccinated	279.17

It is of some interest to note that the incidence in those who had received three doses of Salk vaccine, which was prepared in North America, not in South Africa, was 2.3 per 100,000, two doses 11.23, and unvaccinated 279.

These figures are slightly loaded in favor of the vaccine because of the specific attack rate which is not gone into here, but they do reflect a marked degree of protection.

The attack rate in the various age groups is shown in Table 3. It will be noted that in the 1959 epidemic, as in the previous ones, but even more marked, most cases occurred in the under three-year-old age group.

Vaccination. Dr. Winter arrived on 14 August 1959. After consultation with the Ministry of Health, it was agreed that all children between six months and 10 years should receive the vaccine. Vaccination centers, each in charge of a medical officer, were established and vaccination was started on 24 August. There was a good response and within 10 days, 195,000 children out of an estimated population of 213,000 had been given the vaccine. The course of the epidemic is shown in Table 4. It will be noted that after 29 August there were only three further cases, and there have been no cases since then.

It is apparent that the epidemic was waning before the feeding of the vaccine virus was begun. However, none of the three previous epidemics terminated so abruptly and it is possible that the vaccination campaign played some part in bringing it to an end.

The island was devastated by two cyclones in February and March this year, but no outbreaks of poliomyelitis followed in their wake, as was the case in 1945. Professor Dick went to the island to study the very severe epidemic which occurred in that year. To protect against typhoid fever resulting from the breakdown in sanitation about 250,000 people were inoculated twice. The Director of Medical Services has commented that the contrast of the administration of this vaccine impressed him with the ease of the giving of poliovirus vaccine by mouth.

TABLE 3. POLIOMYELITIS ATTACK RATES (PER 100,000)—MAURITIUS

YEAR	ATTACK RATE/100,000, BY AGE GROUP										
	0–1	1–	2–	3–	4–	5–	6–	7–	8–	9–	10+
1945	400	1490	1230	1230	1300	1190	860	340	170	80	Not given
1952	237	388	422	139	36	Not given					
1959	55	154	115	41	14	15	10	5	11	0	.94

TABLE 4. POLIOMYELITIS EPIDEMIC, 1959—MAURITIUS

	JUNE			JULY			AUGUST				
Week ending	20	27	4	11	18	25	1	8	15	22	29
No. cases	1	0	5	—	17	20	19	16	8	5	4

Subsequent to feeding virus:

	SEPTEMBER				OCTOBER
Week ending	5	12	19	26	
No. of cases	1	—	2	—	nil.

Laboratory Studies. Before the campaign began, samples of stools and blood from family groups and blood from children under six years who had not received Salk type vaccine were taken at random from all parts of the island. The results of the virus isolations, summarized in Table 5, show that 216 stools were tested, with a percentage positive rate of 33 per cent, indicating a very wide spread of infection.

Post-epidemic, but pre-vaccination immunity is given in Table 6, and it will be noted that in the 0 to 5-year-age group, of the 113 tested, every one was immune to Type 1; 40 were immune to Type 2; and 33 to Type 3.

So the conclusion obviously is that the epidemic had immunized the population before the vaccine was given.

Rodrigues. Rodrigues is a small island, a dependency of Mauritius, 300 miles further east. It has a population of about 17,500 and a steamer from Mauritius calls about once a month with mail and supplies.

In an immunity survey carried out in 1957 (see Table 7), it was found that most children under 12 years lacked immunity to Type 1 poliovirus. In January 1958, vaccination with Salk type vaccine was offered. However, many children were not inoculated, and as an epidemic of poliomyelitis was occurring in Mauritius it was considered desirable to immunize the population with live virus vaccine before the epidemic strain was introduced, as seemed likely to occur. It was originally planned to begin this campaign in September 1959, but this was not possible as

TABLE 5. VIRUS ISOLATION—MAURITIUS

NO. OF STOOLS TESTED	NO. POSITIVE	PERCENTAGE POSITIVE
216	71 (Type 1)	33

TABLE 6. PRE-VACCINATION IMMUNITY, AUGUST 1959—MAURITIUS

	TOTAL	TYPE 1		TYPE 2		TYPE 3	
AGE GROUP	TESTED	+	−	+	−	+	−
0—5 yrs	113	113	0	40	73	33	80
6—10 yrs	57	57	0	56	1	56	1
11—15 yrs	6	6	0	6	0	6	0
Over 20	17	17	0	17	0	16	1
	193						

TABLE 7. RODRIGUES—IMMUNITY SURVEY 1957

AGE GROUP	TOTAL TESTED	TYPE 1		TYPE 2		TYPE 3	
		+	−	+	−	+	−
0—3 yrs	45	0	45	0	45	0	45
4—10 yrs	41	3	38	38	3	32	9
11—15 yrs	28	7	21	28	0	23	5
16—20 yrs	8	2	6	7	1	6	2
Over 20	10	8	2	10	0	10	0
Post Salk Vaccine							
0— 5 yrs	50	1	49	34	16	20	30

neither the vaccine nor transportation was available until November.

While waiting, the epidemic struck Rodrigues. I think we are extremely lucky that transportation was not available, since the infection was apparently introduced by passengers arriving by steamer from Mauritius in the middle of August. The first paralytic case was reported on 25 September 1959, and the last case on 6 October. Nine cases were reported. These were scattered over the island. Two had previously been vaccinated with Salk type vaccine, and seven had not been vaccinated.

Trivalent vaccine prepared from Sabin's strains by the Laboratories of the Poliomyelitis Research Foundation arrived on the island on 8 November and a mass vaccination campaign was carried out under the direction of Dr. Chung Hin. Of the total population of 17,603, 16,802 received the vaccine, including the oldest inhabitant, a woman of 103 years. No ill effects were reported after vaccination.

For the assessment of the value of the vaccine, pre- and post-vaccination bloods and samples of pre-vaccination stool were collected. These were refrigerated, taken by steamer to Mauritius, and then flown by air to Johannesburg.

Laboratory Studies. The results of the laboratory studies are summarized in Tables 8 and 9. The post-epidemic but pre-feeding specimens showed that all of those tested in this age group

TABLE 8. VIRUS ISOLATION—RODRIGUES

NO. OF STOOLS TESTED	NO. POSITIVE	NEGATIVE
4	2 (Type 1)	2

TABLE 9. PRE- AND POST-VACCINATION IMMUNITY, NOVEMBER 1959—RODRIGUES

AGE GROUP	TOTAL TESTED		TYPE 1		TYPE 2		TYPE 3	
			+	−	+	−	+	−
0—3 yrs	33	Pre	33	0	23	10	6	27
		Post	33	0	27	6	26	7
4—10 yrs	56	Pre	56	0	48	8	42	14
		Post	56	0	55	1	52	4
11—15 yrs	26	Pre	26	0	26	0	26	0
		Post	26	0	26	0	26	0
16—20 yrs	2	Pre	2	0	2	0	2	0
		Post	2	0	2	0	2	0
Over 20	0		—	—	—	—	—	—

were immune to Type 1, as they are also immune after vaccination.

It will be noted that there was a slight change in immunity status of the Type 2. Some became immune who were not previously immune. There was a more marked change with Type 3.

Observations of some interest, and of possible significance in view of the other findings reported at this Conference, were that after the Salk vaccine given in 1958, before the epidemic, the immunity of 50 children was tested, and of the 50 only one had antibodies against Type 1, 34 had antibodies against Type 2, and 20 against Type 3. The immunity given by the Salk-type vaccine against Type 1 was extremely inadequate.

DISCUSSION

It was apparent from the clinical findings that the wave of infection had swept through most of the population of both Mauritius and Rodrigues by the time the vaccination campaign was begun. This conclusion was confirmed by the laboratory studies. Of interest was the high incidence of positive findings in the pre-vaccination specimens of feces, 33 per cent. The very high proportion of the tested children who had antibodies against Type 1 virus before the administration of the live virus vaccine, was also significant, indicating that natural infection spreads very much more rapidly than the virus vaccine infection. In view of these findings, no conclusion can be drawn in regard to the value of the vaccine in bringing the epidemic to an end. However, it is noteworthy that no ill effects were reported from either Mauritius or Rodrigues following vaccination, and in the latter island, most children who were lacking antibody developed it to Type 3, and a lesser number to Type 2 after the administration of the vaccine.

Kenya. In the last decade the epidemiology of poliomyelitis in Kenya has changed from a disease of high endemicity with sporadic infantile cases to one of epidemicity. There has been a steadily rising number of cases reported each year from 1945, with two major epidemics, one in 1954 and one in 1957. Both were studied in detail and it was noted that Europeans, particularly young children and recent immigrants, were at great risk, with an attack rate of 244 (1954) and 80 (1957) per 100,000. Asians were next with attack rates of 40 and 14 per 100,000. Africans had the lowest rates, of 4.6 and 7.2 per 100,000. In 1957 Koprowski's attenuated Type 1 strain was offered for trial, but after consideration was not used.

In 1959 a slight increase in the number of poliomyelitis cases was noted in Mombasa. This was followed by a sudden occurrence of cases in the Kerugoya and Embu districts of Central Kenya, which had been relatively severely affected in 1957. Nearly all cases were in the under three year olds. Type 1 poliovirus was proved to be responsible.

It was decided to undertake a mass immunization campaign with the attenuated Type 1 poliovirus.

After consultation with Dr. Winter, 60,000 children were given Sabin's Type 1 strain during the week ending 12 December. During the seven weeks preceding this, there had been 25 cases. In the subsequent seven weeks, there were 16 further cases, five of which were in non-vaccinated children, and 11 of which occurred within three weeks of vaccination. Studies since then have shown that Coxsackie A virus infections were common among the vaccinated group of children. This may have had an interference effect on the infection with the vaccine virus. The campaign has since been extended and about 1,500,000 children in the Central Province and in Nairobi have been vaccinated.

The study of the effects is still in progress.

ACKNOWLEDGMENTS

In preparing this paper, I had the help of the reports of Dr. Loconsci of Mauritius and Dr. Fendall of Kenya, to both of whom I am grateful.

DISCUSSION

CHAIRMAN RHODES: These two papers are open for discussion.

DR. DICK: I should like to ask Dr. Plotkin one question. Could we have the age of the patients in Table 8 of his presentation?

With regard to Dr. Gear's presentation it is rather interesting to calculate the attack rates in the Salk vaccinated and non-Salk vaccinated individuals in Mauritius, and it appears that in the Salk-vaccinated individuals the attack rate was 6 per 100,000 while in the non-vaccinated individuals it was 279 per 100,000, which suggests an effectiveness of about 98 per cent.

DR. DULBECCO: I should like to comment on the use of genetic markers, as mentioned in the first report.

I think that I should be very careful in concluding, based on the reported differences between the original strain and some isolated strains, that the latter were different strains not derived from the first one.

In fact, it has been shown that, for instance, a mutation from the inability to grow at 40° C. to the ability to grow at 40° C., would very frequently entail a change in neurovirulence.

Thus, the neurovirulent strains, if they are at all related to the vaccine strain, differ from it because of some mutation, which could be one involving the *T* character. The isolated strains may therefore not have the original *T* character, even if they derive from the administered strain.

I should therefore caution, as I already pointed out on the first day, to the fact that very many markers, like the *T*, the *d*, and others, cannot be used for identification of strains, because they can change when a mutation occurs, which in turn changes the neurovirulence.

The other remark is that I also would be somewhat cautious about the use of the antigenic marker as shown in the first report, because mutations affecting some character, which for instance modifies the speed of virus multiplication in the cells in which the test is carried out, may modify the size of the plaques under antiserum, even if there is no modification of the antigenic character.

And I would suggest that this point be tested by isolating mutants from the original strain, for instance, by selection of some *T*+ at high temperature, which would probably be neurovirulent, and by testing for plaque formation under antiserum, this mutant which certainly derives from the original strain and the original strain itself.

DR. KOPROWSKI: These are very good points made by Dr. Dulbecco, but of course we are dealing with biological phenomena, which do not describe absolute values but their relationships.

There are possibly two approaches to strains isolated from such cases, either to do nothing, or to try using the available markers to distinguish them from other strains.

Now, as far as identity is concerned, I do not think any one of us would identify a strain on the basis of a *T*, *d*, *MS*, or any other such character. We attempted to identify it on the basis of the sero-differentiation test. From the data so far available, we have not encountered a strain which has been obtained after passage of this particular virus through the human intestinal tract which is not neutralized by the anti-CHAT serum.

The test suggested by Dr. Dulbecco is at present being done. We have the very virulent *T*+ mutant of an attenuated strain obtained by Dr. Lwoff after passage at 40° C. and we are checking it against antiserum towards the attenuated strain.

If these results are positive, I believe I would be satisfied that the sero-differentiation test identified excreted virus.

We have done a lot of work plaquing the original virus and checking progenies of virus under serum, and so far, out of 33 or 35 plaque progeny tested, they are all neutralized by the anti-CHAT serum.

DR. PLOTKIN: In reply to Dr. Dick's question,

the ages of the five children referred to in Table 8 are one year, 15 months, one year, three years, and 18 months.

There was also a question asked of me in private which I might answer to clear up any confusion there might be. The calculation of efficacy was made on the basis of considering all cases, regardless of their known or unknown etiology. In other words, Type 3 cases were included in the calculation.

CHAIRMAN RHODES: We shall now proceed with Dr. Smorodintsev's paper on "Material on the Immunological and Epidemiological Effectiveness of Live Poliomyelitis Vaccine." The discussion on this paper will take place during the eighth session.

21. MATERIAL ON THE IMMUNOLOGICAL AND EPIDEMIOLOGICAL EFFECTIVENESS OF LIVE POLIOMYELITIS VACCINE

A. A. Smorodintsev, A. I. Drobyshevskaya, N. P. Bulychev, O. M. Chalkina, G. M. Groisman, V. I. Ilyenko, R. A. Kantorovich, L. M. Kurnosova, K. G. Vasiliev, V. I. Votiakov, and G. P. Zhilova

Virology Department, Institute of Experimental Medicine, USSR Academy of Medical Sciences, Leningrad, USSR

Dr. Smorodintsev *(presenting the paper)*: The extensive use of Salk's inactivated virus vaccine for poliomyelitis control during the last few years, has not eliminated the danger of paralytic forms of the disease developing in triply-vaccinated children, and has had no effect on the circulation of the virus among vaccinated subjects. The limited duration of post-vaccinal immunity has made reimmunization necessary after completion of the schedule of three recommended inoculations.

The fact that 150 monkeys are needed for production of every million dose of killed-virus vaccine, has already created great difficulties in the supply of monkeys for the ever-expanding manufacture of this wasteful preparation.

Nowadays, large-scale production of the complex, expensive, and insufficiently effective Salk vaccine, which involves additional scarring of children because of the repeated injections required, is quite unnecessary in view of the fact that its place can be taken altogether by a more effective, completely harmless live vaccine for oral administration, easy to produce and to use, prepared from the A. B. Sabin or H. Koprowski attenuated strains.[13]

The live vaccine is free from the main defects of the Salk vaccine. It not only establishes long-lasting humoral immunity, which prevents the virus from invading the central nervous system, but also renders the intestinal tract highly insusceptible, thus making it difficult for the causative agent to multiply and spread to other susceptible organisms. The live vaccine provides an opportunity for solving a most important epidemiological problem—the curbing and eliminating of the immense hordes of highly pathogenic wild viruses prevalent in our child population. This is a task quite beyond the powers of the killed vaccine.

The large-scale trials carried out in the USSR in 1959, by the Institute of Experimental Medicine and the Institute for Poliomyelitis Research of the USSR Academy of Medical Sciences for the purpose of assessing the epidemiological effectiveness against poliomyelitis of the live vaccine prepared from the Sabin strains in about 3,000,000 children vaccinated in April-May (before the beginning of the seasonal rise in the incidence of the disease), were made possible by the research carried out by our laboratory in 1956-1958. During those years, we assumed the responsibility of testing the harmlessness and immunogenic properties of the Sabin vaccinal strains on gradually increasing numbers of young children. This made it possible by the end of 1958 to give an affirmative reply to the basic question of whether the live vaccine was harmless and effective on the basis of data obtained from laboratory and clinical examinations of 2,500 healthy children of preschool age inoculated with the live vaccine produced in Leningrad.[15]

The results of this work, carried out in close cooperation with the Nervous Diseases Clinic of the Leningrad Medical Pediatric Institute (Director, E. F. Davidenkova) and the Infectious Diseases Clinic of the Leningrad Institute of Medical Sanitation and Hygiene (Director, V. V. Kosmachevski), showed the complete harmlessness of the live vaccine, both for the children actually inoculated and for uninoculated susceptible children in close contact with them.

Observation of vaccinated and non-vaccinated children over a period of two years showed that

the live vaccine caused no lesions of the central nervous system nor produced even the slightest meningeal forms of poliomyelitis, and caused no damage to the intestinal canal, the respiratory tract, or any other organs. Even fever reactions were very rare in the children vaccinated with the live vaccine and were usually no more frequent than in children of similar age in institutions where the live vaccine had not been used at all.[17, 4, 11]

The observations established that the live attenuated vaccine possesses a high degree of immunogenicity for susceptible children when administered in three doses in the form of monovalent vaccines. A single administration of two and three viruses of different types combined in a divaccine or trivaccine proved immunogenically somewhat less effective.

The immunization of susceptible children in three stages with monovalent preparations of live poliomyelitis vaccine of Types 1, 2, and 3 produces similar rises in antibody level independently of the order in which the various vaccinal serotypes are administered. A relatively lower level of antibodies against Type 3 virus, compared with humoral immunity to Types 1 and 2, was noted in all cases.[12]

The proportion of susceptible children who responded to a single administration of monovalent vaccines by developing humoral immunity made up 90-95 per cent of the total for vaccines of Types 1 and 2, and about 80-90 per cent for vaccine of Type 3. The revaccination with polyvalent vaccine of Types 1, 2, and 3 of children originally vaccinated with monovalent vaccines, eliminates the shortcomings of the first immunizing schedule and gives the majority of originally unresponsive children complete immunity against all three types (Fig. 1).

The ready-to-use polyvalent vaccine containing 100,000 tissue-culture units of each type of virus is the most convenient form of combined preparation for practical use.

A single administration of trivaccine results in a lower level of antibodies to all three types than three administrations of monovalent vac-

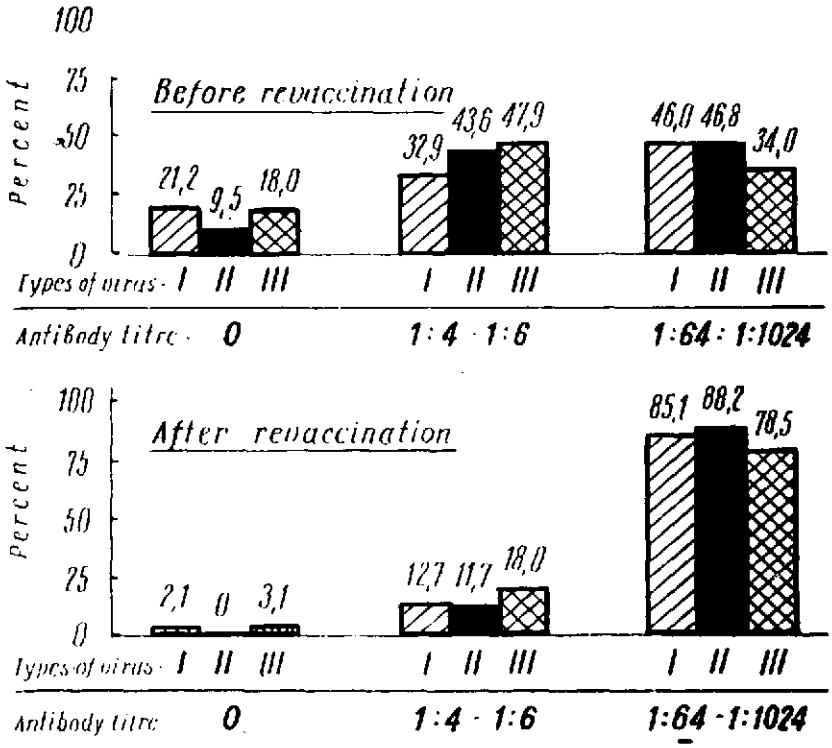


FIG. 1. Immunological changes in children 1-7 years of age vaccinated 8-10 months before with monovalent live vaccine after revaccination with trivaccine.

cines or divaccine, but three administrations of the trivaccine produce immunity in almost 100 per cent of cases (Table 1).

A very convenient method in regard to speed and effectiveness is triple immunization by the following schedule: monovaccine of Type 1, divaccine of Types 2 and 3, and trivaccine of Types 1, 2, and 3, given at intervals of one month. This schedule produces the maximum immunogenic effect and requires the same amount of vaccine as three administrations of monovaccines followed by revaccination with trivaccine after one to three months. Its great advantage lies in the fact that the schedule is completed more quickly and medical personnel are not required to give a fourth revaccination (Table 1).

The high immunogenicity of the live vaccine is due to the multiplication of the vaccinal viruses in the intestinal canal of vaccinated children; this follows a regular cycle with maximum accumulation of the virus between the seventh and the 21st day, followed by a gradual diminution in its concentration during the subsequent 15-20 days.

The multiplication of the virus follows an extremely regular course, with no interference from other viruses, and reaches its greatest intensity in children with previous low level of immunity to poliomyelitis, growing gradually less intense in persons with specific antibodies in their blood prior to vaccination.[7]

The most important feature of the live vaccine has been the formation of intense, long-lasting resistance in the intestinal canal to the subsequent administration of maximum doses of homologous virus, which do not multiply intensively and are quickly eliminated from the intestinal tract.[6]

In rare instances, the later administered virus proves capable of multiplication but at a con-

TABLE 1. THE IMMUNOLOGICAL ACTIVITY OF THE LIVE POLIOVACCINE IN RELATION TO THE SCHEDULE OF IMMUNIZATION OF NEGATIVE CHILDREN

Vaccine used	Type of virus	Number of children tested		4fold and over increase of antibodies	
		total	negative	total	%
Monovaccine (once)	I	227	148	143	96,6
	II	81	68	68	100,0
	III	89	68	65	95,6
Divaccine (once)	II	150	70	57	81
	III	150	55	44	80
Trivaccine (once)	I	189	102	70	68,7
	II	189	129	97	75
	III	189	144	66	45,8
Trivaccine (twice)	I	141	84	76	90,5
	II	141	82	81	98,8
	III	141	108	82	76
Trivaccine (3 times)	I	141	84	84	100
	II	141	82	82	100
	III	141	108	94	87
Monovaccine of Type I + Divaccine of Types II and III (random)	I	860	360	283	78
	II	860	510	463	90
	III	860	432	312	72
Monovaccine of Type I + Divaccine of Types II and III + Trivaccine(random)	I	860	360	352	97
	II	860	510	507	99
	III	860	432	412	95

siderably lower rate than that shown by the initial quantitative multiplication curve in the same child.

Observations over a long period of the immunity of the intestinal canal in children, who had been immunized with the live vaccine between four months and two years beforehand, showed that their revaccination at the prescribed dates after the first administration of vaccine, promotes the regular maintenance of intense and durable immunity of the intestinal canal to subsequent infection with the vaccinal strain.

The resistance of the intestinal tract, in children, to the primary administration of vaccinal strains depends markedly on the state of their humoral immunity to homologous virus. The intestinal tract has been found to possess only weak defensive powers against infection by vaccinal strains when the initial titers of virus-neutralizing antibodies are low (1:4-1:16), whereas at higher antibody titers the vaccinal viruses do not multiply, or else develop at a low rate and for a short time.

In order to establish durable local resistance in the intestinal tract, it is important to ensure vaccination of all susceptibles with a guaranteed high-quality vaccine, in view of the unreliability of the results of immunization which develops after contact with inoculated children.

The results of vaccination depend mainly on the doses of the preparation administered and the level of specific and non-specific immunity in the children to be inoculated.

Liquid vaccine containing $10^{5.0}TCD_{50}$ of virus is a guaranteed way of ensuring oral immunization against poliomyelitis and of obtaining the greatest possible uniformity and standardization of dosage between different children.

The intensive excretion of vaccinal strains from the intestines of inoculated children in the first month after immunization leads to the natural dissemination of vaccinal viruses among susceptible persons in contact with those inoculated.[8]

This process of spread of the vaccinal virus to contact groups is one of the most characteristic features of the live poliomyelitis vaccine, creating conditions in which population groups in contact with the inoculated persons may become immunized, while those already immunized may be reimmunized thereby.

The real epidemiological importance of this blind immunization should not be exaggerated. Thus the data quoted below, obtained in 1959 in epidemiological surveys in the Byelorussian and Moldavian Republics, showed that uninoculated groups of children in potential contact with inoculated ones showed a rather high incidence of paralytic poliomyelitis, varying but little from the incidence among persons not in such contact.

"Blind" infection with the vaccinal virus reaches its highest intensity under primitive sanitary and hygienic conditions or where there is a low level of personal hygiene. This latter feature is particularly common among young children, for example in young children's homes. As the age of the children increases, the intensity of contact infection falls quickly (among children of preschool and school age) and reaches a minimum among susceptible adults.

We paid a great deal of attention to the cardinal problem of the possibility of the Sabin vaccine strains reverting to the initial pathogenic form after 10 passages through the intestinal canal of susceptible children.[15, 16]

A study of changes in the neurotropic activity for monkeys of the vaccinal viruses after 10 artificial or four natural passages in susceptible children, showed a periodic and comparatively slight increase in the neurotropic properties of the vaccinal strains, which did not lead, however, to a progressive intensification of their neurovirulence.

These data will go a long way towards ending the bitter arguments on the dangers of reversion in the group of vaccinal strains we studied.

In November 1958 our data showing the harmlessness of the vaccine enabled the Sera and Vaccines Commission and the Collegium of the USSR Ministry of Health to give permission to the Institute of Experimental Medicine to carry out large-scale epidemiological surveys to study the harmlessness of live poliomyelitis vaccine. At this stage, the Poliomyelitis Research Institute of the USSR Academy of Medical Sciences joined in our activities.

In April and May 1959 we used the reserves of live vaccine in the Virology Department of the Institute of Experimental Medicine, amounting to about two million doses, for the immunization

of 1,700,000 children aged up to 14-18 years in the Byelorussian, Latvian, and Moldavian SSR, and in June-July 1959 in the Pskov and Novgorod regions. The vaccination was carried out in two stages. The first stage in April 1959 consisted of administration of monovaccine of Type 1, and the second stage, in May 1959, of the administration of a divaccine of Types 2 and 3.

The main principles followed in organizing vaccination and studying the epidemiological effectiveness of the live vaccine. The Ministries of the Union Republics responsible for carrying out the vaccination, arranged in good time for the detailed briefing of senior physicians and epidemiologists from the Rayon Sanitational and Epidemiological Centers on the purpose and methods of vaccination, and drew up with them a detailed vaccination plan. Staff was provided for vaccination teams on the basis of 300-500 vaccinations per day per team, a team consisting of a physician or feldsher and two nurses.

Similar work was carried out in every city or rural regions, all medical workers taking part in the immunization campaign being summoned for instructions.

The establishments responsible for the carrying out of vaccination sent permanent representatives to each city or region, who stayed there during the whole vaccination campaign and helped in carrying it out.

Before and during the vaccination campaign, extensive health education work was carried out to acquaint parents and the staff of children's establishments with the objectives and importance of vaccination.

In organized children's communities, vaccination was carried out on the spot; children not attending organized establishments were vaccinated in children's polyclinics near their homes or in the course of domiciliary visits.

The names of all the children to be vaccinated and children exempted from inoculation for various reasons, were entered by the vaccination teams on a list or individual card index, drawn up separately for each children's establishment or center of population in accordance with a standard procedure. The lists contained entries giving basic information concerning the child in question and recording data on previous inoculations against poliomyelitis, as well as reactions registered after vaccination.

The district epidemiologist summarized the data concerning vaccinations carried out in individual establishments or centers of population in standard vaccination reports. Copies of these reports were sent to the Regional Sanitary and Epidemiological Center or the local Institute of Epidemiology and Microbiology, which analyzed the results of the work carried out on each vaccination program.

To study the incidence of poliomyelitis in 1959 and the epidemiological effectiveness of vaccination against the disease, a scientific group was set up by ministerial order in each Republic, consisting of clinicians and epidemiologists for the expert investigation of each reported case of poliomyelitis in the regions where inoculations had been carried out, and to settle questions of diagnosis in doubtful or disputed cases.

To study the epidemiological effectiveness of immunization against poliomyelitis, two control groups were formed in each Republic or Region: the first consisted of children in direct or potential contact with those vaccinated in the territory covered by the live vaccine campaign (internal control); the second consisted of children and young people aged up to 14-18 years living in areas not covered by the campaign (external control).

In selecting the three groups, it was recommended that immunization with the live vaccine should be carried out in areas with higher incidence of poliomyelitis during previous years, leaving as external control areas with a lower morbidity.

In order to leave a sufficiently large internal control group, not more than 60-80 per cent of the population was inoculated in the areas concerned.

In order to provide a correct basis for the evaluation of the epidemiological effectiveness of the live vaccine, we tried to ensure the following main conditions:

I. The receipt of prompt and full information concerning morbidity in all three groups, vaccinated and unvaccinated, mentioned. The following special measures were taken to ensure complete reporting of all cases of paralytic and non-paralytic poliomyelitis:

(1) From the beginning of the vaccination campaign, by decree of the Ministry of the

Union Republic concerned, a system was introduced for the urgent reporting of every case diagnosed as poliomyelitis throughout the whole Republic or Oblast.

The report, with brief information concerning the patient, was given by telephone or telegram to the nearest Republican Sanitational and Epidemiological Center or Institute of Epidemiology and Microbiology;

(2) A member of one of the groups of neuropathologists established, was sent as quickly as possible to the place where each case occurred to check the diagnosis and obtain basic material for virological laboratory examination (the patient's feces and the acute blood specimen; a second sample of blood was taken 3-4 weeks after the beginning of the illness);

(3) Persons who contracted poliomyelitis were sent to the central Oblast and Republic's hospitals immediately after the beginning of the disease, or in cases where this was impossible, after the acute stage had passed. Experienced neuropathologists ensured accurate diagnosis of each case or checked the correctness of the primary clinical diagnosis, taking into account the data obtained by the laboratory examination;

(4) For the analysis of the epidemiological effectiveness of the vaccine, figures for paralytic and non-paralytic cases of poliomyelitis were used separately. The group of patients with paralytic poliomyelitis included those with the more certainly diagnosable forms of spinal and bulbo-spinal poliomyelitis with residual effects. The pontine forms, among which illnesses of a non-poliomyelitic nature predominated, were placed in the non-paralytic group;

(5) The analysis of epidemiological effectiveness was based on morbidity figures taken for the period of the whole epidemic rise in poliomyelitis and covered not less than six months of observation. The material on the effectiveness of vaccination in 1959 was processed on the basis of the period June-December 1959 which began three to four weeks after completion of the second immunization.

II. A proof of the high quality of the work done in assessing the epidemiological effectiveness of vaccination was the completeness of the laboratory examination of notified cases, with full virological examination of the feces of the majority of patients who had been diagnosed as having poliomyelitis. It was recommended that a feces specimen be taken in the first few days of illness, that a further specimen should be taken within 10 days of the beginning of the disease, and that the two specimens should be examined separately or after combination.

In addition to virological examinations of feces, which showed that 50 per cent of the patients investigated were excreting poliovirus of Type 1, and 15 per cent poliovirus of Types 2 and 3, we also carried out a serological examination of the patients by serum titration during the period of convalescence.

In cases where the clinical diagnosis was doubtful, positive laboratory findings made it possible to place the patients concerned in the poliomyelitis group. In the case of negative laboratory data but a positive clinical diagnosis, the cases to be diagnosed were considered to be suffering from poliomyelitis.

III. An important condition for carrying out epidemiological analysis of the effectiveness of vaccination was the obtaining of accurate information concerning the numerical composition by age groups of the children and young people vaccinated with the live vaccine or forming part of the internal and external control groups (Table 2).

For all the groups concerned, we received from the local statistical offices information on the composition of the population by age in areas covered and areas not covered by the vaccination campaign.

In carrying out the vaccinations, the accurate registration of vaccinated children was ensured by means of the data from card indexes and lists and by undertaking primary analysis of the age composition of those vaccinated in the Rayon Sanitational and Epidemiological Centers.

This information, compiled in accordance with a standard procedure, was summarized by the Center or by the Epidemiology Department of the Institute of Epidemiology and Microbiology.

Where the Republic or Oblast contained a sufficiently large group of children who had been given three vaccinations with the killed Salk vaccine, we did not administer the live vaccine to them, but kept them under observation as a separate group, thus providing an opportunity for settling the question of the comparative effec-

TABLE 2. NUMBER OF PERSONS IN THE VACCINATED AND CONTROL GROUPS IN THE BYELORUSSIAN SSR, LATVIAN SSR AND MOLDAVIAN SSR AND IN THE NOVGOROD AND PSKOV OBLASTS IN 1959

REGION	AGE GROUPS	NO. OF PERSONS		
		VACCINATED	INTERNAL CONTROL	EXTERNAL CONTROL
Byelorussian SSR	9 months to 3 years	74,868	77,612	344,076
	3–7 years	119,897	152,353	614,350
	7–14 years	347,754	85,371	977,375
	Total	542,519	315,336	1,935,801
Moldavian SSR	9 months to 3 years	72,274	37,744	107,277
	3– 7 years	80,551	26,870	109,500
	7–14 years	201,782	57,059	164,857
	Total	354,607	121,673	381,634
Latvian SSR	9 months to 3 years	27,840	56,065	32,103
	3– 7 years	55,349	22,652	35,744
	7–14 years	159,751	18,055	73,488
	14–30 years	177,604	10,013	
	Total	420,544	106,785	141,335
Pskov Oblast	9 months to 3 years	49,292	7,133	—
	3– 7 years	45,699	6,526	—
	7–15 years	102,843	21,594	—
	Total	197,834	35,253	—
Novgorod Oblast	9 months to 3 years	41,438	6,979	—
	3– 7 years	39,990	3,970	—
	7–15 years	80,341	13,875	—
	Total	161,769	24,824	—
Grand total		1,677,273	603,873	2,458,770

tiveness of live and killed vaccine under comparable epidemiological conditions.

The distribution of the vaccinated and control groups in the various Republics. In the Byelorussian Republic, 542,519 city children up to 14 years of age were immunized. This corresponded to 63 per cent of the total number of children in the cities. The remaining 37 per cent (315,336) formed the internal control group. The children in the rural areas, who were two-and-a-half times as numerous as the urban child population of the same age, were not given the live vaccine; they constituted the external control group. In previous years, the absolute number of poliomyelitis cases was similar among the rural and urban groups of children, which explains the lower percentage indices for poliomyelitis in rural localities in those years.[10]

In the Moldavian SSR the vaccination campaign covered 13 central rayons of the Republic and the four large cities situated there, which accounts for more than half the population of the Republic and in which, in the previous years, about 50 per cent of the annual number of poliomyelitis cases in the Republic had been recorded.

A total of 354,607 children and young people aged between nine months and 14 years were vaccinated; 106,872 formed the internal control (contact) group. The third group consisted of 381,634 persons of the same age and constituted the external control group.[1]

In the Latvian SSR 420,544 children and adults up to 30 years of age were vaccinated in Riga City and in a number of rayons in the Republic.[18]

The 106,872 children in the contact groups left unvaccinated in the regions where the live vaccine was employed constituted the internal control. The population numbering 41,336 between nine months and 18 years of age in rayons where live vaccine had not been administered represented the external control group.

Somewhat later, in June and July 1959, 360,000 children in the cities and settlements of the Novgorod and Pskov Oblasts up to 14 years of

age were vaccinated, nearly 20 per cent of the total number of that age having been left unvaccinated.

In all Republics the vaccine was administered in two stages at an interval of one month. The first dose—vaccine of Type 1—was given in April, and the second, consisting of divaccine of Types 2 and 3, in May 1959. On 1 June 1959, i.e., two weeks after completion of the second immunization and one-and-a-half months after completion of the first, our study of the epidemiological effectiveness of the vaccination began.

ence of 100 minimum doses of virus and monkey-kidney cells (L. M. Kurnosova and G. P. Zhilova).

More than 2,500 serum tests showed the existence of a quite high percentage of susceptible children in various geographical areas of the USSR. This percentage was at its maximum among children up to two to four years of age (60 per cent and over) and gradually fell towards the age of 10-12 (Table 3).

Parallel examinations of all negative sera by

TABLE 3. IMMUNOLOGICAL STRUCTURE, BY POLIOVIRUS TYPES, OF THE CHILD POPULATION IN LENINGRAD, NOVGOROD OBLAST, LATVIAN SSR, MOLDAVIAN SSR AND BYELORUSSIAN SSR, AS PERCENT OF NEGATIVES IN EACH OF FIVE AGE GROUPS

Age Yrs.	Type I % negative					Type II % negative					Type III % negative					% triple negative				
	Leningrad	Novgorod	Latvian SSR	Moldavian SSR	Byelorussian SSR	Leningrad	Novgorod	Latvian SSR	Moldavian SSR	Byelorussian SSR	Leningrad	Novgorod	Latvian SSR	Moldavian SSR	Byelorussian SSR	Leningrad	Novgorod	Latvian SSR	Moldavian SSR	Byelorussian SSR
0-2	77	63	64	64	60	72	87	78	79,2	65	64	82	66	64,4	65	48	48	41	33,6	36
3-4	41	31	42	28	45	41	60	50	37,9	54	48	47	35	38,7	50	23	15	20	9,1	22
5-6	28	18	25	20	40	38	46	41	25	53	37	35	23	29	26	18	6	6	5	0
7-9	29	18	25	20	31	26	42	39	21	37	29	33	29	26	38	14	6	4	4	13
10-12	26	11	19	15	35	26	33	34	19	58	37	30	27	22	27	7	4	0	3	20

Study of the immunological structure of the population before and after vaccination. The great importance of humoral antibodies in the blood of children and adults for the epidemiological prognosis of poliomyelitis and a properly based evaluation of the results of immunization is well known.

When we began immunization with the live vaccine, we determined the percentage of persons susceptible to poliomyelitis in the various age groups to be immunized, in relation to the different virus serotypes. For this purpose, the sera of the groups of population to be investigated, taken before vaccination, were subjected to the color test in a dilution of 1:4 in the presting neutralization of the cytopathogenic action of the virus in monolayer tissue cultures, showed that the percentage given by the color test was 25-30 per cent too high. Taking into account the fact that a similar correction is necessary for sera examined by the color test after completion of vaccination, we took the percentage of negative sera as a relative index of actual susceptibility.

Re-estimation of the susceptible population based on the computed percentages of negative sera shows that more than 25 per cent of the population in the regions surveyed were susceptible to one of the poliovirus serotypes, and 10-15 per cent belonged to the most highly sus-

TABLE 4. ESTIMATED NUMBER OF SUSCEPTIBLES TO POLIOMYELITIS AMONG A POPULATION FED LIVE VACCINE ON THE BASIS OF THE PERCENTAGE OF SERONEGATIVES TO TYPES 1, 2 AND 3, AND THE NUMBER OF TRIPLE NEGATIVE PERSONS (MOLDAVIAN SSR)

Age groups	Number in group vaccinated	% of susceptible persons on basis of results of color test with serum in dilution 1:4								Probable number of susceptible persons without antibodies to indicated types			
		No investigated	Type I		Type II		Type III		Triple Neg.		I	II	III	Triple negative
			No negative	%	No negative	%	No negative	%	No negative	%				
9 mos. to 3 yrs	72274	138	79	57,2	99	71,7	81	58,7	39	28,2	41340	61820	42425	20381
3 - 7 yrs	80551	107	24	22,4	30	28,0	34	31,8	7	6,0	18043	22554	25615	4833
7 - 10 yrs	120872	81	16	19,6	19	23,4	20	24,6	4	4,9	23690	28284	29734	5922
10 - 14 yrs	80910	95	15	15,5	18	18,9	21	22,1	3	3,1	12541	15291	17881	2508
14 - 18 yrs	52905	84	8	9,5	13	15,4	14	16,6	2	2,3	5025	8147	8848	1216
Total	407512	505	142		179		170		55		100639	136096	124503	34860

ceptible group (triply negative). Table 4 gives an example of this calculation for the vaccinated population in the Moldavian SSR.

With 25 per cent of susceptible children among the 1,700,000 persons we vaccinated, we had no fewer than 400,000 persons susceptible to Type 1, 2, or 3 and more than 100,000 triple-negative children. If a poliomyelitis virus, capable of reverting to one of the existing pathogenic strains, had been administered to this number of children, about 2,000 cases of paralytic poliomyelitis could have been expected among them, caused by the slightly virulent, moderately virulent, and highly virulent strains of Type 1, and about 400 cases of poliomyelitis caused by Types 2 and 3, similar to the naturally encountered viruses. In actual fact, despite the administration to hundreds of thousands of children of a highly active vaccinal virus, which began to circulate intensively among the healthy contacts, no increase in the number of cases of poliomyelitis recorded was observed in the months immediately following immunization in the areas covered by the campaign. On the contrary, a marked decrease in poliomyelitis morbidity was observed, compared with what had been expected on the basis of the incidence recorded among the external control group, among whom the live vaccine had not been used. From February to April 1960, 1,100,000 people under 20 years of age, or 35 per cent of the whole population of Leningrad, were immunized three times with polio vaccine from the Moscow Poliomyelitis Institute. The vaccinated population was under medical control, which was as good, I suppose, as in any other country. The most qualified medical personnel participated in the research concerning the safety of this vaccination. Special departments and hospitals cooperated to accept the children ill with any severe symptoms from vaccinated groups, but no cases were registered during the last four months of observations which could be considered to have any connection with the vaccination campaign.

The two-stage immunization carried out in April-May 1959 brought about essential changes in the immunological structure of the population by decreasing between four-and eight-fold the percentage of susceptible children and young people (Tables 5, 6 and 7).

The third immunization made at the beginning of 1960 in a number of places by means of the trivaccine, containing 100,000 cytopathogenic

TABLE 5. SUSCEPTIBILITY TO POLIOMYELITIS OF THE CHILD POPULATION OF THE BYELORUSSIAN SSR BEFORE, AND 6 MONTHS AFTER, TWO-STAGE IMMUNIZATION WITH LIVE VACCINE

Age of children investigated (years)	Before vaccination					After vaccination				
	N° of children	% without antibodies to polioviruses				N° of children investigated	% without antibodies to polioviruses			
		Type I	Type II	Type III	All three types		Type I	Type II	Type III	All three types
0 - 2	55	60	65	65	36	68	19	11,7	19	1,5
3 - 4	22	45	54	50	22	49	8	6,1	8	2
5 - 6	15	40	53	26	0	67	1,5	1,5	4,5	0
7 - 9	45	31	37	33	13	60	1,5	16,6	10	1,6
10 - 12	48	35	50	27	20	51	11,7	11,7	13	0
13 - 15	35	28	31	17	0	31	9,7	3,2	3,2	0
Total	220					326				

TABLE 6. SUSCEPTIBILITY TO POLIOMYELITIS OF A CHILD POPULATION IN THE LATVIAN SSR BEFORE VACCINATION AND 6 MONTHS AFTER DOUBLE IMMUNIZATION WITH LIVE VACCINE

Age of children (years)	Before vaccination					After vaccination				
	N° of children investigated	% without antibodies to polioviruses				N° of children investigated	% without antibodies to polioviruses			
		Type I	Type II	Type III	All three types		Type I	Type II	Type III	All three types
0 - 2	67	64	78	66	41	33	0	10	3	0
3 - 4	45	42	50	35	20	30	10	13	13	0
5 - 6	44	25	41	23	6	41	10	5	22	0
7 - 9	72	25	39	29	4	67	2	1	12	0
10 - 12	43	19	34	27	0	84	2	5	14	0
13 - 15	25	16	22	4	0	78	1	1	5	0
16 - 20	22	18	18	10	0	56	10	6	16	0
Total	318					389				

TABLE 7. SUSCEPTIBILITY TO POLIOMYELITIS OF A CHILD POPULATION IN THE MOLDAVIAN SSR BEFORE VACCINATION AND 6 MONTHS AFTER DOUBLE IMMUNIZATION WITH LIVE VACCINE

Age groups	No in groups vaccinated	Before vaccination									After vaccination								
		No investigated	type 1		type 2		type 3		all three types		No investigated	type 1		type 2		type 3		all three types	
			No negative	%	No negative	%	No negative	%	No negative	%		No negative	%	No negative	%	No negative	%	No negative	%
9 mos. to 3 yrs	72274	138	79	57,2	99	71,7	81	58,7	39	28,2	91	13	14,2	16	17,5	35	33,3	0	
3 - 7 yrs	80551	107	24	22,4	30	28,0	34	31,8	7	6,5	104	5	4,8	4	3,8	15	14,4	1	0,9
7 yrs-10 yrs	120872	81	16	19,6	19	23,4	20	24,6	4	4,9	148	5	3,3	2	1,3	4	2,7	0	
10 - 15 yrs	80910	95	15	15,5	18	18,9	21	22,1	3	3,1	121	1	0,8	0	0	0	0	0	
15 - 18 yrs	52905	84	8	9,5	13	15,4	14	16,6	2	2,3	80	5	6,2	3	3,7	2	2,5	0	
Total	407512	505	142		179		170		55		544	29		25		56		1	

TABLE 8. THE IMMUNOLOGICAL ACTIVITY OF THE LIVE POLIOMYELITIS VACCINE IN RELATION TO THE SCHEDULE OF IMMUNIZATION (ON THE BASIS OF RANDOM OBSERVATION OF 4006 CHILDREN)

Groups: I - no vaccination
II - twice vaccination (first vaccination with monovaccine of type I, second with divaccine of types II and III)
III - three time vaccination (additional third administration of trivaccine of types I, II, III)

groups tested	Vaccination	Types of poliovirus	Percentage without antibodies to the poliovirus tested in the indicated ages-groups					
			9 months - 2 years	3 - 4 years	5 - 6 years	7 - 9 years	10 - 12 years	13 - 14 years
I	no	I	69	33	21	23	18	20
II	twice		19	5	4	2	3	2
III	three times		1	1	0	0	0	0
I	no	II	70	50	35	40	37	20
II	twice		10	6	1	2	3	2
III	three times		0	0,9	0	0	0	0
I	no	III	70	42	31	30	26	15
II	twice		31	13	5	7	7	3
III	three times		4	2	0	0	0	0
I	no	triple negative	41	13	5	6	6	3
II	twice		1	0,7	0	0,3	0	0
III	three times		0	0,6	0	0	0	0

Figures—average number of sera in 18 groups tested on 200-300 samples in each.

doses of each type, caused a further and very sharp change in the quantitative antibody level, reducing to the maximum the percentage of susceptible children among the worst threatened groups (four years of age and under) (Table 8).

These results demonstrate the high degree of immunogenicity of the live vaccine administered in three stages—a monovaccine of Type 1, a divaccine of Types 2 and 3, and a trivaccine of Types 1, 2, and 3—which almost completely eliminated susceptibility among the population and who acquired protection against all three serotypes of the virus.

The effect of vaccination on seasonal changes in the incidence of poliomyelitis and on the number of cases. The changes in the incidence of paralytic cases of poliomyelitis in the Byelorussian, Latvian, and Moldavian SSR, and also in the Novgorod and Pskov Oblasts of the R.S.F.S.R. where, among the population covered by the mass immunization campaign, for the first time in recent years there was a complete absence of the seasonal rise which usually takes place in the period from June to October, are very significant. The same phenomenon was also found among the contact groups of uninoculated children (internal control) among whom cases were very sharply reduced as a result of the vaccination of a considerable proportion of the child population in the area. These radical

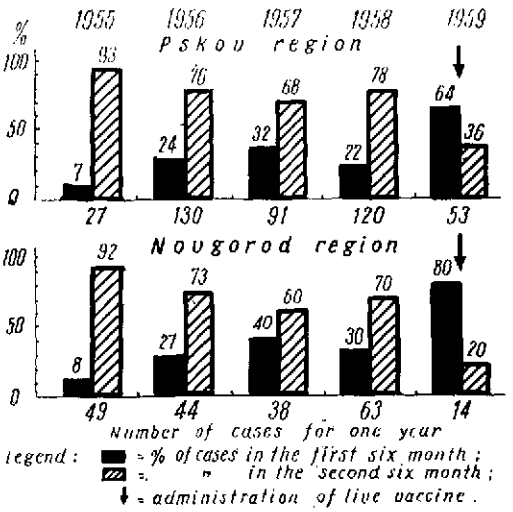


Fig. 2. Distribution of paralytic poliomyelitis in Pskov and Novgorod regions in the first and second half-years 1955-1959 (in % to all cases for corresponding year).

breakdowns in the usual seasonal rise in poliomyelitis in these areas constitute a convincing illustration of the effectiveness of the live vaccine (Table 9 and Fig. 2).

The same thing is demonstrated even more clearly by data on distribution of the paralytic cases of poliomyelitis recorded in the period after completion of vaccination, i.e., in June-December 1959, among children of various ages vaccinated and not vaccinated with the live vaccine in the various Republics (Table 10).

We obtained particularly clear-cut results showing the effectiveness of the live vaccine in May-December 1959 and January-February 1960 in the Byelorussian SSR (Table 11). Among the 74,868 children of up to three years of age to whom the live vaccine was administered, three cases of paralytic poliomyelitis were recorded in the period of 10 months. At the same time, among the 77,612 unvaccinated children of the same age in potential contact with them, 41 paralytic cases were reported. This indicates a reduction in the incidence of the disease among the inoculated of some 13-fold compared with the incidence which could have been expected otherwise and of 6-7-fold compared with the actual incidence among the external control groups.

The effectiveness of the live vaccine among the older groups of preschool and school-age children is expressed by the achievement of a two- to three-fold reduction. This is due to the low incidence of poliomyelitis in the corresponding control groups (4.0 per 100,000 for children aged seven to fourteen). The total morbidity index for the whole of the vaccinated group was 1.5 per 100,000, for the internal control, 22.0 per 100,000, and for the external control, 7.5 per 100,000 (Table 11).

The higher incidence of poliomyelitis among the internal control in the Byelorussian SSR (urban children in contact with those inoculated) as compared with the external control (country children not in contact with the inoculated) may be ascribed to the higher incidence of poliomyelitis among children in the towns which had been observed in all the previous years. By calculating on the basis of the incidence among unvaccinated children of the internal control, we would have expected 54 cases

TABLE 9. SEASONAL DYNAMICS OF PARALYTIC POLIOMYELITIS IN BYELORUSSIA, LATVIA AND MOLDAVIA IN 1957-1959 IN REGIONS COVERED (+) AND NOT COVERED (—) BY VACCINATION

Republic	Year	Area of immunization in 1959	Month of year												Total
			Jan	Feb	Mar	Apr	May	June	July	Aug.	Sept	Oct	Nov.	Dec.	
Byelorussia	1957	+	5	5	3	4	4	14	20	30	29	23	6	18	161
		—	6	8	5	5	6	21	59	58	43	33	18	24	286
	1958	+	11	6	12	6	15	21	62	48	47	35	16	9	288
		—	20	10	26	13	13	45	49	53	82	51	31	20	413
	1959	+	9	5	5	5	12	9	5	8	6	6	6	1	77
		—	8	12	3	12	17	12	19	20	19	11	8	7	148
Latvia	1957	+	11	5	4	2	3	2	6	9	12	13	3	2	72
		—	2	—	1	2	—	3	1	4	2	—	—	2	17
	1958	+	—	3	1	2	2	3	17	28	18	22	17	10	123
		—	2	1	—	—	—	2	3	6	6	1	6	3	30
	1959	+	4	2	5	1	—	1	—	2	—	1	—	—	16
		—	—	1	—	—	—	3	6	3	1	2	—	—	16
Moldavia	1957	+	0	0	0	1	0	0	0	0	3	7	8	7	26
		—	0	3	0	2	2	1	2	16	25	11	2	2	66
	1958	+	1	5	3	3	7	27	30	16	28	12	5	3	140
		—	7	17	33	14	3	23	19	22	17	4	0	2	161
	1959	+	4	5	2	3	1	4	1	2	0	1	1	1	25
		—	5	1	7	1	1	5	3	12	10	13	3	1	62

of paralytic poliomyelitis among those inoculated, instead of the three which actually occurred.

In the Moldavian SSR, among the 354,607 children up to 14 years of age who were inoculated, two paralytic cases of poliomyelitis were recorded. Among the group serving as internal control, which numbered 118,427 persons, equivalent to about one-third of the inoculated group, there were seven cases of paralytic poliomyelitis, which indicates a reduction in morbidity of more than eight-fold. Compared with the unvaccinated groups forming the external control, where there were 41 cases out of 332,033 children or 22.5 per 100,000, the reduction in the incidence of the disease was still greater (Table 12).

In the Latvian SSR in 1959, 242,940 children up to 14 years of age were inoculated. Among these, in June-December 1959 there was one case of paralytic poliomyelitis as against the 15 cases which could have been expected on the basis of morbidity in the control groups in regions in which live vaccination had not taken place.

In all the previous years the number of poliomyelitis cases in these regions used for external control had not exceeded 20 per cent of the number recorded in the areas in which vaccination was carried out in 1959. In six months of the current year, nine poliomyelitis cases were recorded among 141,335 non vaccinated children up to 14 years of age, i.e., 12.8 per 100,000, as compared to 0.8 per 100,000 among the children in the inoculated group, and 3.9 per 100,000 among the 106,872 children of the internal control group.

Thus, in the Latvian SSR also the reduction of morbidity among those inoculated with the live vaccine was more than 10-fold.

According to preliminary figures collected in the Pskov Oblast the incidence among those inoculated with the live vaccine decreased sevenfold (Fig. 3).

The study of the live poliomyelitis vaccine carried out in 1959 under our guidance in the Latvian, Byelorussian, Moldavian, and Russian SSR's in 1,700,000 inoculated persons has established the harmlessness of the product used,

TABLE 10. NUMBER OF PARALYTIC CASES OF POLIOMYELITIS OCCURRING IN THE COURSE OF THE EPIDEMIC SEASON OF 1959 IN AREAS OF BYELORUSSIA, LATVIA, AND MOLDAVIA COVERED AND NOT COVERED BY IMMUNIZATION

REPUBLIC	AGE	UNVACCINATED (EXTERNAL CONTROL)			UNVACCINATED (INTERNAL CONTROL)			PERSONS VACCINATED WITH THE LIVE VACCINE			NUMBER OF CASES OF POLIOMYELITIS ANTICIPATED
		NUMBER OF PERSONS	NUMBER OF CASES		NUMBER OF PERSONS	NUMBER OF CASES		NUMBER OF PERSONS	NUMBER OF CASES		
			TOTAL	INDEX PER 100,000 PERSONS		TOTAL	INDEX PER 100,000 PERSONS		TOTAL	INDEX PER 100,000 PERSONS	
Byelorussia	9 months—3 years	344,076	85	30.0	77,612	41	63.0	74,868	3	4.8	39
	3– 7 years	614,350	24	4.7	152,353	10	8.0	119,897	3	3.0	7
	7–14 years	977,375	13	1.5	85,371	2	2.8	347,754	1	0.3	8
	Total	1,935,801	122	7.5	315,336	53	22.0	542,519	7	1.5	54
Moldavia	9 months—3 years	79,066	36	78.2	37,744	7	31.8	72,274	2	4.1	33
	3– 7 years	66,886	4	10.5	26,870	0	0	80,551	0	0	4
	7–14 years	164,857	1	1.0	24,239	0	0	201,782	0	0	1
	0–7 years*	70,825	6	14.7	32,820	1	5.1				
	Total	381,634	47	21.9	121,673	8	6.5	354,607	2	0.8	38
Latvia	9 months—3 years	32,103	1	6.2	56,065	2	7.1	27,840	0	0	1
	3– 7 years	35,744	3	16.8	32,752	0	0	55,349	0	0	4
	7–14 years	73,488	5	13.6	18,055	0	0	159,751	1	1.2	10
	Total	141,335	9	12.8	106,872	2	3.9	242,940	1	0.8	15

* Inoculated with Salk vaccine.

TABLE 11. ANALYSIS OF THE INCIDENCE OF POLIOMYELITIS IN BYELORUSSIA IN 10 MONTHS OF 1959 AND 1960

Age groups	Groups	Number of persons in the group	No. of cases of paralytic poliomyelitis in the months indicated										Total for the 10 months	Mean annual index per 100 000 children
			V	VI	VII	VIII	IX	X	XI	XII	I	II		
6 mos-3 yrs	live vaccine	74868	1	–	–	1	–	–	–	–	–	1	3	4,8
	internal control	77612	10	4	3	6	4	4	3	2	4	1	41	63,0
	external control	344076	13	8	16	14	14	7	4	5	4	–	85	30,0
3 - 7 yrs	live vaccine	119897	–	–	–	–	1	2	–	–	–	–	3	3,0
	internal control	152353	1	5	2	1	–	–	1	–	–	–	10	8,0
	external control	614350	3	3	3	4	4	2	2	2	1	–	24	4,7
7 - 14 yrs	live vaccine	347754	–	–	–	–	1	–	–	–	–	–	1	0,3
	internal control	85371	–	–	–	–	–	–	2	–	–	–	2	2,8
	external control	977375	1	1	–	2	1	2	4	–	2	–	13	1,5
6 mos-14 yrs	live vaccine	542519	1	–	–	1	2	2	–	–	–	1	7	1,5
	internal control	315336	11	9	5	7	4	4	6	2	4	1	53	22,0
	external control	1935801	17	12	19	20	19	11	10	7	7	–	122	7,5

which was made from attenuated Sabin strains, and proved its high degree of epidemiological effectiveness.

In all Republics and Oblasts where the live vaccine was administered to the bulk of the susceptible child population under 14 years of age, radical changes took place in the seasonal dynamics of poliomyelitis morbidity. In areas of mass immunization the seasonal rise in the incidence of poliomyelitis did not occur at all, while it was observed in the same months among non-vaccinated children in areas without live vaccine administration.

The number of paralytic cases of poliomyelitis, among children to whom the live vaccine had been administered, proved quite negligible compared with the number among the uninoculated groups of children of the same age. The indices of the effectiveness of the live vaccine were highest (15-20-fold) among very young children, among whom most cases occurred in the control groups, and fell in the older age groups parallel with the fall in incidence in the control groups. The mean minimum index of effectiveness varied in the different Republics from six- to 15-fold.

The live vaccine proved considerably more effective than three injections of the Salk inactivated vaccine when both the preparations were studied in the most highly comparable conditions.

The fairly high incidence of paralytic poliomyelitis among children in the internal control groups in 1959, i.e., among children in potential contact with those to whom the live vaccine had been administered, demonstrates the unreliability and irregular nature of "blind" immunization by means of the circulating vaccinal virus. This emphasizes the need for universal and compulsory immunization of all children with the live vaccine, a procedure that will produce the best immunological effect and may lead to the maximum displacement of the "wild" pathogenic viruses.

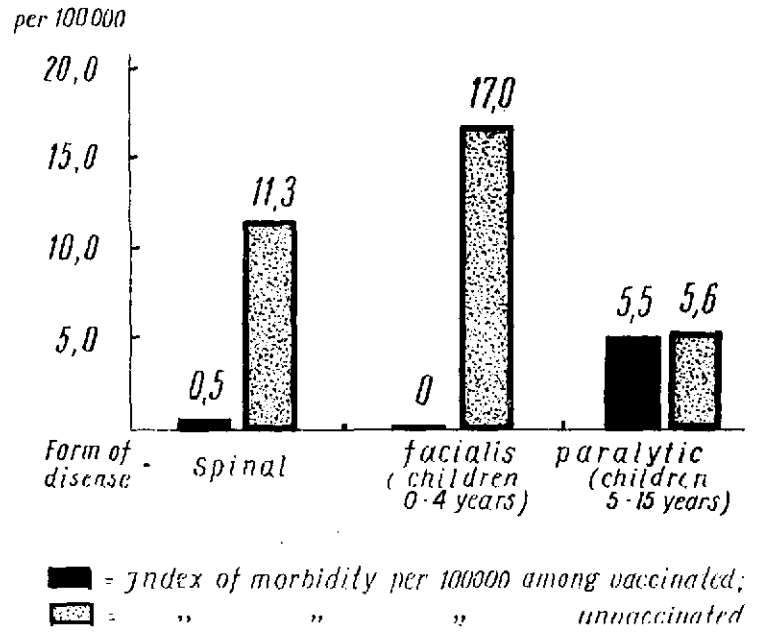


FIG. 3. Distribution of 24 spinal paralytic and facialis paralytic cases of poliomyelitis among vaccinated with live vaccine and unvaccinated persons in Pskov region.

CONCLUSIONS

1. The live poliomyelitis vaccine made from attenuated Sabin strains which we studied is completely harmless and carries no threat of the vaccine strains reversion to a more virulent state.

2. Mass immunization by this method is extremely convenient and simple and ensures the widest possible coverage of the population within a very short period—two or three weeks in each cycle of vaccination.

3. The absence in the vaccinated children of any general or local reactions, which differ in frequency from similar symptoms in the uninoculated external control groups, provides every basis for sharply reducing the list of contra-indications to the use of live vaccine when other inoculations are being carried out.

4. The high degree of epidemiological effectiveness of the live vaccine, shown by our observations in 1959 in four Republics, fully justifies the proposal to cease further use of the less effective Salk vaccine and to turn completely to immunization with the live vaccine from 1960 onwards.

Only the live vaccine is capable of meeting quickly in practice the country's demands for an effective vaccine, in view of its cheapness and the possibility of the largest production.

5. The quite high incidence of poliomyelitis among contact groups of unvaccinated children indicates the need to make administration of the live vaccine to the largest percentage of children, as in the case of smallpox vaccination or diptheria immunization. Only this method of total immunization, carried out under strict State control, will enable us to solve the most important task of the gradual elimination of the reservoir of wild pathogenic strains, and the suppression of their circulation, leading inevitably in these circumstances to the eradication of poliomyelitis as a dangerous, large-scale infection of children.

6. For the forthcoming mass immunization, it will be advisable to make three administrations of the live vaccine in accordance with the following optimum schedule: the first inoculation with a monovaccine of Type 1, the second with a divaccine of Types 2 and 3, the third with a trivaccine of Types 1, 2, and 3, administered

TABLE 12. THE DISTRIBUTION OF PARALYTIC CASES OF POLIOMYELITIS IN JUNE-NOVEMBER 1959 IN AREAS COVERED AND NOT COVERED BY THE LIVE VACCINATION CAMPAIGN IN THE MOLDAVIAN SSR IN 1959

GROUP	VACCINES USED	AGE GROUPS	NUMBER IN THE GROUP		NUMBER OF PARALYTIC CASES OF POLIOMYELITIS IN THE MONTHS INDICATED								MEAN ANNUAL INDEX PER 100,000 PERSONS	NUMBER OF CASES ANTICIPATED
			TOTAL VACCINATED	PER CENT	JUNE	JULY	AUG.	SEPT.	OCT.	NOV.	DEC.	TOTAL		
I	Live vaccine	9 months—3 years	72,274	20.4	—	—	2	—	—	—	—	2	4.1	30
		3– 7 years	80,551	22.7	—	—	—	—	—	—	—	—	—	4
		7–14 years	201,782	56.9	—	—	—	—	—	—	—	—	—	1
		Total	354,607	100	—	—	2	—	—	—	—	2	0.8	35
II Internal control	3 times with Salk vaccine	9 months—3 years	14,622	12.0	1	—	—	—	—	—	—	1	11.6	
		3– 7 years	15,611	12.7	—	—	—	—	—	—	—	—	—	
		7–14 years	2,587	2.1	—	—	—	—	—	—	—	—	—	
		Total	32,820	26.8	1	—	—	—	—	—	—	1	5.1	
III Internal control	Unvaccinated	9 months—3 years	37,744	31.1	3	1	—	—	1	1	1	7	31.8	
		3– 7 years	26,870	22.2	—	—	—	—	—	—	—	—	—	
		7–14 years	24,239	19.9	—	—	—	—	—	—	—	—	—	
		Total	88,853	73.2	3	1	—	—	1	1	1	7	13.5	
Total Groups II and III			121,673	100	4	1	2	0	1	1	1	10	13.9	

IV External control	Unvaccinated	9 months—3 years	79,066	20.7	4	2	9	9	10	1	1	36	78.2	
		3– 7 years	66,886	17.3	1	—	1	1	1	—	—	4	10.5	
		7–14 years	164,857	43.4	—	—	—	—	—	1	—	1	1.0	
		Total	310,809	81.4	5	2	10	10	11	2	1	41	22.5	
V External control	3 times with Salk vaccine	9 months—3 years	28,211	7.4	—	—	1	—	2	—	—	3	18.0	
		3– 7 years	42,614	11.2	—	1	1	—	—	1	—	3	11.8	
		7–14 years		0	—	—	—	—	—	—	—	—	—	
		Total	70,825	18.6	—	1	2	—	2	1	—	6	14.7	
Total Groups IV and V			381,634	100	5	3	12	10	13	3	1	47	21.9	

at intervals of four to six weeks. This schedule ensures two administrations of each virus serotype in the course of the triple immunization, is convenient in practice and is more effective, to judge from immunological findings, than the administration of monovalent vaccines if it is not followed by a fourth revaccination with trivaccine.

7. In 1960 the age groups to be immunized can be restricted to those up to 16-20 years, whose need for immunization is greater than that of the other age-groups. The comparatively rare cases of illness among older people are caused by infection with viruses circulating among children and young people.

8. The elimination of the main reservoir of the virus among children and young people up to 20 years of age will probably eliminate it among the older population in our country, who may be included in the immunization campaign at a later stage.

BIBLIOGRAPHY

1. Bulichev, N. I., Groisman, G. M., Chalkina, O. M., Dyakova, V. S., Yezhov, N. N., Kovalev, A. A., Steklova, P. M., Sharapov, B. I., and Shlyakov, E. N.: Results of study of the epidemiological effectiveness of active immunization with live poliomyelitis vaccine in the Moldavian SSR. In: *Live Poliomyelitis Vaccine*, Leningrad, 1960.
2. Chumakov, M. P., *et al.*: Preliminary communication on the mass peroral immunization of the population against poliomyelitis with a live virus vaccine made from attenuated Sabin strains. *Vopr. virus.* **5**: 520-533, 1959.
3. ————: Comparative description of the characteristics of the live poliomyelitis vaccine. *Ibidem.* **5**: 533-537, 1959.
4. Davidenkova, Y. F. and Savelyeva-Vasilyeva, Y. A.: Results of two years' clinical observations of children immunized with the live attenuated vaccine against poliomyelitis. *Zh. nevropat. psikhiat.* **59**: 790-795, 1959.
5. Drobyshevskaya, A. I., Klyucharcva, T. Y., Kolesnikov, L. V., and Smorodintsev, A. A.: The characteristics of the live vaccine used in 1959. In: *Live Poliomyelitis Vaccine*, Leningrad, 1960.
6. Gorev, N. Y.: The behavior of vaccinal strains of poliovirus in the intestinal canal of children vaccinated more than once. In: *Live Poliomyelitis Vaccine*, Leningrad, 1960.
7. Ilyenko, V. I. and Alekseyev, B. P.: The dynamics of poliovirus multiplication in the intestines of children vaccinated with the live attenuated vaccine. In: *Live Poliomyelitis Vaccine*, Leningrad, 1960.
8. ————, Kurnosova, L. M., Zhilova, G. P., and Radionova, R. N.: Material obtained from virological and serological examinations of children in contact with children vaccinated with the live attenuated poliomyelitis vaccine. In: *Live Poliomyelitis Vaccine*, Leningrad, 1960.
9. Kantorovich, R. A., Zhilova, G. P., Ostrovski, G. D., Rykushina, B. P., Bregantseva, P. V., and Golubeva, L. P.: Experience in the mass immunization of the child population of the Pskov and Novgorod oblasts with live attenuated poliomyelitis vaccine. In: *Live Poliomyelitis Vaccine*, Leningrad, 1960.
10. Kardash, I. B., Votyakov, V. I., Ilyenko, V. I., Monakhova, O. I., Selivanov, Y. M., Feldman, E. V., Bondareva, N. V., Mar, G. I., and Sheremetyeva, L. G.: Study of the epidemiological effectiveness of the live attenuated vaccine in the Byelorussian SSR. In: *Live Poliomyelitis Vaccine*, Leningrad, 1960.
11. Kosmachevski, V. V., Glaznova, G. S., and Aleksandrova, V. R.: Clinical observation of children of pre-school age after enteral vaccination with the live attenuated poliomyelitis vaccine. In: *Live Poliomyelitis Vaccine*, Leningrad, 1960.
12. Kurnosova, L. M. and Zhilova, G. P.: Immunological changes in the blood of children vaccinated with live poliomyelitis vaccine. In: *Live Poliomyelitis Vaccine*, Leningrad, 1960.
13. Smorodintsev, A. A.: The theoretical basis for the use of live poliomyelitis vaccine. In: *Live Poliomyelitis Vaccine*, Leningrad, 1960.
14. ————: Instructions on methods of organizing and carrying out peroral immunization with live attenuated poliomyelitis vaccine. *Ibidem.*
15. ————, *et al.*: Live poliomyelitis vac-

cine. 1957 Yearbook of the Institute of Experimental Medicine, pp. 301-315, 1958.

16. ————: Live poliomyelitis vaccine. Sovremenniye problemy immunologii (Contemtemporary problems of immunology) pp. 292-309, 1959.
17. Vasilyev, K. G. and Glinskaya, Y. V.: An analysis of the reaction-causing properties of the live poliomyelitis vaccine according to observations in Riga. In: *Live Poliomyelitis Vaccine*, Leningrad, 1960.
18. ————, Glinskaya, Y. G., Kibitkina, Y. N., Kukain, R. A., Kurnosova, L. M., Khvatova, A. Y., and Khvatov, P. P.: Provisional results of vaccination against poliomyelitis by means of attenuated live vaccine in the Latvian SSR. In: *Live Poliomyelitis Vaccine*, Leningrad, 1960.

EIGHTH SESSION

THURSDAY, 9 JUNE 1960, 2:00 P.M.

Chairman

DR. CHARLES H. STUART-HARRIS
Professor of Medicine
University of Sheffield
Sheffield, England

TOPIC III. EFFICACY. (B) FIELD EVIDENCE
(continuation)

(DISCUSSION)

Presentation of Papers by:

Dr. V. Skovránek

Dr. F. Przesmycki

(DISCUSSION)

Dr. N. Oker-Blom

Dr. J. J. Alcocer

Dr. Oscar Vargas-Méndez

Dr. J. M. Quirce

(DISCUSSION)

Dr. V. M. Zhdanov

DISCUSSION

CHAIRMAN STUART-HARRIS: We shall reconvene this afternoon first with a discussion on Dr. Smorodintsev's paper.

DR. KITAOKA: I should like to ask Dr. Smorodintsev how the live vaccine which was used in his country was prepared. I should also like to address the same question to Dr. Gear, in connection with the preparation of the live vaccine in his laboratory.

I myself have not yet dared to prepare the vaccine in my laboratory, until some procedure is developed to eliminate the possibility of reversion due to mutation in culture media used for vaccine production, as mentioned by Dr. Dulbecco this morning. The seed stock supplied by Dr. Sabin and Dr. Koprowski is very safe and avirulent, but during the preparation of the vaccine there is still a possibility for a mutant to come out.

DR. SMORODINTSEV: The live vaccine was prepared in the Leningrad Institute of Experimental Medicine in accordance with the standards established by Dr. Sabin. These lots were used in all field trials and consisted of one batch totalling approximately 20 liters of each type. This vaccine was not only tested in Leningrad but in Sabin's laboratories with results comparable to the standards set for this type of vaccine. Our lots represented the second passage of the original vaccine made in the United States.

DR. GEAR: In reply to Dr. Kitaoka's question, our vaccine was prepared from Sabin's strains. The preparation was made from single monkey-kidney lots. The neurovirulence of the original vaccine sent to us by Dr. Sabin was tested intracerebrally and also intraspinally, as were also the derived lots. We derive one lot, which we set aside as a seed lot, and from that we make the production lots for issue.

The lot I was discussing this morning has been tested on 120 monkeys, 60 intracerebrally and 60 intraspinally, and in no instance in the intracerebral inoculation was there any sign of paralysis. The intraspinal inoculation gives somewhat wider lesions than Dr. Sabin has described in connection with his monkeys, but this undoubtedly is due to the method of inoculation.

As far as neurovirulence goes, we are satisfied that it has not gained as a result of passage.

We also tested the vaccine in baby mice, in guinea pigs, in rabbits, as well as in monkeys, in tissue culture of non-primate tissues, and in tissue culture of primate tissues, after the virus had been neutralized to ascertain that there was no other virus present in the vaccine.

DR. BODIAN: Prof. Smorodintsev's study is so impressive that I am sorry we have so little time to discuss it. I have been impressed by the fact that there was an attempt to set up some type of control groups. I think the advantages of this are, or should be, obvious.

Prof. Smorodintsev did mention two things that seemed to me to be somewhat contradictory, and I would very much appreciate an explanation or an interpretation of this. First of all, it was emphasized that the expectation of what was referred to as "blind immunization," that is, by secondary spread, was very low. Yet I thought I heard a statement that in the control groups there was evidence of diminishing polio rates, parallel with the decline in the vaccinated group. I also thought I detected, at least in some of the areas, that the external control group showed a decline of incidence parallel with the decline in the vaccinated group. I realize that there may be several factors going along together here.

Finally, I wish to congratulate Prof. Smorodintsev, among other things, on having cleared up, in my opinion, some of the problems of scheduling, because it does appear that he has very convincingly demonstrated the virtues of repeated immunization.

DR. SMORODINTSEV: The total decline of polio cases in the whole vaccinated group depended on the extent of immunization, which included not less than 65 per cent of the population. Under these conditions we removed the most significant portion of cases expected and ex-

hausted the morbidity rate so much that final results in the whole group were very favorable and did not produce the usual seasonal increase.

We observed quite a good correlation between immunological changes in internal control groups. The protective effect of this blind immunization was very striking and evident in our previous observations made in such children's institutions where the young children were living under conditions providing very close contact. But in children living at home the same mechanism of immunization is not as striking as may be observed under other conditions.

I, personally, am sure that the practice of immunization which is now used in the USSR under conditions of mass immunization involving as much of the whole population as possible, is the only reasonable thing which should be recommended.

Under these circumstances, the extent of vaccination was much less in the preschool than in school children where the percentage of vaccinated was usually 86 per cent and more.

DR. BELL: I still do not quite understand exactly how the internal control group was selected. I understand that persons in certain health stations or regions were vaccinated and that certain were not within the city of Leningrad. If that were the case, then why should there be such a big difference in the age distribution of the vaccinated and internal controls?

I appreciate the administrative and scientific difficulties of getting controls for this kind of study. It would be nice to have a little more explanation as to how the control group was selected, as contrasted with the vaccinated group. This would help us evaluate the effectiveness of the vaccine.

DR. SMORODINTSEV: The internal control group was composed of all children who did not participate in the mass immunization program, which was undertaken on a strictly voluntary basis in 1959, as well as of children who were not present at the beginning of immunization, or who had some temporary or other contraindications for immunization.

The comparative immunological data showed that in the beginning of immunization we had similar distribution of negative children in both groups. From an epidemiological standpoint, I suppose we are inclined to think that this internal control group was in less danger of poliomyelitis because most of the vaccinated children belonged to the groups which were more inclined to participate in this program and which belonged to the more intelligent segment of the population.

So the distribution of this group was not made with any pre-selection of children which may be considered as more susceptible; on the contrary, they are probably less susceptible. But immunological data had showed pretty similar distribution of the percentage of negative children in both groups.

The statistical data on this internal control group was compiled from very complete records kept by the children's polio clinic, which records information on the children population from the moment of birth to the age of sixteen.

The census taken in our country in January 1959 corresponds very closely with these data, which were used for all calculations and were corrected on the basis of this census data.

We did not register these children immediately, but after vaccination was accomplished, all children who did not participate in the beginning of the vaccination were registered, and thus this information is quite complete.

CHAIRMAN STUART-HARRIS: I shall now call on Dr. Skovránek to present his paper on "Further Observations in Conjunction with the First Field Trial with Live Poliovirus Vaccine in Czechoslovakia." It will be followed by Dr. Przesmycki's paper on "Vaccination against Poliomyelitis in Poland with Koprowski's Live Attenuated Strains."

TOPIC III. EFFICACY. (B) FIELD EVIDENCE
(continuation)

22. FURTHER OBSERVATIONS IN CONJUNCTION WITH THE FIRST FIELD TRIAL WITH LIVE POLIOVIRUS VACCINE IN CZECHOSLOVAKIA. EPIDEMIOLOGICAL STUDY

VILÉM SKOVRÁNEK, M.D.

Ministry of Health, Prague

DR. SKOVRÁNEK: At the First International Conference on Live Poliovirus Vaccines last year, I had the opportunity to report on the first results of the extensive work of Czechoslovak scientists, compiled during the first field trial with Sabin's vaccine, which was conducted in a defined part of our country in approximately 140,000 children aged two to eight years, who had been previously vaccinated three times, intradermally, with Salk's vaccine.[1]

The purpose of this paper is not merely to report on further results obtained in the course of the above trial, but also to discuss from a somewhat broader aspect, the reasons why we (including myself as the responsible public health service officer*), took the decision to use the live vaccine in the spring of this year on a very large scale for mass vaccination of the entire child population aged two months to 14 years in the entire territory of our State†. The short time which has elapsed since this extensive vaccination program was completed, less than one month ago, permits me so far to say only that nothing remarkable occurred in the course of this extensive vaccination, which would arouse the attention of the health services and which could be interpreted as having any causal relationship with the vaccination. We were thus able to confirm, on more than the 20-fold number of vaccinated individuals, the conclusion reached during the first field trial, i.e., that vaccination with the live poliovirus vaccine from Sabin's strains is safe.

The main purpose of my paper, as mentioned before, is to explain the reasons why, after analyzing the results compiled in 1959, we took the decision to carry out this extensive mass vaccination.

Before evaluating the 1959 results, I should like to say, by way of introduction, that during that year the vaccination program with the live vaccine was not extended further. This gave us an opportunity to conduct observations throughout the year in strictly defined areas (four orally vaccinated regions—Ustí, Liberec, Jihlava, and Ostrava; and in one region—Plzeň, where a fourth dose of Salk's vaccine was administered

* According to Czechoslovak regulations it is within the competence of the "Chief Hygienist," i.e., the Chief Medical Officer of the sanitary and epidemiological services, to decide on any type of special mass vaccinations, if justified on epidemiological grounds.

† The mass vaccination program with live vaccine produced in Czechoslovakia from Sabin's strains, and partly supplemented by Types 2 and 3 prepared in the Soviet Union, was implemented in two stages. Type 1 was administered between 28 March and 11 April 1960; a mixture of Types 2 and 3 was administered between 2-11 May 1960.

In four regions, where a portion of the child population had received live vaccine during the first field trial in the winter of 1958-1959, a single dose of trivalent vaccine was administered between 11-20 April of this year to all children aged from two months to 14 years (including children already vaccinated in the first trial). A total of about 3,500,000 children aged from two months to 14 years (about 94 per cent of the child population or about 26 per cent of the entire population of Czechoslovakia), was vaccinated by both of the above-mentioned methods.

TABLE 1. VACCINATION STATUS IN CZECHOSLOVAKIA IN 1959

(a) Number of children vaccinated with inactivated vaccine

(Report through 1 July 1959)

AGE GROUP	NO. OF INOCULATIONS	CZECH REGIONS		SLOVAKIA		TOTAL	
		NUMBER	%	NUMBER	%	NUMBER	%
1–9	0	160,833	11.0	130,380	16.0	291,263	12.8
	1	31,256	2.1	37,117	4.6	68,373	3.0
	2	128,197	8.7	87,590	10.8	215,787	9.5
	3	1,144,343	78.2	556,876	68.6	1,701,219	74.7
	2+3	1,272,540	86.9	644,466	79.4	1,917,006	84.2
10–16	0	413,479	34.8	169,756	35.0	583,235	34.9
	1	12,710	1.1	10,995	2.3	23,705	1.4
	2	37,258	3.1	25,523	5.3	62,781	3.8
	3	725,941	61.0	277,782	57.4	1,003,723	59.9
	2+3	763,199	64.1	303,305	62.7	1,066,504	63.7

(b) Number of children vaccinated with the fourth dose of Salk's vaccine in the region of Plzeň

(April-May 1959)

AGE GROUP	NUMBER OF VACCINATED	% FROM CHILDREN VACCINATED THREE TIMES
2—10 years	49,058	68.7

(c) Number of orally vaccinated children

(December 1958-February 1959)

AGE GROUP	TYPE 1		TYPES 1–3		TYPES 1–3–2	
	NUMBER	%*	NUMBER	%*	NUMBER	%*
2—6	114,064	73.0	101,487	64.9	90,386	57.8
6—8	29,313	34.0	25,803	29.9	24,124	28.0
Total	143,377	59.1	127,290	52.5	114,510	47.2

* Percentage from number of children vaccinated three times with inactivated vaccine.

on a mass scale). This strict delimitation of the experimental areas has the disadvantage that it does not permit (in view of the relatively small area of the regions) more general epidemiological conclusions. I feel, however, that for the purpose of our investigations, this method was more suitable than the very variable experimental conditions in the entire population, which would not be appropriate, particularly when using the live vaccine.

RESULTS OF INVESTIGATIONS MADE IN 1959

Our results and conclusions are based on a confrontation of different facts assembled in the course of 1959, i.e., the confrontation of the number of vaccinated individuals with the results of morbidity investigations, investigations of the seroimmunity, and repeated investigations of the spread of polioviruses in different parts of our country.

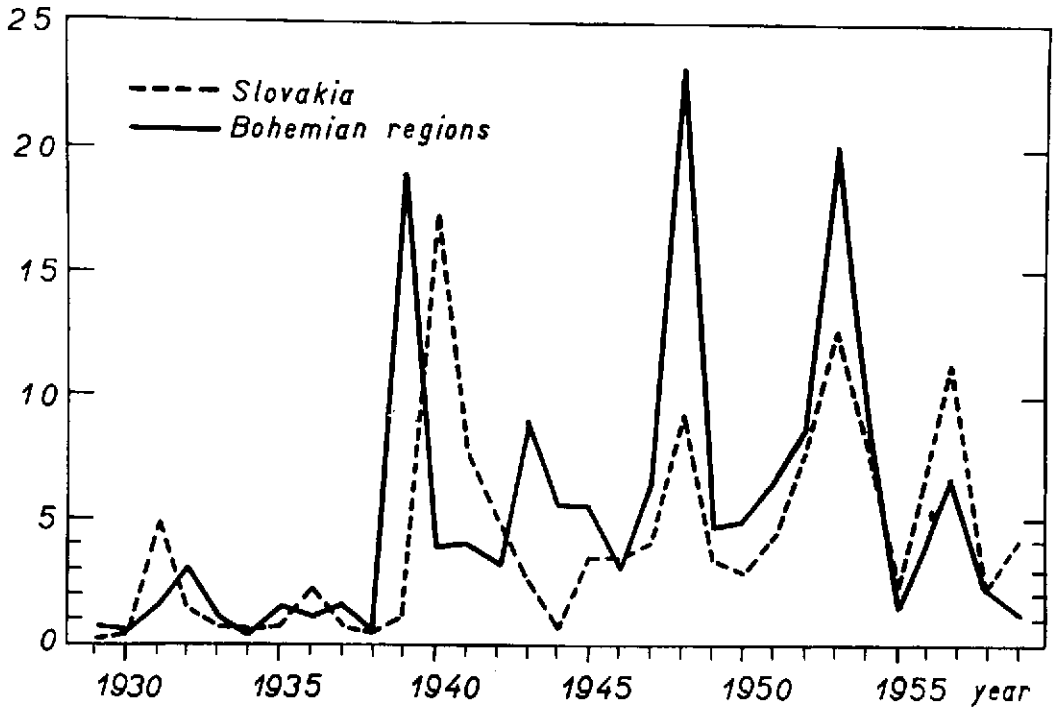


FIG. 1. Poliomyelitis in Czechoslovakia per 100,000 population.

The number of individuals vaccinated by different methods in Czechoslovakia up to 1 July 1959 (before the onset of the usual polio season), is given in Table 1. One can see from the table that the relatively high vaccination ratio, particularly by the intradermal route (twice at 0.125 ml. per dose), is apparent.* Mass vaccination with a fourth dose of Salk's vaccine in the Plzeň region (1 ml. of vaccine administered subcutaneously) was made approximately one year after the third intradermal dose. The table also shows the number of individuals vaccinated with the live vaccine in four regions during the winter of 1958-1959.

1. *Results of Morbidity Investigations from Paralytic Poliomyelitis in 1959.* From the figure summarizing the poliomyelitis morbidity in Czechoslovakia (Fig. 1), based on the number of reported cases, it appears that the morbidity in 1959 was relatively low. The number of cases was 288 (rate 2.1 per 100,000), i.e., it was lower than in 1958 when the absolute number was 302 (rate 2.2 per 100,000). A more detailed analysis of the morbidity in 1959 (based on reported cases) reveals certain differences in the two parts of our State (see Fig. 2); in the eastern part (Slovakia), not only was a substantial rise of morbidity recorded, but what is even more serious, an atypical shift of the morbidity curve occurred toward the end of the year. As far as the morbidity of the entire population of the four regions is concerned, where the live vaccine has been used (dotted line), we cannot draw any definite conclusions so far, except that

* The change to subcutaneous vaccination with 1 ml. of vaccine in small children over six months of age was made only at the end of 1958 and manifested itself particularly during 1959.

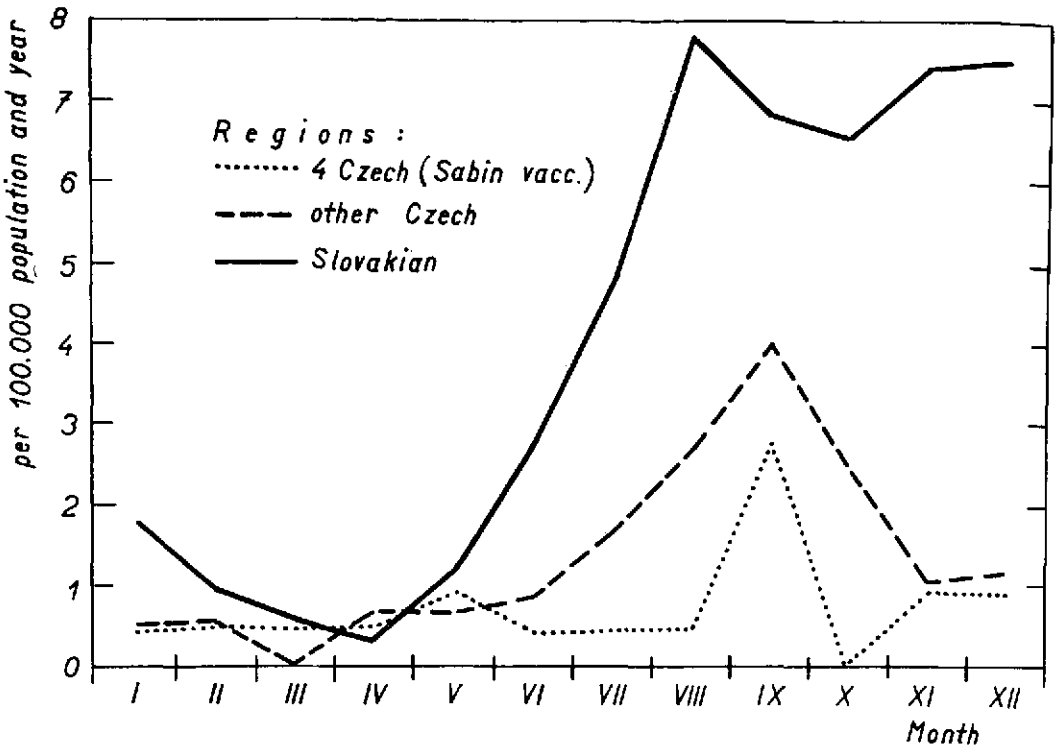


FIG. 2. Poliomyelitis morbidity in Czechoslovakia, 1959.

the attenuated viruses, fed to part of the population, did not cause (during the observation period which so far is one year) an increase in the morbidity. In poliomyelitis, it is, of course, very difficult to rule out whether a certain reduction of the morbidity in certain parts of the country is not due to the commonly observed local variations; nevertheless, a more detailed analysis of the results in the four orally vaccinated regions gives the impression that some differences exist, particularly as regards the age groups of vaccinated children (Table 2 and Fig. 3). The table indicates that in the group of children aged two to eight years in 1958 and three to nine years in 1959, respectively, there was no case of poliomyelitis among vaccinated individuals; in non-vaccinated individuals, the morbidity was lower than in other regions; this can be explained by contact transmission of attenuated viruses.

As far as the effectiveness of the commonly used doses of inactivated vaccine in the remaining parts of the country is concerned, certain differences were observed in the specific age incidence among vaccinated and non-vaccinated individuals in 1959 (Fig. 4).

In general, the analysis of the poliomyelitis incidence in 1959 should be no special cause for alarm, except for the increased morbidity in Slovakia. On the contrary, the relatively low general morbidity and the large number of vaccinated individuals could, without more detailed investigations, lead to a favorable evaluation of the situation.

2. *Results of Seroimmunity Investigations.* The low morbidity figures, particularly in the western part of the country, contrast considerably with the results of repeated investigations of the seroimmunity of population groups and individuals subjected to different methods of vaccination.

In view of the fact that a considerable portion of our paper last year was devoted to the methods and main results of these investigations, and because they are the subject of a separate report (Dr. Vonka)*, I should like to refer

* See pp. 228-239.

TABLE 2. DISTRIBUTION OF PARALYTIC POLIOMYELITIS BY AGE GROUPS IN CZECH REGIONS IN 1959
(Cases virologically and clinically confirmed)*

REGIONS	AGE GROUPS IN YEARS									TOTAL		
	0—2			3—9			10+					
	POPULATION	C	RATE 100,000	POPULATION	C	RATE 100,000	POPULATION	C	RATE 100,000	POPULATION	C	RATE 100,000
Ustí, Liberec, Jihlava, Ostrava (vaccinated)	123,772	8	6.46	143,377†	0	0	2,231,062	5	0.22	2,637,266	18	0.68
				139,055‡	5	3.60						
Other Czech regions (not vaccinated)	289,602	17	5.87	689,543	43	6.24	6,019,022	24	0.40	6,998,167	84	1.20
Total	413,374	25	6.05	971,975	48	4.94	8,250,084	29	0.35	9,635,433	102	1.06

* Incl. seventh-nerve palsy virologically confirmed.
† Number of vaccinated children.
‡ Number of controls, non-orally vaccinated.
C = cases.

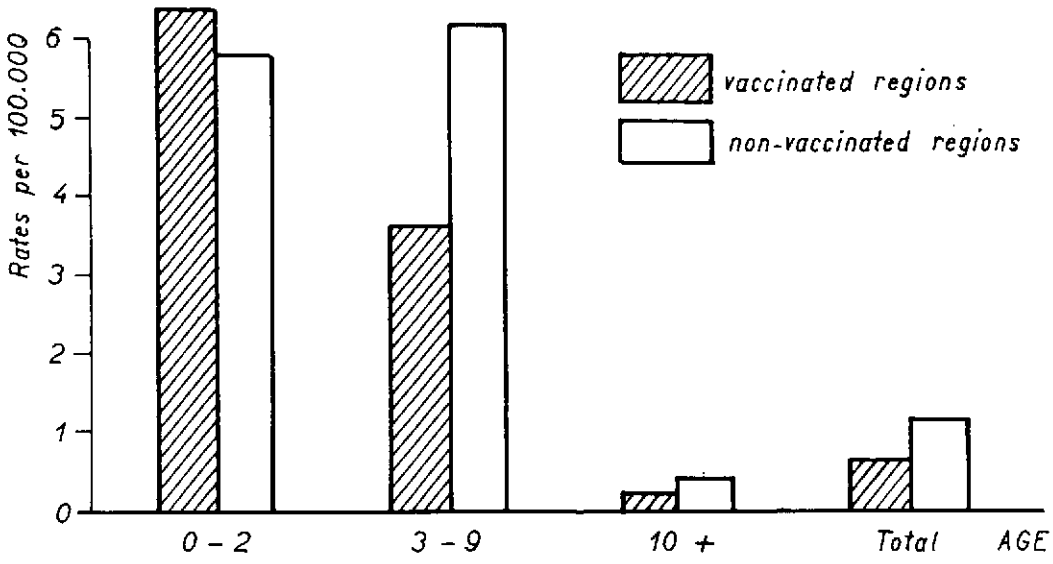


FIG. 3. Poliomyelitis case rates in orally vaccinated and non-vaccinated (Czech regions in 1959).

only briefly to those results which bear any impact on our own study.

(a) Repeated seroimmunity studies were made in the general population prior to the first mass administration of Salk's vaccine at the beginning of 1957 (Zácek,[2] Pesek[3]), and then about eight months and one year after the administration of three intradermal doses of vaccine, i.e., at the end of 1958 and the beginning of 1959, respectively. Results revealed a certain increase in the proportion of higher antibody titers against Type 1, which may be accounted for by the natural spread of Type 1 polioviruses in the observation period, and a more marked increase of antibodies against Type 2, which can be partially explained by the presence of these viruses in the population. However, in view of the lower titers, mainly in younger children, they can also be interpreted by the effect of the antigen component of Type 2 of Salk's vaccine. Besides the differences described above, no substantial changes in the antibody level of sera taken before vaccination with inactivated vaccine and eight to 12 months after it, were found in children aged two to 10 years. Investigations made at the end of 1958 (before the administration of the live vaccine) and in the spring of 1959 (before the administration of a fourth dose of Salk's vaccine in the Plzeň region), revealed that about *40 per cent of the most threatened child population under 10 years of age lacked detectable antibodies against poliovirus Types 1 and 3*, while in some areas antibodies against Type 2 were absent in about 20 per cent of the children in these age groups.

(b) Investigations of paried sera of children vaccinated with a fourth dose of Salk's vaccine (1 ml. subcutaneously) and live oral vaccine, revealed significant differences in the antibody response between the two types of vaccination, particularly as regards Type 1. In children lacking homologous antibodies against Type 1, after the fourth dose of inactivated vaccine, conversion occurred in 50 per cent, while after the live vaccine conversion was recorded in 95 per cent. (Significant, though less marked differences, were recorded also in the remaining types). The main results of these investigations are summarized in Table 3, which corrects the preliminary results presented in our paper last year.

(c) More detailed analyses of these results made by Zácek and Vonka, [4,5] revealed further significant differences in the quality and amount of antibodies obtained by the two vaccination

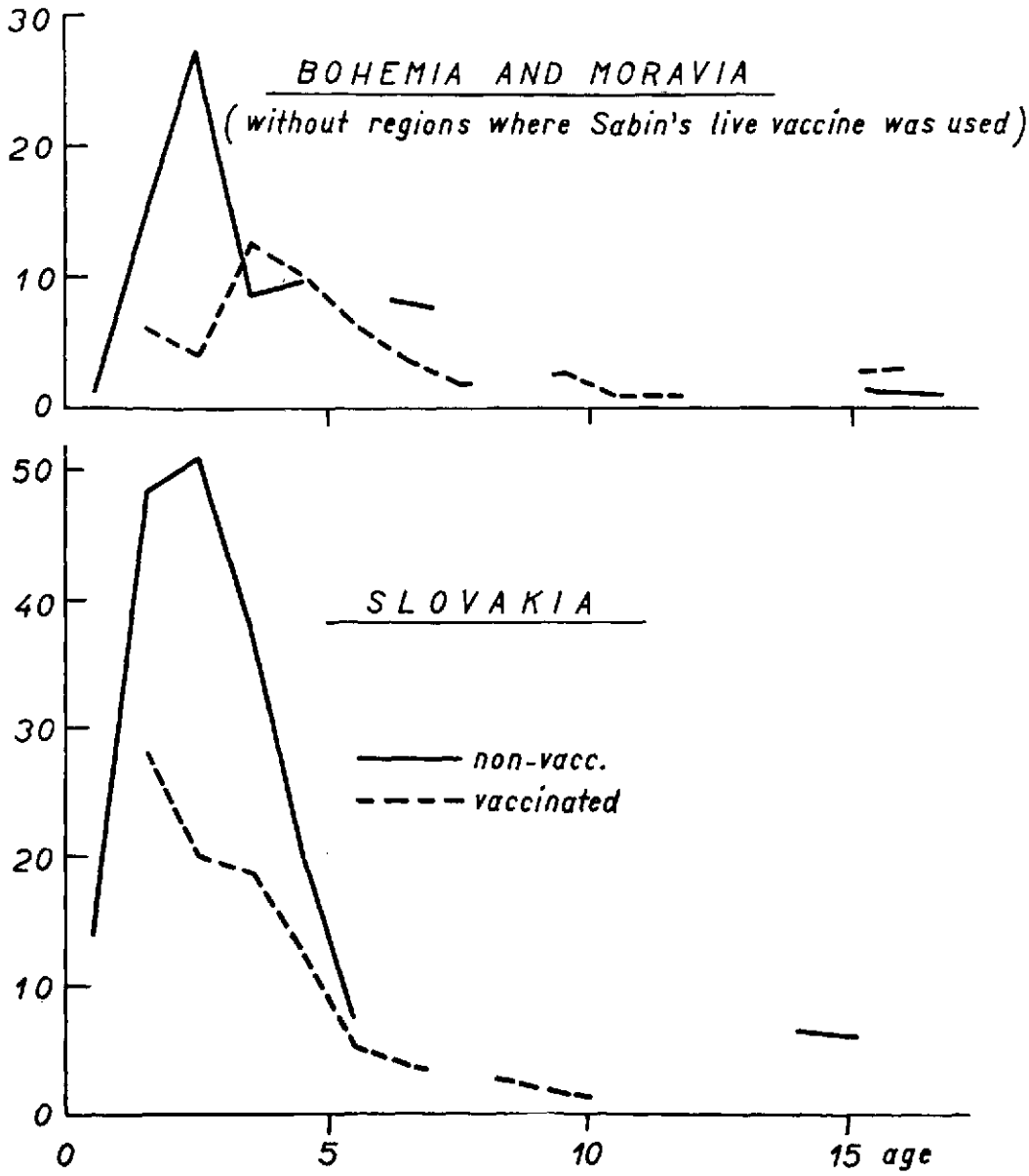


FIG. 4. Poliomyelitis morbidity in children in 1959, by age.

methods. While after the live vaccine, predominantly higher titers were obtained, particularly of Type 1 antibodies, the antibody responses after the fourth dose of inactivated vaccine are quantitatively weaker (Table 4). Frequently, particularly in children also lacking antibodies against Type 2, no booster effect was recorded. The weak antibody response resembled rather the primary immunization reaction* (Table 5).

* For the basic intradermal vaccination with inactivated vaccine the Canadian vaccine (Connaught Laboratories) was used. For the fourth dose administered in Plzeň region one lot of locally produced vaccine was used (Institute for Sera and Vaccines, Prague).

TABLE 3. POLIOMYELITIS ANTIBODY RESPONSE IN 159 CHILDREN AGED 2-10 YEARS, VACCINATED WITH THE FOURTH DOSE SALK VACCINE (IN THE REGION OF PLZEŇ) AND IN 93, 82, AND 88 CHILDREN AGED 2-8 YEARS VACCINATED WITH SABIN LIVE POLIOVIRUS VACCINE (IN THE REGION OF ÚSTÍ AND JIHLAVA)

FOURTH DOSE OF SALK VACCINE (PLZEŇ)						LIVE VACCINE–SABIN (ÚSTÍ AND JIHLAVA)					
TYPE	NUMBER OF CHILDREN INVESTIGATED	ANTIBODY STATUS PRIOR VACCINATION (pH TEST)		SIGNIFICANT RISE IN ANTIBODY*		TYPE	NUMBER OF CHILDREN INVESTIGATED	ANTIBODY STATUS PRIOR VACCINATION (pH TEST)		SIGNIFICANT RISE IN ANTIBODY*	
		TITER	N°	N°	%			TITER	N°	N°	%
1	159 (39% neg.)	Negative	62	31	50	1	93 (39% neg.)	Negative	36	34	95
		4–32	36	29	80			4–32	21	14	67
		64–>512	61	36	58			64–>512	36	23	64
2	159 (13% neg.)	Negative	21	14	67	2	82 (17% neg.)	Negative	14	13	93
		4–32	81	67	83			4–32	36	30	83
		64–>512	57	32	56			64–>512	32	21	66
3	159 (41% neg.)	Negative	65	54	83	3	88 (39% neg.)	Negative	34	32	94
		4–32	48	34	71			4–32	27	20	74
		64–>512	46	22	48			64–>512	27	12	44

* These data are based on:
(a) 4x increase in pH and CPE test, or
(b) 4x increase in CPE test without rise in pH test, or
(c) 8x increase in pH or CPE test.

TABLE 4. ANTIBODY RESPONSE IN CHILDREN LACKING HOMOLOGOUS ANTIBODIES PRIOR TO VACCINATION (NEGATIVE CHILDREN) TO THE FOURTH DOSE OF SALK VACCINE AND/OR TO THE SABIN LIVE POLIOVIRUS VACCINE

(More Detailed Data from Table 3)

FOURTH DOSE OF SALK VACCINE						LIVE VACCINE–SABIN					
TYPE	N° OF NEGATIVES (pH TEST)	N° OF CHILDREN WHICH DEVELOPED ANTIBODIES AFTER BOOSTER INJECTION	HOMOLOGOUS ANTIBODY RESPONSE*			TYPE	N° OF NEGATIVES (pH TEST)	N° OF CHILDREN WHICH DEVELOPED ANTIBODIES AFTER VACCINATION	HOMOLOGOUS ANTIBODY RESPONSE*		
			TITER	N°	%				TITER	N°	%
1	62	31 (50% positive)	4–32 64–>512	28 3	90 10	1	36	34 (95% positive)	4–32 64–>512	11 23	32 68
2	21	14 (64% positive)	4–32 64–>512	5 9	36 64	2	14	13 (93% positive)	4–32 64–>512	2 11	15 85
3	65	54 (83% positive)	4–32 64–>512	33 21	61 39	3	34	32 (94% positive)	4–32 64–>512	18 14	56 44

* These data are based on pH and CPE antibody tests.

TABLE 5. TYPES 1 AND 3 ANTIBODY RESPONSE IN CHILDREN TO THE FOURTH DOSE OF SALK VACCINE IN RELATION TO THE STATUS OF HOMOLOGOUS AND TYPE 2 ANTIBODY PRIOR TO VACCINATION

N° OF CHILDREN INVESTIGATED	TYPE	WITHOUT HOMOLOGOUS ANTIBODIES PRIOR TO VACCINATION (pH TEST)	STATUS OF TYPE 2 ANTIBODIES PRIOR TO VACCINATION (pH TEST)	HOMOLOGOUS ANTIBODY RESPONSE		
				TITER	N°	%
282	1	107 (38% *negative*)	Positive 80 children	Negative	21	26
				4–32	50	62
				64–>512	9	12
			Negative 27 children	Negative	23	85
				4–32	4	15
				64–>512	0	0
	3	114 (40% negative)	Positive 89 children	Negative	6	7
				4–32	41	46
				64–>512	42	47
			Negative 25 children	Negative	8	32
				4–32	17	68
				64–>512	0	0

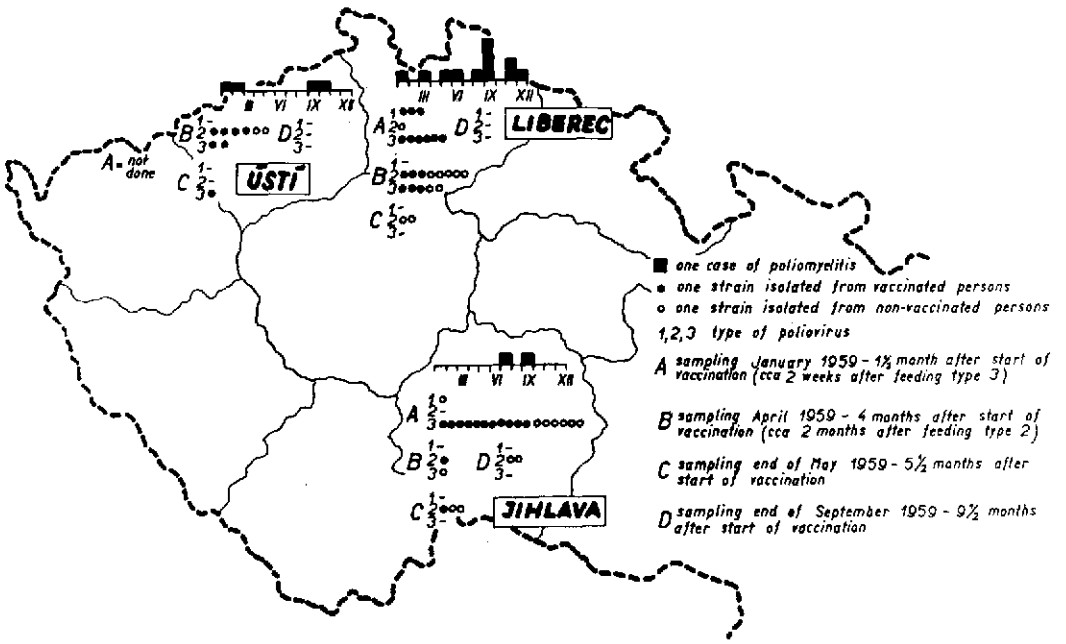


FIG. 5. Spread of polioviruses in orally vaccinated Czech regions and number of paralytic polio cases, 1959.

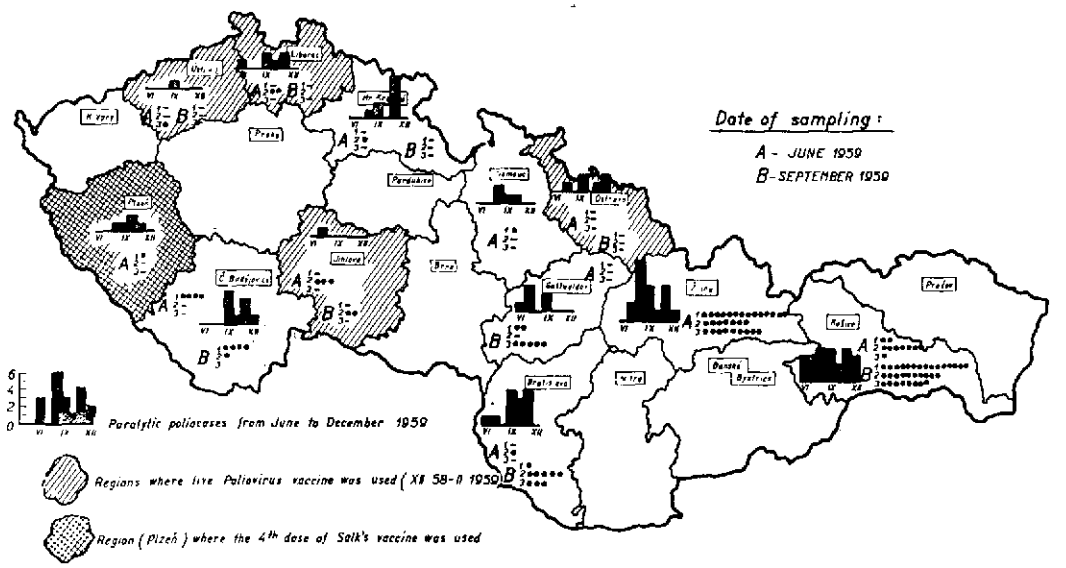


FIG. 6. Paralytic poliocases and spread of polioviruses in some Czechoslovak regions, 1959 (Cases virologically and clinically confirmed).

These results, particularly in conjunction with investigations of the spread of polioviruses in the population, influenced our final decision in a significant manner.

3. *Results of Investigations of the Poliovirus Spread in the Population*: In 1959 great attention was paid to these investigations in Czechoslovakia. The fate of attenuated polioviruses

in the population of the four experimental and neighboring regions was investigated and repeated investigations on the spread of wild polioviruses (and other enteroviruses) were conducted in several Czech and Slovak regions. The main attention was paid to relations between the amount of detected strains and the poliomyelitis morbidity*. The results, obtained before the usual onset and at the peak of the usual polio season (June-September), are of particular importance. From the results, discussed in greater detail elsewhere (Burian, Vojtová and others),[6] I should like to demonstrate the following main facts shown on two maps (Figures 5 and 6):

(a) Repeated investigations in four regions, where in the winter of 1958-1959 the live vaccine was administered (Fig. 5), revealed that *attenuated viruses disappear relatively rapidly from the population.* Isolated viruses, if detected later in non-vaccinated individuals (white circles on the map), were most probably wild viruses imported to these regions from elsewhere, or wild viruses occurring in the non-vaccinated part of the population. (It must be emphasized that the first field trial with the live vaccine was conducted in a relatively small part of the population—in less than 50 per cent of children aged two to eight years). The polio-morbidity in these four regions (black squares on the map; one square stands for one case of poliomyelitis) does not have any peculiar features.

(b) Very important results are shown on the map summarizing the results of two investigations of the poliovirus spread in Czechoslovakia in 1959 during June (A) and September (B), and which gives also the number of cases of poliomyelitis in the second half of 1959 (Fig. 6). The results indicate that in regions neighboring upon those where the live vaccine was administered, the incidence of polioviruses was low and that thus, most probably, these viruses did not penetrate into neighboring regions. (It must also be taken into account that the vaccination was carried out in winter). If later there was a higher incidence of viruses in some localities (such as in the Gottwaldov region), this was more probably due to the penetration of wild viruses from neighboring Slovak regions, particularly the Zilina region, which is very close to the Gottwaldov region. (It is interesting to note that in 1959 there was an extensive poliomyelitis epidemic in neighboring Hungary, which borders on Slovakia along the entire frontier.)

The most important conclusion from these investigations is that, when there was a higher incidence of polioviruses in the population, which had been practically evenly vaccinated with the inactivated vaccine, there was also a higher incidence of poliomyelitis. In other words, we may say that not even the previous high vaccination rate with inactivated vaccine was able to prevent the spread of polioviruses and that to a certain degree, we have to rely on chance whether an unexpected spread of viruses will occur or not. If we add to this the defects of seroimmunity, it is evident why in 1959 there was a substantially higher morbidity in Slovakia, as shown in Fig. 2 and why we considered the future epidemiological prognosis of poliomyelitis in our country uncertain.*

The main purpose of my report was to give an idea of the trend of our deliberations and to demonstrate at least some fundamental facts on which we based our decision to use the live vaccine for mass vaccination. I think that our results indicated clearly *the necessity to improve the state of immunity of our most susceptible population.* A comparison of the results of the first field trial with the live vaccine with those after the administration of a fourth dose of inactivated vaccine on a mass scale, however, revealed also the *method of how to achieve this improvement most expediently.* I have tried to summarize the balance of our deliberations on the advantages of either method in Table 6.

The objection could certainly be raised that our decision was premature. I dare not, however, answer the second aspect of this objection, and I doubt that anybody will be able to do so. It is my opinion that it would be much more difficult to explain to our people in the case of an epidemic, why we did not undertake every-

* Specimens of feces, proportional to the population density, were collected by random sampling, predominantly from children. In child communities, never more than two specimens were taken from one community.

* The results assembled during the first few months of 1960 confirmed to a certain extent that these fears were justified. The number of patients between 1 January 1960 and 28 March 1960 was, as compared with the same period in 1959, three times greater (66:22), and several small local epidemics occurred.

TABLE 6. BALANCE OF DELIBERATIONS AND ACTUAL EXPERIENCE WITH BOTH METHODS OF VACCINATION AGAINST POLIOMYELITIS IN CZECHOSLOVAKIA AT THE END OF 1959

Inactivated Vaccine

Advantages	*Disadvantages*
1. Large number of vaccinated individuals.	1. Intradermal route of administration.
2. Confirmed effectiveness of vaccine in 1957 (66 and 74%, respectively).	2. Unsatisfactory state of seroimmunity in vaccinated population, particularly against Types 1 and 3 (practically the same as prior to vaccination).
3. Low poliomyelitis morbidity in 1958 and 1959.	3. Unsatisfactory antibody response after a fourth dose of vaccine as regards the number of reacting individuals and the titers, particularly in Type 1. (The need for a larger number of doses of vaccine to improve the state of immunity?).
	4. It was confirmed that in the event of a greater spread of polioviruses in the population the morbidity rises, regardless of previous vaccination.

Live Vaccine

Advantages	*Disadvantages*
1. Simple administration.	1. Relatively small number of individuals vaccinated in first field trial which does not permit any general conclusions on the epidemiological effectiveness of the vaccine.*
2. High antibody response against all types; particularly Type 1.	2. Problems of reversion?†
3. Natural, but chronologically limited spread of attenuated polioviruses in population, rendering possible a certain increase of the percentage of immune individuals.	
4. Resistance of the intestinal tract as a further prerequisite for the prevention of the disease (prevention of the spread of viruses).	
5. Longer duration of immunity?	
6. The possibility of eradicating wild viruses from the population.	

* This "disadvantage" is balanced by the much more extensive experience in the USSR.
† Evidence against this "disadvantage" is provided, among other facts, by the short-term excretion of viruses by vaccinated individuals.

thing we could have done which resulted from findings of our own research.

I do not, of course, feel justified to generalize. The results and the decisions are based on our actual investigations, making use of long-term experience, and results of systematic field surveys in which many specialists from different branches participated. The purpose was to demonstrate the methods we have used. And we feel that these might have a more general validity, though the final results may obviously differ in different countries.

Within the last few hours I have tried to sketch some simplified schedules applicable to situations which could be found when using this method of investigation (Fig. 7). The figure shows four different possible situations in the population, with a relatively high number of children lacking antibodies.

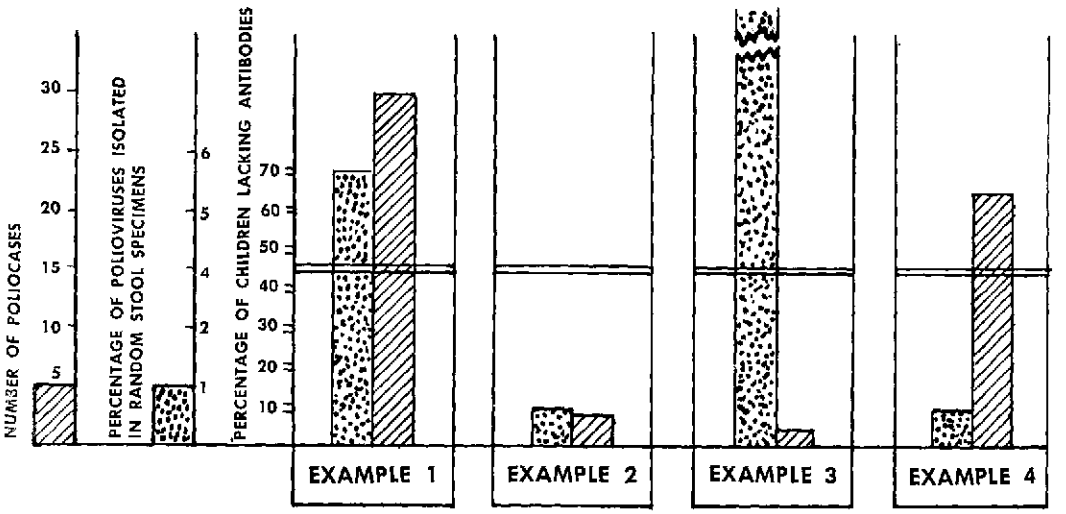


FIG. 7. Examples for possible relationship between seroimmunity, spread of polioviruses and poliomorbidity in different conditions

Example 1 shows the situation with high spread of polioviruses in the population, resulting in high morbidity. This was our situation in the eastern part of Czechoslovakia last year.

Example 2 shows quite a different situation: low spread of polioviruses, low polio morbidity. This was our situation in the western part of our Republic. I assume that this is the case of many countries with contemporary low morbidity, perhaps in the Netherlands too.

I am of the opinion that from the epidemiological point of view, these situations are very dangerous and the epidemiological prognosis of these countries, or parts thereof, is unclear.

Example No. 3 shows the typical situation which can be found immediately after feeding of attenuated vaccine: an extraordinary high spread of viruses and low or no polio morbidity.

The last example is quite atypical. It shows low spread of polioviruses and a high number of paralytic diseases. Theoretically, two explanations can be taken into consideration: either incorrect sampling was carried out, or the recorded cases are not poliomyelitis.

It is quite evident that an analogous situation with different consequences may exist under different conditions. This, however, is not the aim of this small philosophical consideration. The main reason was to emphasize the necessity for studying the relationship between different phenomena occurring in the population under *different conditions of human existence.*

Based on the results of similarly conducted extensive field trials, and a comparison of various methods of investigation, it would be easier to evaluate another very important aspect of the problem which we were unable to evaluate ourselves, i.e., whether our actual position was *solely* due to the intradermal route of vaccination used up to the end of 1958.

ADDENDUM

Finally, I should like to submit the following recommendation to the leading officials of the WHO: It is beyond doubt that not even for us—who had at our disposal a well established network of health services, virological laboratories staffed with competent workers able to prepare with all precautions a vaccine of such a degree of safety, as rendered possible by all available methods—was it an easy matter to decide on the mass administration of such large amounts of a live virus preparation to the entire population. I think that it is not a problem of one country, but a world-wide problem what sort of new agents are administered to the population. I recommend therefore the following concrete provisions:

1. That the WHO should establish a world reference laboratory, responsible for the selection and provision of tested virus strains, suitable for the production of vaccine; and

2. That the WHO should, in collaboration with governments of Member Countries, appoint a certain small number of laboratories entitled to prepare controlled live vaccine for certain areas of the world.

Only then will it be possible to preventively avoid possible damage and utilize fully the advantages of the new prophylactic preparation against the development of poliomyelitis epidemics.

REFERENCES

1. Skovránek, V., Zácek, K., *et al.:* Field trial with Sabin's live poliovirus vaccine in Czechoslovakia, 1958-1959. In: *Live Poliovirus Vaccines, First International Conference.* Scient. Pub. no. 44 of the Pan American Health Organization, pp. 530-571, 1959.
2. Zácek, K., Vonka, V., *et al.:* The state of seroimmunity for poliomyelitis in Czechoslovakia. *J. Hyg. Epidem.* (Praha) **2**:423-437, 1958.
3. Pesek, J.: Incidence of antibodies against polioviruses in the population of Slovakia prior to the 1957 vaccination scheme. *J. Hyg. Epidem.* (Praha) **2**:443-447, 1958.
4. Zácek, K., Vonka, V., and Adam, E.: The antibody response in children vaccinated with (a) live attenuated polioviruses and (b) the fourth dose inactivated poliovaccine. Paper presented at the International Epidemiological Symposium, Prague, February 1960.
5. Burian, V., Vojtová, H., and Zácek, K.: The spread of polioviruses in the population vaccinated with live polioviruses in comparison with the nonvaccinated population. Paper presented at the International Epidemiological Symposium, Prague, February 1960.

23. VACCINATION AGAINST POLIOMYELITIS IN POLAND WITH KOPROWSKI'S LIVE ATTENUATED STRAINS

F. PRZESMYCKI, H. DOBROWOLSKA, B. MIRSKI,
R. STANCZYK, H. WIOR, AND A. ZALESKA
Department of Virology of the State Institute of Hygiene, Warsaw, Poland

Dr. PRZESMYCKI (*presenting the paper*): In 1959 trials of live attenuated vaccine, prepared from Koprowski's Type 1 CHAT strain, were completed. Vaccination was performed in a small town and in three adjacent villages, with a total population of 8,716. In all, 2,888 persons from six months to 16 years of age were vaccinated, or 95 per cent of the registered population in this age group. There were no complications among the vaccinated subjects, and an increased antibody level was observed after vaccination. The vaccination campaign was initiated on 20 October 1958; since then no case of poliomyelitis has been recorded in these localities over a period of 18 months.

On the basis of the results of this introductory trial, a special committee was appointed by the Ministry of Health, which recommended the inauguration of a mass vaccination campaign according to the following scheme:

(1) Children from six months to seven years of age were to be vaccinated at least twice with Salk's inactivated vaccine before being inoculated with the live virus.

(2) Children over seven years of age might be vaccinated with the live vaccine, without prior immunization with Salk vaccine.

On the basis of these recommendations, a plan was worked out for the vaccination campaign. The management of the campaign was entrusted to the Provincial Public Health Laboratories. The vaccines—Type 1 and Type 3—were supplied by Dr. H. Koprowski and were tested by the Department of Virology of the State Institute of Hygiene to establish: (1) the titer; (2) the neuropathogenic properties of the strains for monkeys; and (3) the proper dose of vaccine by vaccinating small groups of children.

RESULTS OF TESTS

The titer for Type 1 was $10^{7.6}$ $TCID_{50}$; that for Type 3 was 10^{8} $TCID_{50}$ per ml.

The Type 1 vaccine (CHAT 18 GI) was inoculated intracerebrally in 53 monkeys. No clinical symptoms were observed. In two cases, however, histopathologic lesions could be seen. The same vaccine was inoculated intraspinally in 24 monkeys. In seven cases, definite clinical symptoms could be discerned, and in 10 monkeys, histopathologic lesions were demonstrated (42 per cent) (see Tables 1 and 2).

The Type 3 vaccine (Fox) was inoculated intracerebrally in 19 monkeys. No clinical symptoms were observed. On the other hand, atypical histopathologic lesions were noted in one monkey, and typical lesions in a second animal. Thirty monkeys received an intraspinal injection of the same vaccine. Out of this total number, typical clinical symptoms were observed in three cases, doubtful symptoms in four, and histopathologic lesions in nine (30 per cent) (see Tables 3 and 4).

Studies were also carried out in children in order to determine the proper dosage.

The investigation was conducted in a semi-isolated children's home, where 37 children from six months to three years of age were observed. Examinations of stool and blood specimens were made before the vaccine was administered. Koprowski's live attenuated strain, Type 1 (CHAT) was then administered orally. The following dosages were used: 1,000,000, 200,000, and 100,000 $TCID_{50}$. Subsequently, stool specimens were collected from the vaccinated children after three, seven, and 30 days. Specimens of blood were also collected again after 30 days.

At the same time, i.e., 30 days after the first vaccination with Type 1 CHAT and Koprowski's Type 3, Fox strain was given to the same children. The dosages amounted to 200,000 and 100,000 $TCID_{50}$. Stool specimens were again collected three and seven days after the administration of the Type 3 vaccine. Blood was also re-examined after 30 days. The last blood con-

TABLE 1. INTRACEREBRAL INOCULATION OF MONKEYS WITH CHAT TYPE 1 POLIO VACCINE

BATCH OF VACCINE*	REACTION OF MONKEYS	
	CLINICAL SIGNS	HISTOPATHOLOGIC LESIONS
WCH 18	0/5	0/5
WCH 18 GI	0/14	2/14
WCH 18 GI	0/25**	0/25
WCH 18 GI	0/9	0/9
Total	0/53	2/53

* Tested on 4 different days.
** Five groups of 5 monkeys each were inoculated with a dilution of 10^{0}, 10^{-1}, 10^{-2}, 10^{-3} or 10^{-4}.

TABLE 2. INTRASPINAL INOCULATION OF MONKEYS WITH CHAT POLIO VACCINE

Dilutions of Vaccine →	10^{0}*		10^{-1}*		10^{-2}†		All	
Reactions	Clin. Signs	Hist. Lesions	Clin. Signs	Hist. Lesions	Clin. Signs	Hist. Lesions	Clin. Signs	Hist. Lesions
	2/9	3/9	4/10	5/10	1/5	2/5	7/24	10/24

* Vaccine batch: WCH 18.
† Vaccine batch: WCH 18 GI.

trol test was performed 110 days after the administration of Type 1 virus.

The results of the virologic investigation are given in Table 5. As the table indicates, the highest percentage of isolated strains was obtained with a dosage of 1,000,000 $TCID_{50}$. The percentage isolation decreased with reduction of the $TCID_{50}$.

In general, disregarding the dosage, Type 1 virus was isolated in 80 per cent of the cases. It should be added that in two cases it was not possible either to isolate the virus or to demonstrate an increase in the antibodies. In a certain number of cases, Type 1 virus was isolated after 30 days.

The results obtained after vaccination with Type 3 virus are similar to those achieved when Type 1 virus was used. The virus was isolated in 76 per cent of the cases in all.

It should be pointed out that isolation of the virus was attempted only on the third and seventh days after vaccination. One cannot exclude the possibility that more positive results might be obtained over a prolonged observation period.

As a result of our investigations, we were able to establish that the strains used for vaccination have a definite affinity for the intestinal tract and can multiply there.

Serologic examinations performed prior to

TABLE 3. INTRACEREBRAL INOCULATION OF MONKEYS WITH FOX STRAIN

Batch of Vaccine	Reaction of Monkeys			
	Dilution of Vaccine and Reaction			
	10^{0}		10^{-1}	
	Clinical Signs	Histopathologic Lesions	Clinical Signs	Histopathologic Lesions
WF X	0/9	0/9*		
WF X	0/5	0/5	0/5	1/5
Total	0/14	0/14	0/5	1/5

* One questionable lesion.

TABLE 4. INTRASPINAL INOCULATION OF MONKEYS WITH FOX STRAIN POLIO VACCINE

Batch of Vaccine	Dilution of Vaccine and Reaction							
	10^{0}		10^{-1}		10^{-2}		10^{-3}	
	Clin. Signs	Hist. Lesions	Clin. Signs	Hist. Lesions	Clin. Signs	Hist. Lesions	Clin. Signs	Hist. Lesions
WF X	2/5*	2/5	0/5*	0/5				
WF X	0/5	2/5	1/5	3/5	1/5	1/5	0/5	1/5
Total	2/10	4/10	1/10	3/10	1/5	1/5	0/5	1/5

* Two others had doubtful signs.

Summary:

Doubtful clinical signs	4/30
Typical clinical signs	3/30
Histopathological changes	9/30

vaccination indicated that, with the exception of three children, no child in the group had antibodies to all three types of poliovirus.

The antibody concentration in two children amounted to 1:8 for Types 1 and 3, and 1:4 for Type 2. In a third child, the concentration of antibodies to Type 1 only was 1:8.

The results of the blood studies made after vaccination are given in Tables 6 and 7.

As the tables show, the antibodies against Type 1 increased maximally after 110 days. No antibodies were detected in 16 per cent of the children; in 84 per cent, however, the presence of antibodies was proved. In 72 per cent of the children, antibodies were present in concentrations of 1:16 to 1:1024.

It should be added that the level of antibodies showed variations, or even a decrease, after 30 and 60 days. The highest percentage of positive results for Type 3 virus was obtained after 80 days. All the sera investigated showed titers from 1:64 to 1:2048.

TABLE 5. EXCRETION OF CHAT AND FOX AFTER FEEDING OF DIFFERENT DOSES

DOSE OF VACCINE	HOMOTYPIC VIRUS ISOLATIONS AFTER VACCINATION*			
	CHAT		FOX	
(log $TCID_{50}$)	RATIO	PER CENT	RATIO	PER CENT
6.0	11/12	92		
5.3	9/11	82	12/15	80
5.0	9/13	69	8/10	80

* Stool specimens collected 3, 7, and 30 days after feeding of CHAT and 3 and 7 days after Fox.

TABLE 6. SEROLOGIC RESPONSE TO CHAT AND FOX STRAINS

STRAIN	PRESENCE OF HOMOTYPIC ANTIBODIES				
	BEFORE VACCINATION	DAYS AFTER VACCINATION →			
		30	60	80	110
CHAT	0/33 (0%)	26/33 (79%)	17/24 (71%)		21/25 (84%)
FOX	0/25 (0%)	21/24 (88%)		25/25 (100%)	

It follows from our experiments that vaccine prepared with the Koprowski strains has definite immunogenic properties.

In the initial stage of the oral vaccination trial, a nurse's mistake resulted in the subcutaneous injection of 13 children with live vaccine, each child receiving a 1-ml. amount, containing 200,000 $TCID_{50}$. Eight of these 13 children were seven to eight years old and had been vaccinated twice before with Salk vaccine. The remaining five were eight months to six years old, and two of them had been vaccinated once with the Salk vaccine. Three children aged eight to 12 months had had no previous immunization. All 13 children were given gamma-globulin five days after the erroneous inoculation. None of them showed any symptoms of illness. Material from five children (the youngest ones) was taken for viral and serologic investigations. Of four children tested after vaccination and gamma-globulin inoculation, one still lacked antibody to Type 1 virus.

FIELD TRIALS

In June 1959, 642,930 children six months to 15 years of age were fed the Type 1 (CHAT) attenuated strain. This initial trial was carried out in two provinces only.

The campaign proper started on 20 October 1959. In March 1960, vaccinations with Type 1 were completed, and those with Type 3 ended in April 1960. The vaccination campaign usually

TABLE 7. DEVELOPMENT OF ANTIBODIES IN INDIVIDUALS FED CHAT AND FOX

ANTIBODY TITER	CHAT DAY OF EXAMINATION				FOX DAY OF EXAMINATION		
	0	30	60	110	0	30	80
>512			4	6		3	16
256–512		4		6		6	6
64–128		5	3	3		7	3
16–32		14	4	3		3	
4–8		3	6	3		2	
0	33	7	7	4	25	3	
All	33	33	24	25	25	24	25

lasted six to 12 days in each province. It was carried out in separate provinces on fixed days.

Management of the vaccination campaign. On predetermined days, the vaccine, packed in containers with dry ice, was dispatched by car to the provincial capitals. The instructions recommended that the dilution of the vaccine in saline be performed at the Provincial Public Health Laboratories. For the Type 1 vaccine, the dilution was 1:500, i.e., 200,000 $TCID_{50}$ per ml.; for Type 3, it was 1:1000, i.e., about 100,000 $TCID_{50}$ per ml. The diluted vaccine was then placed in containers with ordinary ice and sent by car to each vaccination point. It was advised that the diluted vaccine be refrigerated and used for inoculation during the first 48 hours after dilution. Samples of the diluted vaccine were collected at the vaccination points, and the titer of the virus was examined in our laboratory. Eight samples were examined, all with satisfactory results.

The dose of vaccine to be given orally was 1 ml. It was added to 4 ml. of distilled water with 10 per cent glucose. The proportion of persons vaccinated varied in the different provinces from 63 to 99 per cent. Altogether, 7,130,000 people were vaccinated with Type 1, i.e., 83.5 per cent of the registered children in the age group from six months to 15 years. A total of 6,250,000 persons was vaccinated with Type 3, corresponding to 75% of those registered (85-94 per cent of those who received the Type 1 vaccine).

It was decided that those who did not receive the vaccine during this campaign would be vaccinated at a later date. All children from six months to 15 years will be vaccinated with Type 2 in November of this year.

In connection with the vaccination campaign, I would like to discuss some of the results of our virologic and serologic investigations carried out in three provinces.

The investigations in Warsaw were conducted at the State Institute of Hygiene. The material was collected in three homes for children. The group was composed of 160 children, aged one to three years. Samples of feces were collected and examined before vaccination. The procedure was repeated 10 to 14 days after vaccination with Type 1 virus, as well as 10 to 14 days after the administration of Type 3 virus. The results obtained are shown in Table 8.

The table indicates that before vaccination Types 1 and 3 virus were isolated in 3.1 per cent. Type 2 virus has not been isolated at all. A cytopathogenic agent was isolated in 0.6 per cent. After the administration of the vaccine containing Type 1 strain, the virus was isolated from feces in 55.1 per cent. This percentage amounted to 66.9 when Type 3 virus was given.

It should be emphasized that the percentage of isolated strains varied rather considerably in the different homes. In general, the Type 3 virus was isolated in a higher percentage of cases than Type 1 virus. Serologic investigations have not been completed as yet; hence the results are not given here. Analogous studies were carried out in the Public Health Laboratory at Lublin.

The material was collected from children aged

six months to three years, three to seven years old, and in a small group over seven years of age. In all, 215 samples of feces were collected. The samples were collected before vaccination and from three to four weeks after administration of Type I virus. The same procedure was used after the administration of Type 3 virus. The results obtained are presented in Table 9.

Before vaccination, Type 1 virus was isolated in 1.86% of the samples; no Type 2 or 3 virus was isolated. In addition, a cytopathogenic agent was isolated in 1.86 per cent of the cases.

After the vaccination of children with Type 1 virus, the virus was isolated from feces in 27.44 per cent; the corresponding figure for Type 3 virus was 21.1 per cent. The comparatively low

TABLE 8. EXCRETION OF VIRUS IN STOOLS OF CHILDREN FED THE CHAT AND FOX STRAINS AT THE AGE OF 1-3 YRS. IN WARSAW

CHILDREN'S HOME	NUMBER OF STOOL SPECIMENS EXAMINED	ISOLATION OF VIRUS FROM STOOL SPECIMENS*				
		BEFORE IMMUNIZATION			AFTER IMMUNIZATION	
		NOT POLIO %	TYPE 1 %	TYPE 3 %	TYPE 1 %	TYPE 3 %
A	78	1	1	4	56	67
B	38	0	8	5	76	61
C	44	0	2	0	36	73
Total	160	<1	3	3	55	67

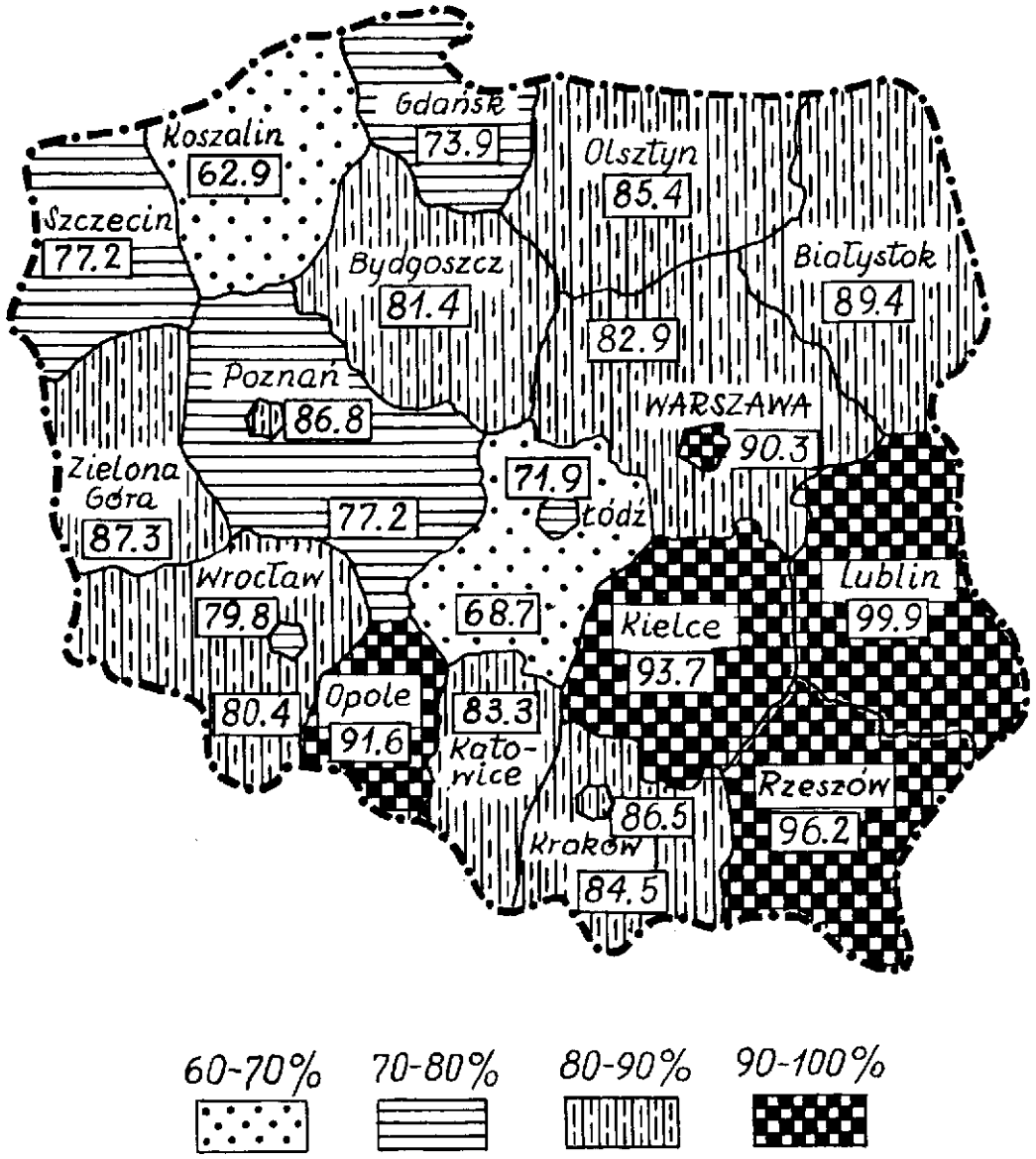
* Collected 10-14 days after administration of the vaccine.

TABLE 9. EXCRETION OF VIRUS IN STOOLS OF CHILDREN FED THE CHAT AND FOX STRAINS IN LUBLIN†

AGE OF CHILDREN IN YEARS	NUMBER OF STOOL SPECIMENS EXAMINED	ISOLATION OF VIRUS FROM STOOL SPECIMENS*			
		BEFORE IMMUNIZATION		AFTER IMMUNIZATION	
		NOT POLIO %	TYPE 1 %	TYPE 1 %	TYPE 3 %
1/2–3	120	2	3	35	34
3–7	76	3	0	19	17
>7	19	0	0	16	6
Total	215	2	2	27	25

* Collected 21-28 days after administration of the vaccines.
† Data collected by Dr. Mirkowski.

MAP 2. THE PERCENTAGE OF CHILDREN VACCINATED IN EACH PROVINCE WITH LIVE ATTENUATED KOPROWSKI'S CHAT STRAIN (TYPE 1).



MAP 2. THE PERCENTAGE OF CHILDREN VACCINATED IN EACH PROVINCE WITH LIVE ATTENUATED KOPROWSKI'S FOX STRAIN (TYPE 3)

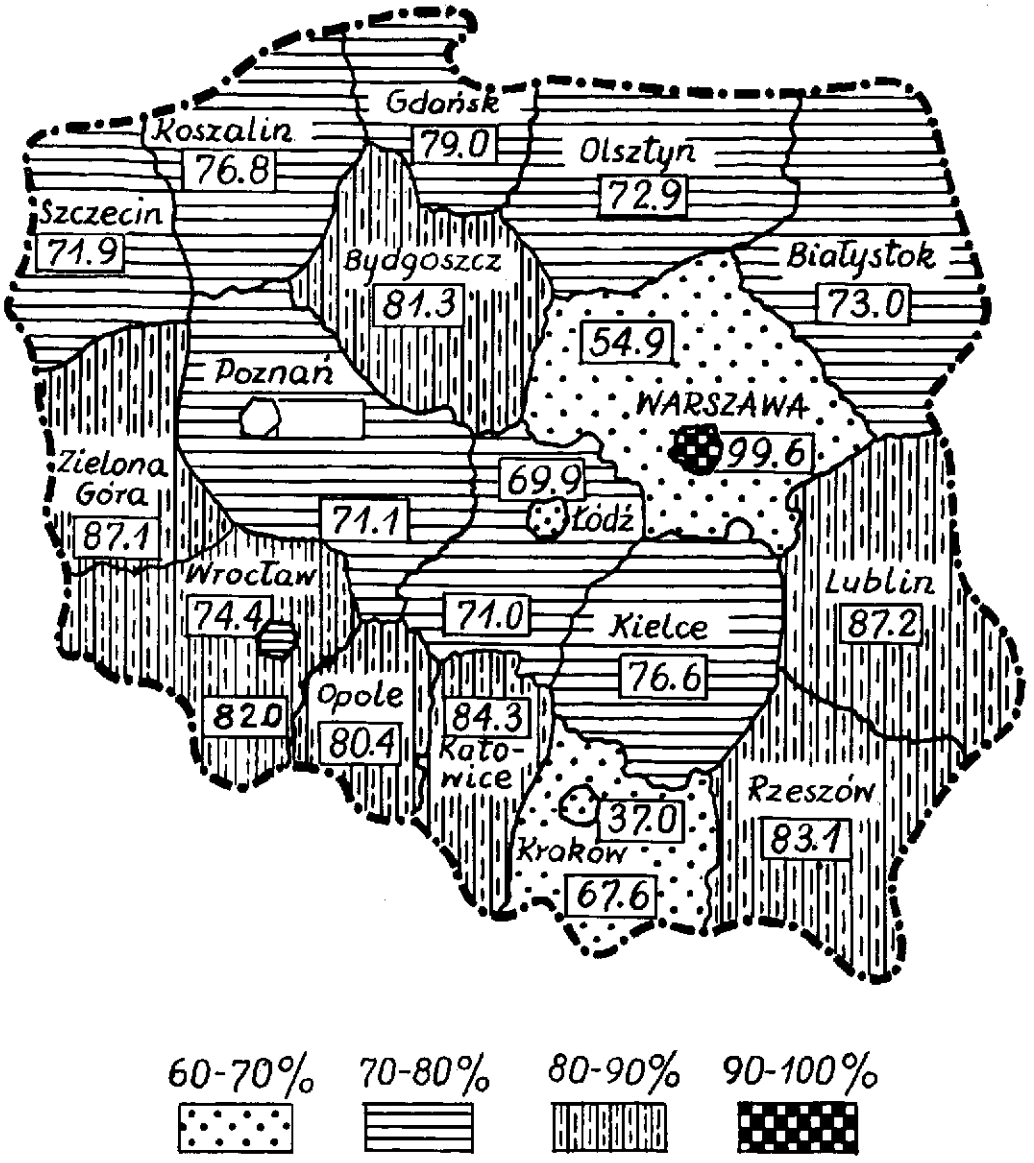


TABLE 10.† PRELIMINARY SEROLOGICAL RESULTS IN CHILDREN FED CHAT AND FOX STRAINS IN LUBLIN*

Type of Virus Fed	Blood Samples Obtained	Sera with no Antibodies Against Type Fed Before Immunization	Ratios of Sera with Antibodies Against Type Fed After Immunization	
			Ratio	%
1	178	36	35/36	97
2	178	23	10/23	44
3	178	39	32/39	82

* Metabolic inhibition test in monkey-kidney tissue culture.
† Data collected by Drs. Mirkowski and I. Buch.

percentage of strains isolated might be explained by the fact that the feces were collected only 21-28 days after vaccination. Serologic investigations are still under way.

The color test on MK-cells was used in our study. Thus far, 176 sera have been examined. They were collected before vaccination and 30 days after the administration of Type 3 virus, i.e., 60 days after the administration of Type 1. The results obtained are presented in Table 10.

The analysis of the immunogenic properties of the vaccine was rendered difficult by the fact that all the children had previously received at least two vaccinations with the Salk vaccine. In the 36 persons examined who had no antibodies against Type 1 before vaccination, these antibodies developed after the procedure.

Of 23 persons who had no antibodies against Type 2, the antibodies developed after vaccination in 10 cases. Of 39 persons who had no antibodies against Type 3, 32 persons developed these antibodies after vaccination. About 50 per cent of persons who had antibodies before vaccination showed an approximately fourfold increase to all three types of poliovirus. The Institute of Naval Medicine at Gdańsk was also engaged in similar investigations.

In order to ascertain the dissemination of enteroviruses in the province of Gdańsk, feces of 1,544 persons aged from one to seven years were examined before the beginning of the vaccination campaign. For results see Table 11.

TABLE 11. EXCRETION OF VIRUS IN STOOLS OF CHILDREN FED THE CHAT AND FOX STRAINS IN GDAŃSK†

TYPE OF VIRUS ISOLATED	PER CENT POSITIVE STOOLS*		
	BEFORE VACCINATION (No. = 1544)	AFTER TYPE 1 (No. = 682)	AFTER TYPE 3 (No. = 160)
1	11	29	10
2	3	2	1
3	2	2	43
Not polio	13	26	27

* Collected on the 5th and 9th days after administration of the respective viruses.
† Data collected by Drs. Georgiades and Morzycka.

The table indicates the isolation from the samples collected before vaccination of: Type 1 virus in 11 per cent; Type 2 virus in 2.8 per cent; and Type 3 virus in 2.2 per cent. A cytopathogenic agent was isolated in 12.8 per cent. Feces of 682 children vaccinated with Type 1 virus were re-examined after an interval of five to 14 days. In this material, poliovirus Type 1 was isolated in 28.6 per cent of the samples; Type 2 virus in 2.3 per cent; and Type 3 in 1.9 per cent. A cytopathogenic agent was isolated from 23.7 per cent of the samples.

Following the administration of the Type 3 strain, samples of feces were collected from the vaccinated children, after an interval of five to 14 days. From the 160 samples examined, Type 1 virus was isolated in 10 per cent; Type 2 in 0.63 per cent; and Type 3 in 43.1 per cent. A cytopathogenic agent was isolated in 27 per cent of the cases.

It should be noted that in the province of Gdańsk the percentage of viruses isolated was lower than in Warsaw. This may be due to a relatively high percentage of persons with a cytopathogenic agent in the feces.

Thus far, serologic investigations (the color test) have been carried out on 254 paired sera collected before vaccination and 30 days after vaccination with Type 3 virus. The results obtained are presented in Table 12. The table indicates that among the persons examined, there were 92 who had no antibodies against Type 1 before vaccination. After administration of the vaccine, antibodies developed in 77 persons, i.e., in 78.2 per cent. So far as Type 3 was concerned, 127 persons had no antibodies before vaccination; of these 86, or 67.7 per cent, developed antibodies after vaccination. Of those who had antibodies before vaccination, a four-fold titer rise occurred in approximately 50 per cent for both types.

The effects of the virologic and serologic investigations, cited above, were obtained after a mass vaccination campaign. It seems safe to assume, therefore, that the results obtained may serve as a proof of the immunogenic properties of the vaccine used. Only when the necessary epidemiologic data are collected and the results of the virologic and serologic investigations are in, will it be possible to evaluate the ultimate effect of this rather large field trial.

TABLE 12.† PRELIMINARY SEROLOGICAL RESULTS IN CHILDREN FED CHAT AND FOX STRAINS IN GDAŃSK*

TYPE OF VIRUS FED	BEFORE IMMUNIZATION		AFTER IMMUNIZATION	
	ANTIBODY ABSENT	ANTIBODY PRESENT	ANTIBODY PRESENT	ANTIBODY RISE
	No.	No.	No.	No.
1	92		→ 72 (78%)	
		176		→ 89 (51%)
3	127		→ 86 (68%)	
		137		→ 76 (55%)

* Metabolic inhibition test on HeLa cells.
† Data collected by Dr. Georgiades.

DISCUSSION

CHAIRMAN STUART-HARRIS: These two papers are now open for discussion. I should like to ask Dr. Skovránek a question. He mentioned in his closing remarks that there were cases in Slovakia, I believe this year, at a higher level of incidence compared with last year.

Would he clarify whether this area was also included in the mass campaign of 1959?

DR. SKOVRÁNEK: The higher morbidity in Slovakia occurred last year (in 1959) and this year, as well. The first field trial with Sabin's vaccine was not carried out in Slovakia last year—all the four orally vaccinated regions in 1959 were in the western part of Czechoslovakia.

DR. LÉPINE: If I have not misunderstood Dr. Skovránek, he has stated that in Czechoslovakia the use of an inactivated vaccine of the Salk type had given a conversion rate of only 50 per cent.

If this is the case, it must have been a bad vaccine. For comparison purposes, I should like to point out that in France, using the French type of Beta-propiolactone inactivated vaccine, the conversion rates obtained in 1959, as reported by Dr. R. Martin and associates, on more than 5,000 serological tests, were 98.6 per cent for all three types, and individually, 99.5 for Type 1, 99 for Type 2, and 99.3 for Type 3. For the same year, the actual protection rate, calculated by Dr. Langmuir's method, for a group of 2,700,000 vaccinees, was 98.4.

Similarly, in Belgium, from the report of Dr. Prenzie, as reported in Jerusalem last year, an inactivated polio vaccine gave a protection rate of 98.2 per cent for a group of 1,450,000 vaccinees.

There are certainly many reasons which militate in favor of live polio vaccines, but I do not think that the failure of inactivated vaccines is one of these reasons, if we stick to the good ones.

DR. PLOTKIN: I merely wish to point out that Table 10 of Dr. Przesmycki's paper* presents, and we have discussed this, a very good example of the possible heterotypic responses which occur after vaccination, especially when using the metabolic inhibition tests.

On a mere conversion rate basis, there was a 44 per cent conversion to Type 2, which was not fed. If all three types had been fed, this 44 per cent would have contributed to the apparent conversion for Type 2 vaccine.

DR. SKOVRÁNEK: I should like to answer Dr. Lépine; I agree perfectly with him that very good inactivated vaccines can give better effects than the ones we have had. However, the inactivated vaccines vary; in many cases we can find good lots and poor lots. This is one of the reasons why we have used live vaccine.

DR. PRZESMYCKI: In my country we vaccinated four and one half million children with Salk vaccine. The conversion rate was not more than about 60 per cent. You will recall Dr. Gard's investigation which shows that several lots of vaccines had low antigenic potency.

We had the same experience. We tested Salk vaccine samples from different countries and we had a very low, at least less than one extinction limit for several vaccines.

DR. PAYNE: I believe that when we discuss this question of the Salk vaccine, quite apart from the potency of the individual lots, we must pay attention to the route by which it was applied.

I understand that in Czechoslovakia primary immunization, including the first booster dose, was given by the intradermal route, which I think most of us will agree is not nearly as effective as the subcutaneous route.

CHAIRMAN STUART-HARRIS: We shall now proceed with Dr. Oker-Blom's paper on "A Small-Scale Trial with Live Poliomyelitis Vaccine in an Isolated Island Community." This will be followed by Dr. Alcocer's paper on "Vaccination and Challenge—Poliomyelitis in Nicaragua." Next on the program will be Dr. Vargas-Méndez who will present Section I of the paper entitled "Vaccination with Attenuated Polioviruses in Costa Rica." Section II. on the "Surveillance Program" will be presented by Dr. Quirce.

* See p. 530.

24. A SMALL-SCALE TRIAL WITH LIVE POLIOMYELITIS VACCINE IN AN ISOLATED ISLAND COMMUNITY

N. OKER-BLOM, HELENA STRANDSTRÖM, AND A. W. ERIKSSON
Department of Virology, University of Helsinki, Finland

Dr. OKER-BLOM (*presenting the paper*): After several days of listening to the results of large-scale trials and mass vaccinations with all the three types of live attenuated poliovirus strains, one wonders whether it is at all worthwhile to present the results of a trial including only 200 persons.

However, some preliminary data concerning the 1958 Sottunga trial were presented here last year, and as the trials have now been extended and finished, it may be justifiable to give the final results of these trials now, although these may probably be merely of academic interest.

MATERIALS AND METHODS

Selection of Study Population

The region chosen for the trial was the fairly isolated Sottunga Island in the middle of the Aland Archipelago (Fig. 1), which lies west of the Finnish mainland and east of Sweden, with the gulf of Botnia to the north and the Baltic sea to the south.

The whole Aland Archipelago has a population of 21,000 persons. Sottunga Island has a registered population of only 253 persons and

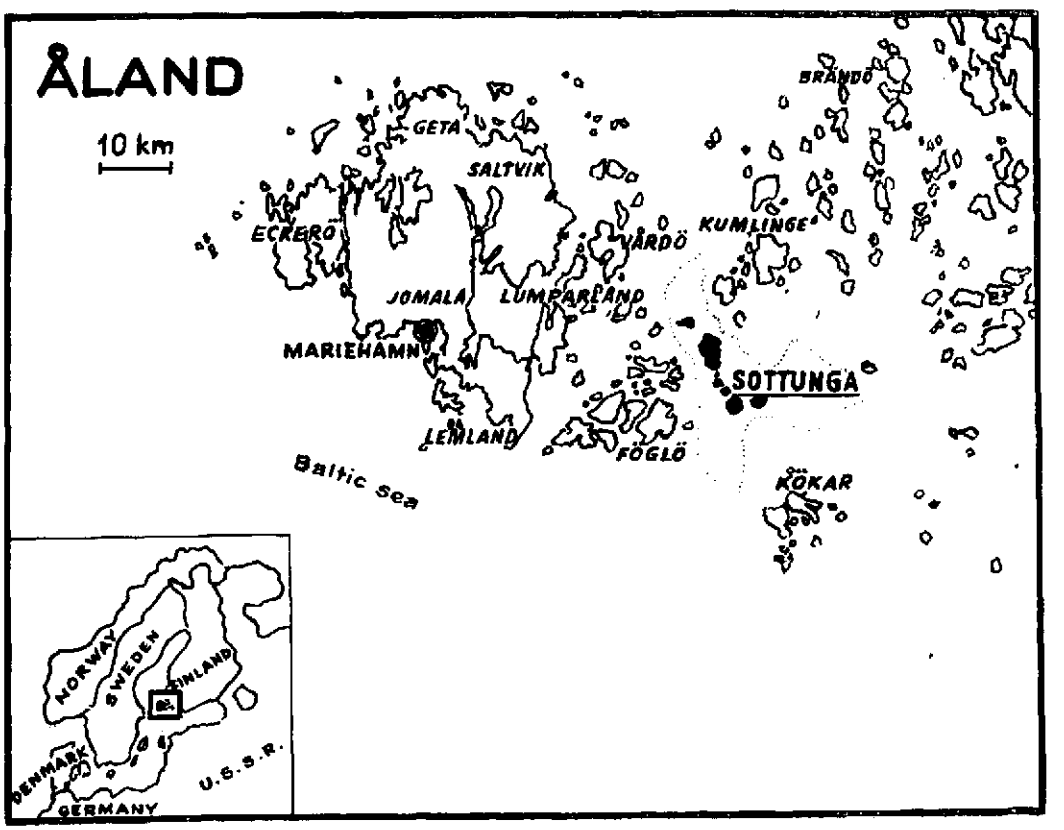


FIG. 1

a still smaller actual population, composed mainly of farmers, fishermen, and sailors, mostly concentrated in a small village in the center of the island (Fig. 2). Modern conveniences are uncommon and most households have outdoor privies.

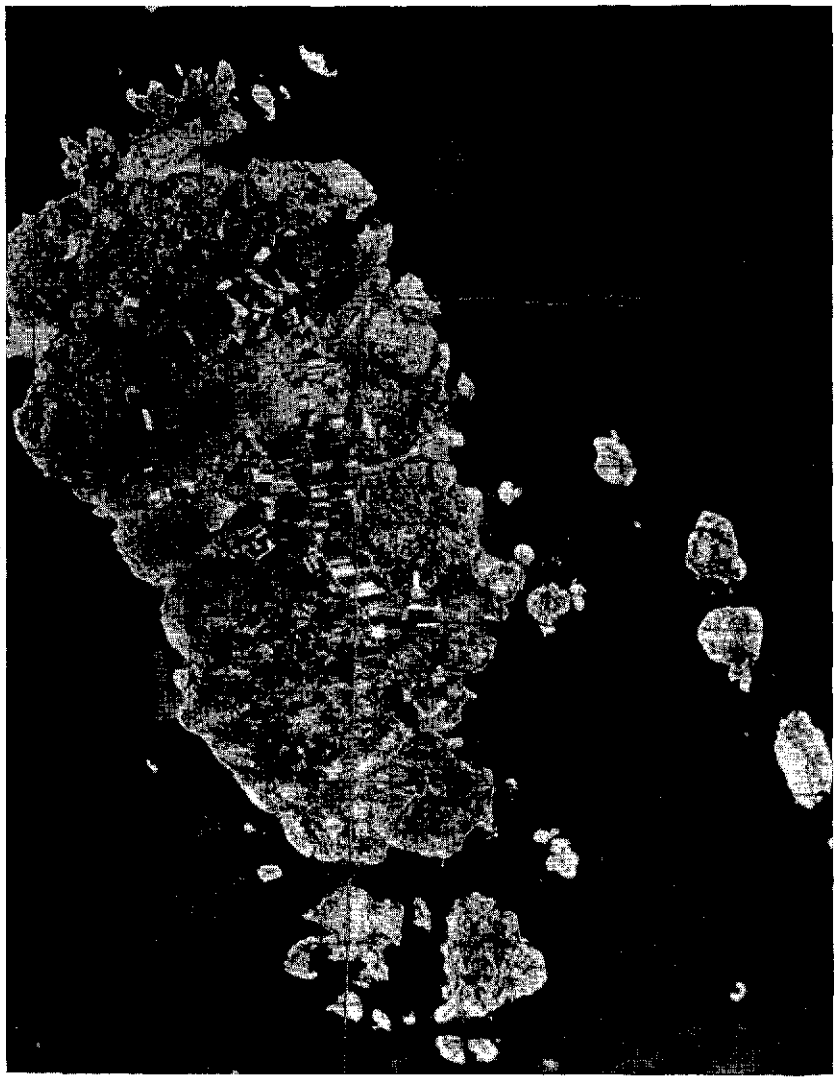


FIG. 2. Aerial photograph of Sottunga Island, Aland Archipelago, Finland.

During the winter, transportation to other islands is very restricted, but during the summer there is a bustling traffic between all the islands in the archipelago.

At the start of the trial the population was told about the purpose of the study and on different occasions was informed about its progress. Cooperation of the authorities on the island, as well as of the whole population, was extremely good.

The Vaccine

The vaccine, developed by the Lederle Laboratories Virus and Rickettsial Research Section, was placed at our disposal by Dr. Herald R. Cox.* For the first part of the trial only Type 1

* We wish to express our thanks to Dr. Cox and to the Lederle Laboratories for help in connection with the accomplishment of the trials.

poliovirus was used and administered in capsules containing 10^5 tissue-culture doses of virus. For the second part of the trial all the three types of poliovirus were used. In this case 1 ml. of a fluid vaccine containing approximately 10^6 tissue-culture doses per ml. of each type was given mixed in about 10 ml. of plain water.

Plan of the Trial

The trial was initiated in January 1958. The immunity status of the population was determined before the start of the trial, and the entire population received two injections of inactivated poliovirus vaccine at three-week intervals. One ml. doses of vaccine were given intramuscularly. Six weeks after the second vaccination, blood and stool samples were collected from the population, and 10 families from different parts of the island comprising 43 persons or about 20 per cent of the population were given live Type 1 poliovirus vaccine. Six and 12 weeks later blood and stool samples were collected from the whole population. In March 1959 blood samples were again collected from the population and the second part of the trial was begun by feeding the whole population all three types of the vaccine as described above at four-week intervals and in the following order: Type 2, Type 1, and Type 3. About six weeks after the last feeding, blood and stool samples were again collected and the trial finished (Table 1).

TABLE 1. TIME SCHEDULE FOR THE SOTTUNGA TRIALS

	Date	Approximate Interval in Weeks	Type of Vaccine	Type of Specimens Collected
1958	January		Inactivated	Blood
	February	3	Inactivated	-
	April	7	Live type 1	Blood, stool
	May	6	-	Blood, stool
	July	6	-	Blood, stool
1959	March		Live type 2	Blood, stool
	April	4	Live type 1	-
	May	4	Live type 3	-
	June	6	-	Blood, stool

Control Measures: Because of its location the island is visited only once a month by a local doctor from the neighboring island, except in emergency cases. Between visits a specially trained nurse is responsible for medical care on the island. During the period in question the entire population was medically examined six times by one of the authors, and the nurse was asked to make careful notes about any signs of infections which might occur in the community. Thus, practically all illnesses were recorded although few of the patients were examined by a doctor except on the occasions mentioned above.

Close contact was kept with the central hospital on the main island. Stool samples from all patients with central nervous system symptoms were tested for the probable presence of poliovirus and in some cases for Coxsackie. Sera were examined for the presence of polio and RSSE antibodies.

Collection of Specimens

It was not possible to obtain specimens from the entire population (the absence of some persons during the days of sampling, transportation difficulties, and similar), and thus at every sampling only about 90 per cent of the specimens were obtained, altogether 1,280 sera and 1,049 stool samples.

Blood specimens were collected in venules and kept at room temperature for one or two days and thereafter for one or two days in the re-

frigerator depending on shipping difficulties to Helsinki. Sera were separated from clots, inactivated at 56° C., and stored at 4° C. until tested.

Stool specimens were collected in about 50 ml. screw cap bottles by the persons themselves and brought by them to the sampling place. They were usually stored at outdoor temperature (in summertime about +20° C. and in wintertime about −30° C.) for one or two days before shipping to Helsinki where they were stored at −20° C.

Testing of Specimens

Virus isolations and identification from stool specimens were done at the Department of Virology, University of Helsinki, and the monkey virulence tests were done by the Virus and Rickettsial Research Section of the Lederle Laboratories.

Of the 1,280 sera collected, 500 sera representing the first four blood specimens from a total of 125 persons, were tested at the Lederle Laboratories for neutralizing antibodies against the three types of polioviruses. The rest of the sera collected in 1958 as well as those collected in 1959 were tested at the Department of Virology, University of Helsinki.

Virus isolations and identification technic. Ten per cent stool suspensions were prepared in bovine amniotic fluid containing 500 units of penicillin, 500 μg. of streptomycin and 100 IU of mycostatin per ml. The stool suspensions were shaken in centrifuge tubes containing glass beads and were then centrifuged in a PR 1 angle centrifuge at 2,500 rpm. at 5° C. for 20 minutes. The supernatant fluid was centrifuged once more in a Spinco Model L preparative centrifuge at 18,000 rpm. for 15 minutes. The supernatant from the second centrifugation was used for inoculation of three HeLa cell-culture tubes, each tube receiving 0.5 ml.

The tubes were inspected microscopically every third day for cytopathic changes. As soon as such changes occurred second passages were made by inoculating 0.2 ml. of tissue-culture fluid into each of three HeLa cell tubes. Agents from the second passage were typed with specific polio immune sera. A pool of the three type sera was included to each test. The sera used were hyperimmune rabbit or guinea pig sera.

Serum neutralization tests. Neutralization tests at the Department of Virology, University of Helsinki, were done as tube tests based on inhibition of the cytopathic effect, using about 100 TCD_{50} of virus and fourfold serum dilutions beginning with a dilution of 1:4. Corresponding tests at the Lederle Laboratories were done by using the color tests.* Serum titers are expressed as the inverse value of the last neutralizing serum dilution.

Virulence tests in monkeys. Ten percent stool suspensions were made in distilled water and then shaken in the cold for about 12 hours. Centrifugation at 5,000 rpm. for one hour followed. To the supernate 10,000 units of penicillin and 10 mg. of streptomycin per ml. were added. Further centrifugation for two hours at 12,000-14,000 rpm. was then carried out and the pH of the supernate adjusted to seven.

Each stool preparation was inoculated into groups of two to three Cynomolgus monkeys, each animal receiving 2 × 0.5 ml. injections intrathalamically. The animals were observed for 21 days for clinical signs of the disease, at which time they were sacrificed for histologic examination of brain and spinal cord specimens.

RESULTS

Immunity status of the population before the trial. Result of determinations of neutralizing antibodies of the Sottunga population against all three types of polioviruses prior to the trial (Table 2), shows that most of the children under 10 years of age were triple negative as estimated by the method used. Also some of the adults were triple negative and the poliovirus antibody pattern corresponded to that in the whole archipelago before the great 1953 polio epidemic in this region, and is thus one of low infection rate. Accordingly, Sottunga island must have escaped the infection in 1953 and apparently, there has been no polio on the island during the last 10 years.

Presence of poliovirus in the community at the start of the trial. Since HeLa cells were used for the isolations, probably only polioviruses, Coxsackie B viruses, and a few Coxsackie

* Cox, H. R., Cabasso, V. J., Markham, F. S., Moses, M. J., Moyer, A. W., Roca-García, M., and Ruegsegger, J. M. "Immunological Response to Trivalent Oral Poliomyelitis Vaccine." *Brit. M. J.*, **2**:591-597, 3 October 1959.

TABLE 2. INCIDENCE OF NATURALLY OCCURRING NEUTRALIZING ANTIBODIES AGAINST THE THREE TYPES OF POLIOVIRUS, SOTTUNGA ISLAND, FINLAND

AGE GROUPS	TOTAL NO. OF PERSONS TESTED	NUMBER WITH NEUTRALIZING ANTIBODIES AGAINST:						NUMBER OF TRIPLE NEGATIVE	
		TYPE 1		TYPE 2		TYPE 3			
		NUMBER	PER CENT	NUMBER	PER CENT	NUMBER	PER CENT	NUMBER	PER CENT
0— 1	3	1		—		1		2	
1— 2	3	—		—		—		3	
2— 3	3	—	6	—	6	—	6	3	90
3— 4	5	—		1		—		4	
4— 5	5	—		—		—		5	
5—10	12	1		1		1		11	
10—15	27	12	44	6	21	13	48	8	30
15—20	15	12	80	4	27	11	73	1	7
20—30	10	6	60	3	30	5	50	1	10
30—40	31	19	61	22	71	17	55	1	3
40—50	29	26	90	20	72	24	83	—	—
50—30	15	14	93	10	71	15	100	—	—
60 & over	35	24	69	29	83	22	63	2	6
Total	193	115	60%	96	45%	109	56%	41	21%

A viruses, as well as some ECHO virus types, could possibly be traced. From stool samples collected in April 1958, on the day of feeding live Type 1 virus to about 20 per cent of the population, no cytopathic agents could be isolated. Thus, it may be stated that there probably were no polioviruses circulating in the community at the start of the trial (Table 3), which corresponds with results of the antibody pattern study mentioned above. No conclusions regarding probable occurrence of other enteroviruses can, however, be made, except that it seems improbable that any Coxsackie B viruses or adenoviruses should have occurred.

Effect of the Salk vaccination. The two injections of Salk vaccine resulted in a conversion to seropositive in 26 per cent of those negative to Type 1, in 54 per cent of those negative to Type 2, and in 24 per cent of those negative to Type 3. After the Salk vaccination 21 persons remained triple negative as estimated by the method used.

Effect of orally administered live Type 1 virus in 1958. The effect of a single dose of Type 1 vaccine was tested on 43 persons from 11 families. All members of the families received the vaccine. In this group nine children under 10 years of age were triple negative in spite of vaccination with inactivated vaccine. Of persons over 10, one was triple negative and one negative to Type 1 only.

Six weeks after the feeding, five of these persons excreted virus. Four of the five persons were negative to Type 1 before feeding (Table 4). Another six or twelve weeks after feeding no virus could be isolated.

A fourfold or greater increase in Type 1 antibodies was seen in all 11 children under 10 years of age and in eight of 28 persons over 10 from whom appropriate blood samples were obtained (Table 5). If antibody increase is regarded as proof of infection, a take was observed in 19 of 39 persons tested or about 50 per cent. Ten of these 19 were triple negative before feeding.

During the period July 1958 to March 1959 an antibody increase was seen in some additional persons fed virus which, assuming that no wild virus was present in the community, may possibly be interpreted as a re-infection with attenuated virus circulating in the community.

TABLE 3. VIRUS ISOLATIONS FROM SUBJECTS ON SOTTUNGA ISLAND AT DIFFERENT STAGES OF THE TRIALS IN 1958 AND 1959

	1958			1959	
	APRIL	MAY	JULY	MARCH	JUNE
NUMBER OF SPECIMENS TESTED	201	186	184	191	196
Type 1 virus isolations	—	11*	1†	—	—
Type 2 virus isolations	—	—	—	—	—
Type 3 virus isolations	—	—	—	—	13‡

* Five persons fed virus, six contacts.
† One contact.
‡ Twelve persons fed virus, one contact.

Spread of attenuated Type 1 virus in the community. Six weeks after feeding live virus to the above mentioned 43 persons, six of 149 other persons tested on the island excreted Type 1 poliovirus. One of these persons still excreted virus 12 weeks after the introduction of the live virus into the community. From two of these six persons serologic data are lacking. Two of the remaining four were negative to Type 1 (Table 4).

A fourfold or greater increase in Type 1 antibodies during the period April 1958 to March 1959, was seen in 66 of 158 persons tested, or 42 per cent. These 66 persons repre-

TABLE 4. AGE AND IMMUNITY STATUS OF SUBJECTS EXCRETING POLIOVIRUS TYPE 1, SOTTUNGA TRIAL 1958

	NUMBER OF FAMILY MEMBERS NON-IMMUNE/ TOTAL	AGE OF VIRUS EXCRETORS	ANTIBODY TITER OF VIRUS EXCRETORS					
			BEFORE FEEDING			6 WEEKS AFTER FEEDING		
			TYPE 1	TYPE 2	TYPE 3	TYPE 1	TYPE 2	TYPE 3
Fed Live Type 1 Virus Vaccine	3/6	15	<8	16	256	256	256	1024
		2	<8	<8	<8	1024	16	16
		13	<8	<8	1024	256	64	1024
	3/4	7	64	64	<8	256	64	<8
	2/7	1	<8	<8	<8	128	<8	<8
Not Fed Live Type 1 Virus Vaccine	2/5	5	<8	<8	<8	256	64	<8
	1/4	12	<8	64	<8	16	64	<8
	?/3	2	No serum obtained			No serum obtained		
	0/2	64	1024	256	256	256	64	256
		33	32	32	8	32	32	32
	1/3	2	No serum obtained			No serum obtained		

TABLE 5. TYPE 1 ANTIBODY RESPONSE IN SUBJECTS FED LIVE TYPE 1 POLIOVIRUS VACCINE (LEDERLE) IN 1958. SIX OR TWELVE WEEKS AFTER FEEDING

Age Group	Prefeeding Immunity Status	Number in Group	Positive Response Fourfold or Greater	
			Number	Per Cent
< 1 - 10	< 8	9	9	100
	⩾ 8	2	2	100
	Total	11	11	100
10 -	< 8	2	2	100
	⩾ 8	26	6	23
	Total	28	8	28

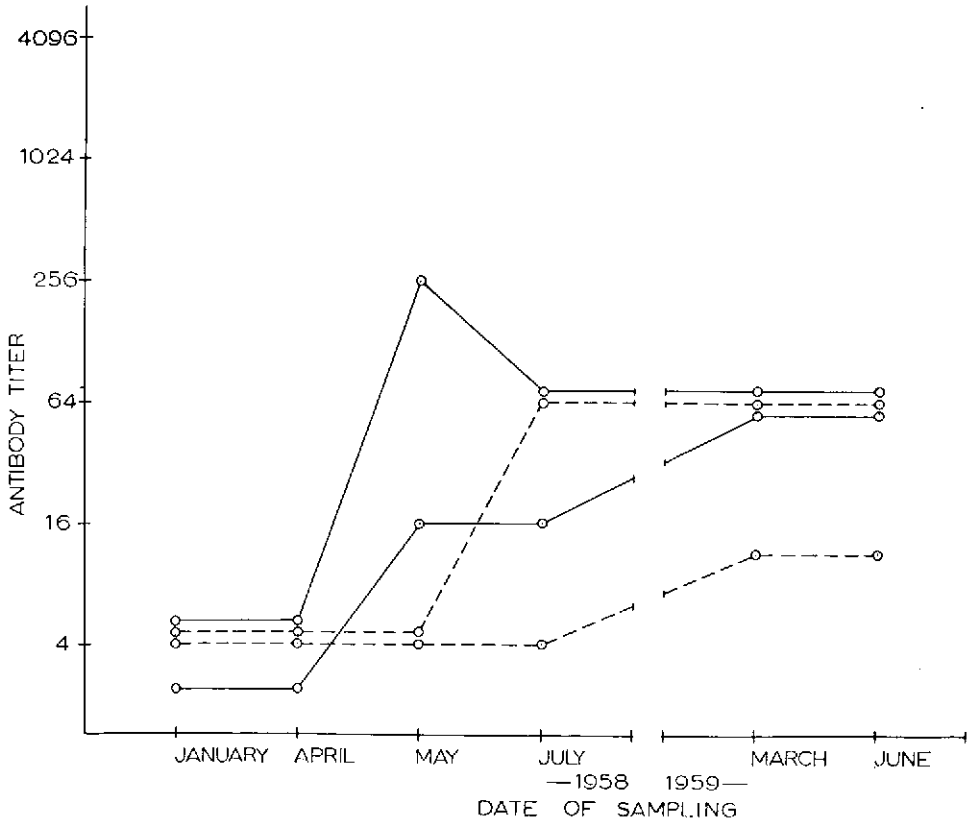


FIG. 3. Type 1 poliovirus antibody pattern of members of a family not fed live Type 1 poliovirus in 1958.

sented 30 households. In some cases an antibody increase during the period April-May was seen in one household member only, whereas other members of the household showed a corresponding antibody increase during the period May-July or July 1958-March 1959 (Fig. 3). This indicates that the extra-familial spread in fact was lower than shown by these figures and that part of the spread, as expected, can be accounted for by subsequent intra-familial spread of the virus.

Changes in Type 2 and Type 3 seroimmunity during 1958 trial. In persons fed live virus and showing an antibody increase to Type 1, or 19 in all, simultaneous increase of Type 2 antibodies was seen in 53 per cent and of Type 3 antibodies in 30 per cent. Of 23 persons fed and showing no antibody increase against Type 1, an increase of Type 2 and Type 3 antibodies was observed in four and five persons respectively, or in 17 and 20 per cent (Table 7).

TABLE 6. NEUROVIRULENCE IN MONKEYS OF TYPE 1 POLIOVIRUS STRAINS ISOLATED FROM PERSONS FED AND NOT FED ATTENUATED LIVE TYPE 1 POLIOVIRUS IN 1958*

GROUP	FAMILY, NAME	STOOL TITER (TCD_{50}/ML.)	PARALYTIC(†) RATIO	HISTOPATHOL. RATIO
Persons Fed Type 1 Virus	1. P.H.	$10^{4.5}$	0/2	0/2
	K.H.	$10^{3.3}$	2?/2	2/3
	E.H.	$10^{2.8}$	0/3	0/3
	2. T.L.	$10^{5.7}$	1/1	1/1
	3. A.M.H.	$10^{3.3}$	0/2	0/2
Persons Not Fed Type 1 Virus	1. H.S.	$<10^{-1.0}$(‡)	NT**	NT
	I.S.	10^{5}	2?/2	2/2
	2. K.G.E	$<10^{-1.0}$(‡)	NT	NT
	3. H.J.	$10^{2.2}$	1?/2	0/2
	H.J.(§)	$10^{3.3}$	0/3	0/3

* Obtained through the courtesy of Dr. V. J. Cabasso, Lederle Laboratories.
† Each monkey received 2 × 0.5 ml. of 10 per cent stool suspension.
‡ Virus recovered only on second tissue-culture passage. Not tested in monkeys.
** NT = Not tested.
§ Second isolation from the same person six weeks later.

Monkey neurovirulence of isolated Type 1 polio strains. Nine of 12 Type 1 strains isolated from the persons fed attenuated virus and from contacts were tested for intracerebral neurovirulence in monkeys.* Of the 20 inoculated monkeys, one developed paralysis and six some weakness. In two of the latter animals the histopathology did not suggest polio (Table 6). The findings seem to be in accordance with previous experience with this Type 1 strain and do not suggest any difference in neurovirulence between strains isolated from persons fed virus or from contacts.

In the sera of 66 persons who were not fed live virus but who showed an increase in Type 1 antibodies, and who thus apparently were infected, an increase in Type 2 antibodies was seen in 28, or 42 per cent. An increase in Type 3 antibodies was seen in 23 of these persons, or 34 per cent. The corresponding numbers for the remaining 92 persons who did not respond with a Type 2 antibody increase were six persons, seven per cent, for Type 2 and 12 persons, or 13 per cent, for Type 3.

These changes in Types 2 and 3 antibodies are difficult to interpret. Assuming, however, on the basis of the increase in Type 1 antibodies, that Type 1 virus circulated in the community

* Our thanks to Dr. V. J. Cabasso of the Lederle Laboratories who performed the tests in monkeys.

TABLE 7. OCCURRENCE OF A FOUR-FOLD OR GREATER INCREASE IN TYPE 2 AND TYPE 3 ANTIBODIES IN PERSONS WITH AND WITHOUT A CORRESPONDING INCREASE IN TYPE 1 ANTIBODIES

Groups		Number in Group	Antibody Response for			
			Type 2		Type 3	
			Number	Per cent	Number	Per cent
Group with Type 1 Antibody Response	Fed Type 1	19	10	53	6	30
	Not Fed Type 1	66	28	42	23	34
	Total	85	38	45	29	34
Group without Type 1 Antibody Response	Fed Type 1	23	4	17	5	22
	Not Fed Type 1	92	6	7	12	13
	Total	115	10	9	17	12

and that no Type 2 or Type 3 viruses could be isolated, the Type 2 and 3 antibody increases may be regarded as heterotypic antibody responses.

Effect of oral administration of all three types of live poliovirus in 1959. When the second part of the trial was initiated in March 1959, blood samples were obtained from 201 persons. Among 41 persons from whom blood samples were obtained and who had been fed live Type 1 vaccine in 1958 there were no triple negatives and no persons negative to Type 1, but nine of these 41 were still negative to Type 3 only, and three to Types 2 and 3. Among the remaining 160 who had not received live Type 1 vaccine, three were triple negative, and 34 were negative to one or two types.

Six weeks after the last feeding of Type 3, 13 persons excreted Type 3 virus. Only four of these 13 were negative to Type 3, and only one was triple negative before feeding. However, only one of the remaining nine had a titer exceeding 1:4. No type 1 or Type 2 virus could be isolated (Table 8). The isolated strains have not been tested for monkey neurovirulence.

Of those who received the vaccine and from whom appropriate serum samples were obtained, a conversion from seronegative to seropositive was seen in all but two of those previously vaccinated with live Type 1 vaccine and in all except one of those not previously vaccinated with live Type 1 vaccine (Table 9). The numbers are extremely small, but on the basis of them, the conversion rates were 93, 93, and 96 per cent for Types 1, 2, and 3 respectively.

Combined effect of the various vaccination schedules. At the end of the trials there were 109 persons from whom all six blood specimens and all five stool samples had been obtained and who had participated in the whole vaccination program. Twenty-nine of these persons had received live Type 1 vaccine in 1958 and 80 had not. All of them had received two injections of inactivated vaccine in 1958 and all three types of live vaccine in 1959.

This selected group, including 21 children under 10 years of age and 88 persons over 10, gives a fairly good picture of the changes in the immunity status of the population of Sottunga (Figs. 4-8). Thus, after the last feeding there were among the children under 10, most of whom were initially triple negative, only one negative to Type 2 and one negative to Type 3. The over-all increase in measurable antibodies is shown by the change in geometric mean titer.

A somewhat similar picture is obtained for the 88 persons over 10 except that among those not fed virus there seems to be an increase in Type 3 antibodies which is difficult to explain. If, however, as previously mentioned, it is assumed that no Type 3 virus was present, the increase may be explained as a heterotypic response to circulating attenuated Type 1 virus.

The combined effect of the various vaccination schedules may also be studied by estimating the total number of negatives to any of the three types during the trial (Table 10).

Thus, of the 196 persons tested at the last sampling in June 1959, only three persons remained negative to Type 1 only, three persons

TABLE 8. AGE AND IMMUNITY STATUS OF SUBJECTS EXCRETING POLIOVIRUS TYPE 3, SOTTUNGA TRIAL 1959

	NUMBER OF FAMILY MEMBERS NON-IMMUNE/ TOTAL	AGE OF VIRUS EXCRETORS	ANTIBODY TITER OF VIRUS EXCRETORS					
			BEFORE FEEDING			AFTER FEEDING		
			TYPE 1	TYPE 2	TYPE 3	TYPE 1	TYPE 2	TYPE 3
Fed Live Type 3 Virus Vaccine	1/8	6	<8	<8	8	8	32	32
		2	<8	32	8	128	512	2048
		5	8	16	8	8	512	32
	0/7	13	64	512	8	128	2048	8
	1/7	1	<8	<8	<8	128	128	128
	0/5	5	<8	32	8	32	512	512
	0/6	1	8	<8	8	128	128	512
	1/4	8	64	32	8	128	256	32
		6	64	256	<8	512	1024	8
	0/6	50	256	256	1024	512	64	2048
	0/4	28	1024	32	8	1024	32	32
	1/7	4	128	8	<8	512	2048	512
Not Fed Live Type 3 Virus Vaccine	1/5	15	128	8	<8	512	8	<8

negative to Type 2 only, four persons negative to Type 3 only and one person negative to Types 2 and 3. Eight of these 11 persons had not received the live vaccine.

CONCLUSIONS

The vaccination of a small isolated, previously Salk-vaccinated, and partly naturally immune population group with all three types of Lederle live attenuated poliovirus vaccine resulted in a high degree of serologic immunity in the population group in question.

The spread of the attenuated virus was studied for Type 1 only and results show that about 40 per cent of persons not receiving the live virus may have been infected under these conditions. Some of the persons infected were intrafamilial contacts.

No harmful effect of any of the three strains of live poliovirus, of which Type 1 was given on two occasions, could be observed clinically in persons on Sottunga receiving the vaccine or in those apparently infected with the virus excreted by these persons.

Some of the results suggest that the attenuated virus may circulate in the community for some time and studies to elucidate this question as well as the question of the duration of the induced immunity are in progress.

TABLE 9. PRE- AND POST-VACCINATION STATUS OF 40 ANTIBODY NEGATIVES AMONG 201 PERSONS FED THE THREE TYPES OF ORAL POLIOMYELITIS VACCINE WITH 4-WEEK INTERVALS (TYPE 2, TYPE 1, TYPE 3) IN 1959

Pre-Vaccination Antibody Negatives		Post-Vaccination Status			
Negative to Type	No. of Subjects	Negative to Type			Converted
		1	2	3	
1, 2, 3	2	-	-	-	2
1, 2	2	-	-	-	2
1, 3	2	-	-	-	2
2, 3	7	-	1	1	6
1	8	1	-	-	7
2	3	-	-	-	3
3	16	-	-	1	15
Totals	40	1	1	2	37

TABLE 10. FREQUENCY OF NON-IMMUNE SUBJECTS ON SOTTUNGA ISLAND AT VARIOUS STAGES DURING THE TRIALS IN 1958 AND 1959

Date of Sampling	Polio Vaccine Used	Number Tested	Per Cent Non-Immune		
			Type 1	Type 2	Type 3
January -58	Inactivated	196	39	53	46
April -58		195	25	19	31
	Live Type 1				
May -58		199	15	11	28
July -58		176	14	10	30
March -59		201	11	6	17
	Live Type 2,1,3	196	1.5	2.0	2.5
June -59		180[x]	0.5	0.5	1.0

[x] Corrected figures for persons who received all the three types in 1959.

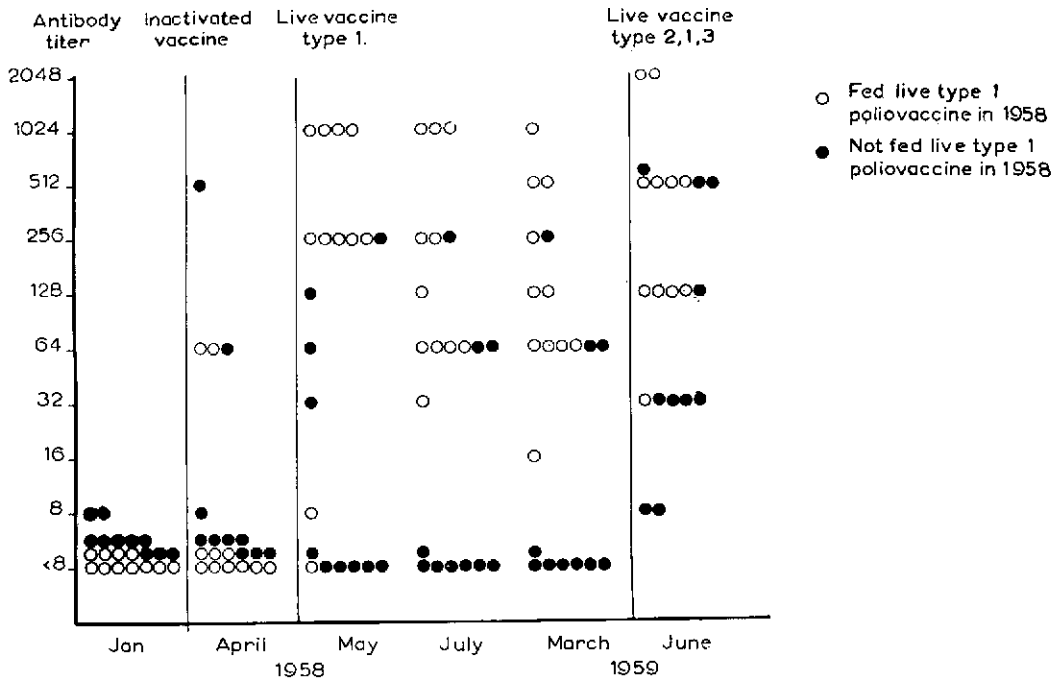


FIG. 4. Type 1, antibody response in children age <10, fed live Type 2, Type 1, and Type 3 poliovirus vaccine in 1959.

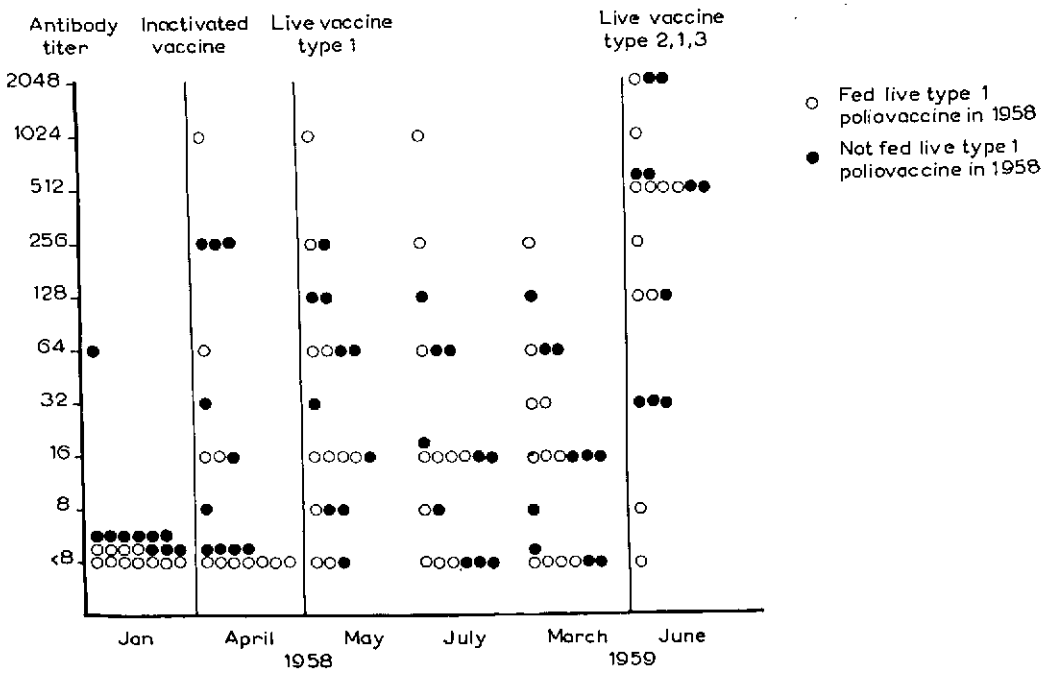


FIG. 5. Type 2, antibody response in children age <10, fed live Type 2, Type 1 and Type 3 poliovirus vaccine in 1959.

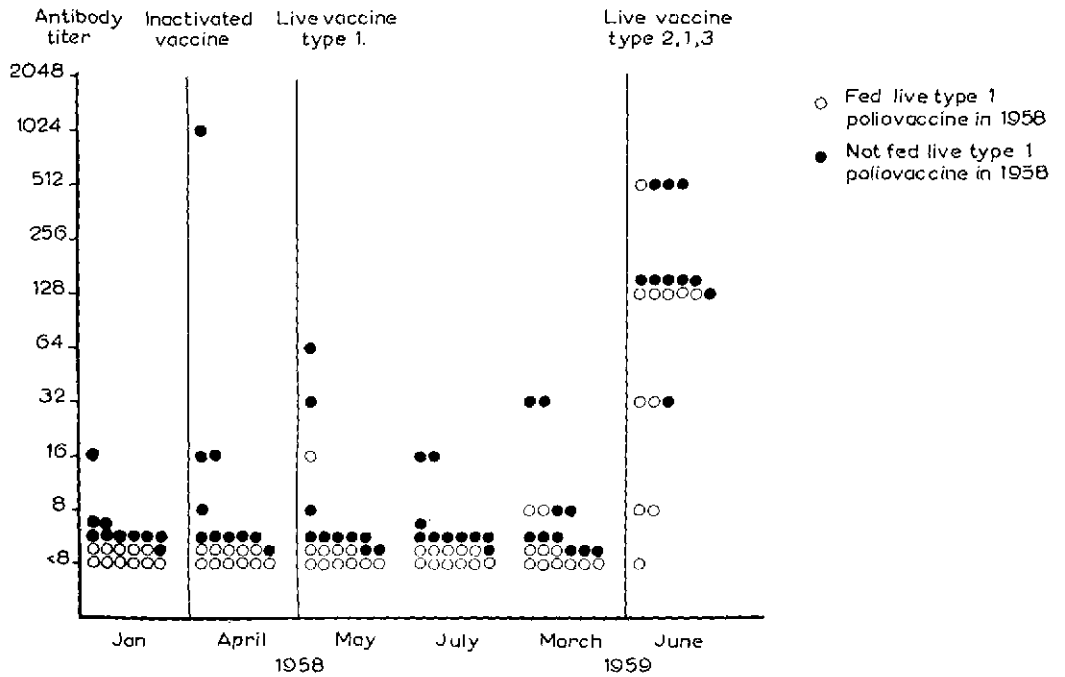


FIG. 6. Type 3, antibody response in children age <10, fed live Type 2, Type 1 and Type 3 poliovirus vaccine in 1959.

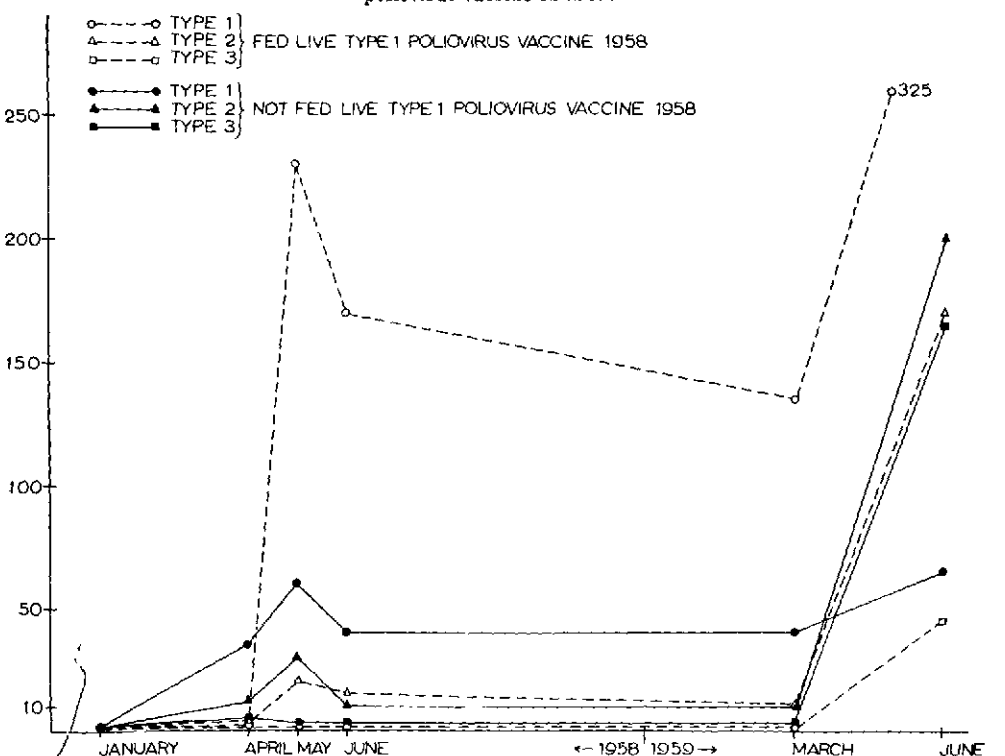


FIG. 7. Geometric mean titers of children age <10 fed live Type 2, Type 1 and Type 3 live poliovirus vaccine in 1959.

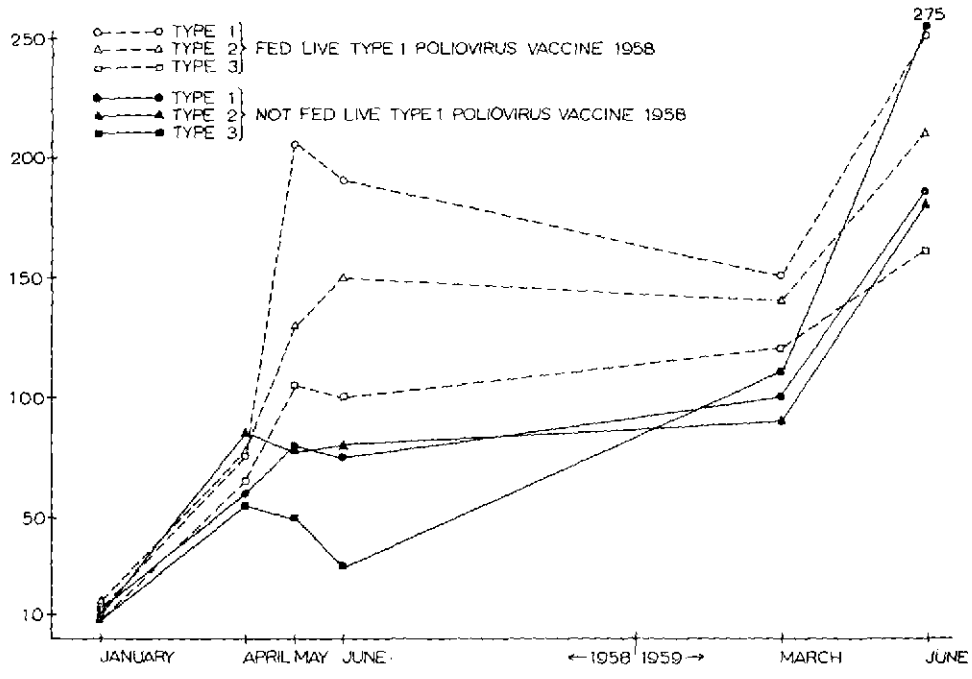


FIG. 8. Geometric mean titers of 88 persons age >10 fed live Type 2, Type 1 and Type 3 poliovirus vaccine in 1959.

25. VACCINATION AND CHALLENGE—POLIOMYELITIS IN NICARAGUA, 1959-1960

JUAN JOSÉ ALCOCER, M.D., ROBERTO ARMIJO, M.D., AND MAURICIO MARTINS DA SILVA, M.D.*

Dr. ALCOCER (*presenting the paper*): In a report presented at the First International Conference on Live Poliovirus Vaccines in Washington, D. C., 22-26 June 1959,[1] we gave an account of the history of poliomyelitis in Nicaragua, and described in detail the oral vaccination program in the City of Managua, carried out during an epidemic of poliomyelitis in 1958. The epidemic started in Managua in May and extended to 14 of the 16 departments of the country. It lasted five months, during which time 237 cases were reported, 98 of which were from Managua. The attack rate in Nicaragua in 1958 was 18.4 per 100,000 population with a case fatality rate of 7.1 per cent. It was established that the epidemic was due to poliovirus Type 2.

The vaccination program started in September 1958 and included children between two months and 10 years of age in the City and the Department of Managua. Mass vaccination was followed by a maintenance program, which was intended to immunize newborn infants in maternity hospitals and health centers in the City of Managua with a liquid trivalent vaccine. Up to 15 May 1959, a total of 59,855 children had received the Type 2 virus, 54,732 the Type 3 virus, and 49,585 all three types. On the basis of information available at the time of the vaccination campaign it was estimated that 98, 91, and 82 per cent of the eligible population under 10 years of age, in Managua, had received vaccine for Types 2, 3, and 1 respectively. However, using the revised population and age distribution data for the Department of Managua in 1959,[2] the actual coverage was 67, 61, and 56 per cent for Types 2, 3, and 1, respectively. The serologic studies showed that of those children who were seronegative at the time of vaccination, 74 per cent responded to Type 1, 58 per cent to Type 2, and 80 per cent to Type 3 polioviruses. No illness attributable to the vaccine was observed and it was gratifying that during the eight months following the vaccination program, no cases of paralytic poliomyelitis were reported in Managua.

POLIOMYELITIS OUTBREAK IN 1960

The maintenance program continued throughout 1959 and 1960 using the trivalent vaccine which contained approximately $10^{6.1}$ TCD_{50} of each of the three types of poliovirus in a 2 ml. dose. By 15 April 1960 a total of 73,533 children under 10 years of age, including 5,344 newborns, had received the oral vaccine (Table 1) on page 48. In mid-1959, in anticaption of the seasonal epidemic exacerbation of the disease, which has been experienced in Managua since 1938, the health authorities of Nicaragua expanded their surveillance activities, in the course of which three unreported paralytic cases of poliomyelitis in 1959 were uncovered in Managua; one had onset in January, one in May, and one in June (Fig. 1) on page 47. This finding requires correction of the previous statement that no cases occurred in Managua between 15 October 1958 and 15 June 1959. From 23 August to 10 October 1959, seven additional cases were notified in the City of Managua. After a quiet period of 10 weeks a new upsurge in notified cases prompted the health authorities to request the cooperation of the Pan American Sanitary Bureau for diagnostic assistance and additional supplies of vaccine. Reports of cases continued to increase in Managua and epidemic outbreaks appeared successively in the departments of Granada, Masaya, Chinandega, León, and Rivas,

* Dr. Alcocer and Dr. Martins da Silva (Pan American Health Organization/World Health Organization); and Dr. Armijo (Ministry of Public Health, Managua, Nicaragua).

TABLE 1. AGE DISTRIBUTION OF CHILDREN FED VACCINE CONTAINING THREE TYPES OF LIVE POLIOVIRUS, SEPARATELY OR COMBINED, IN NICARAGUA AND IN THE DEPARTMENT OF MANAGUA, 1958-1960

AGE IN YEARS	POPULATION RECEIVING VACCINE					
	TOTAL		FIRST STAGE: 7 SEPTEMBER 1958—15 MAY 1959		SECOND STAGE: 16 MAY 1959—15 APRIL 1960	
	NICARAGUA	MANAGUA	NICARAGUA	MANAGUA	NICARAGUA	MANAGUA
Total	73,533	58,884	56,160	49,585	17,373	9,299
<1	10,514	8,713*	4,999	4,414†	5,515	4,299‡
1	7,383	5,910	5,729	5,058	1,654	852
2	7,691	6,100	6,177	5,454	1,514	646
3	8,392	6,486	6,570	5,801	1,822	685
4	8,165	6,216	6,346	5,603	1,819	613
5-9	31,388	25,459	26,339	23,255	5,049	2,204

* Including 5,344 newborns.
† Including 2,418 newborns.
‡ Including 2,926 newborns.

with sporadic cases in Matagalpa, Carazo, Estelí, Chontales, Boaco, and Zelaya (Figures 1 and 2).

In Managua the epidemic lasted 24 weeks and reached its peak during the 19th week.

During the epidemic period from the week beginning 22 November 1959, to 14 May 1960, 92 cases were reported in Managua and 114 in the rest of the country (Table 2 and Figures

TABLE 2. REPORTED CASES OF PARALYTIC POLIOMYELITIS AND DEATHS ASCRIBED TO POLIOMYELITIS IN NICARAGUA 1959-1960

PERIOD	TOTAL CASES	DEPARTMENT OF MANAGUA	OTHER DEPARTMENTS	DEATHS
Total	225	103	122	4*
January-November—1959 (up to November 21)	18	10	8	—
Epidemic Period				
November 22-30, 1959	1	1	—	—
December 1959	4	4	—	—
January 1960	20	12	8	—
February	37	22	15	2
March	71	31	40	—
April	64	21	43	2
May 1–14	10	2	8	—

* Case fatality rate: 1.8 per cent.

FIGURE 1

NOTIFIED CASES OF PARALYTIC POLIOMYELITIS IN NICARAGUA, BY DEPARTMENTS AND BY WEEK
(4 JANUARY, 1959 - 14 MAY, 1960)

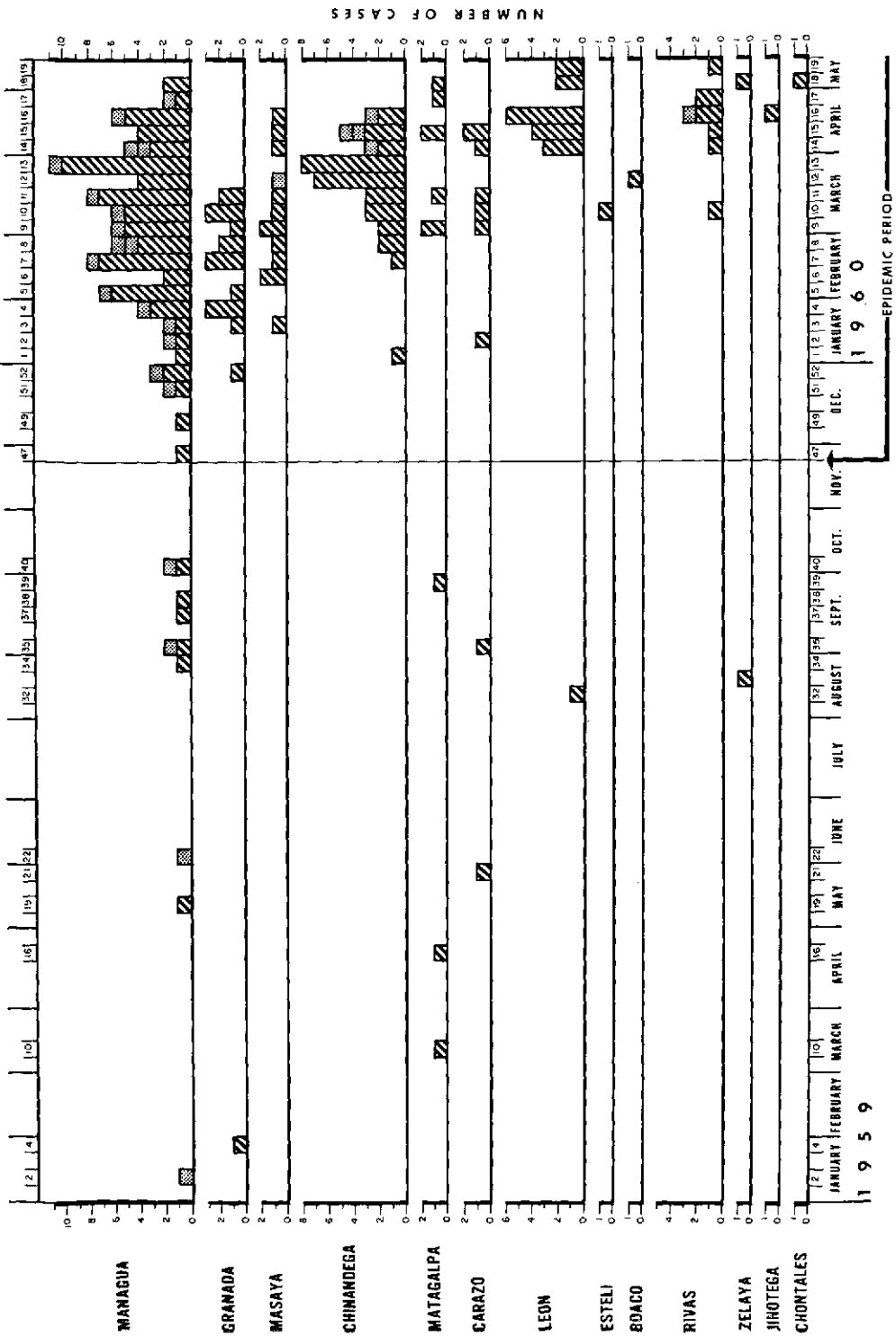


FIGURE 2

DISTRIBUTION OF NOTIFIED CASES OF PARALYTIC POLIOMYELITIS IN NICARAGUA, BY DEPARTMENTS (22 NOVEMBER, 1959 - 14 MAY, 1960)

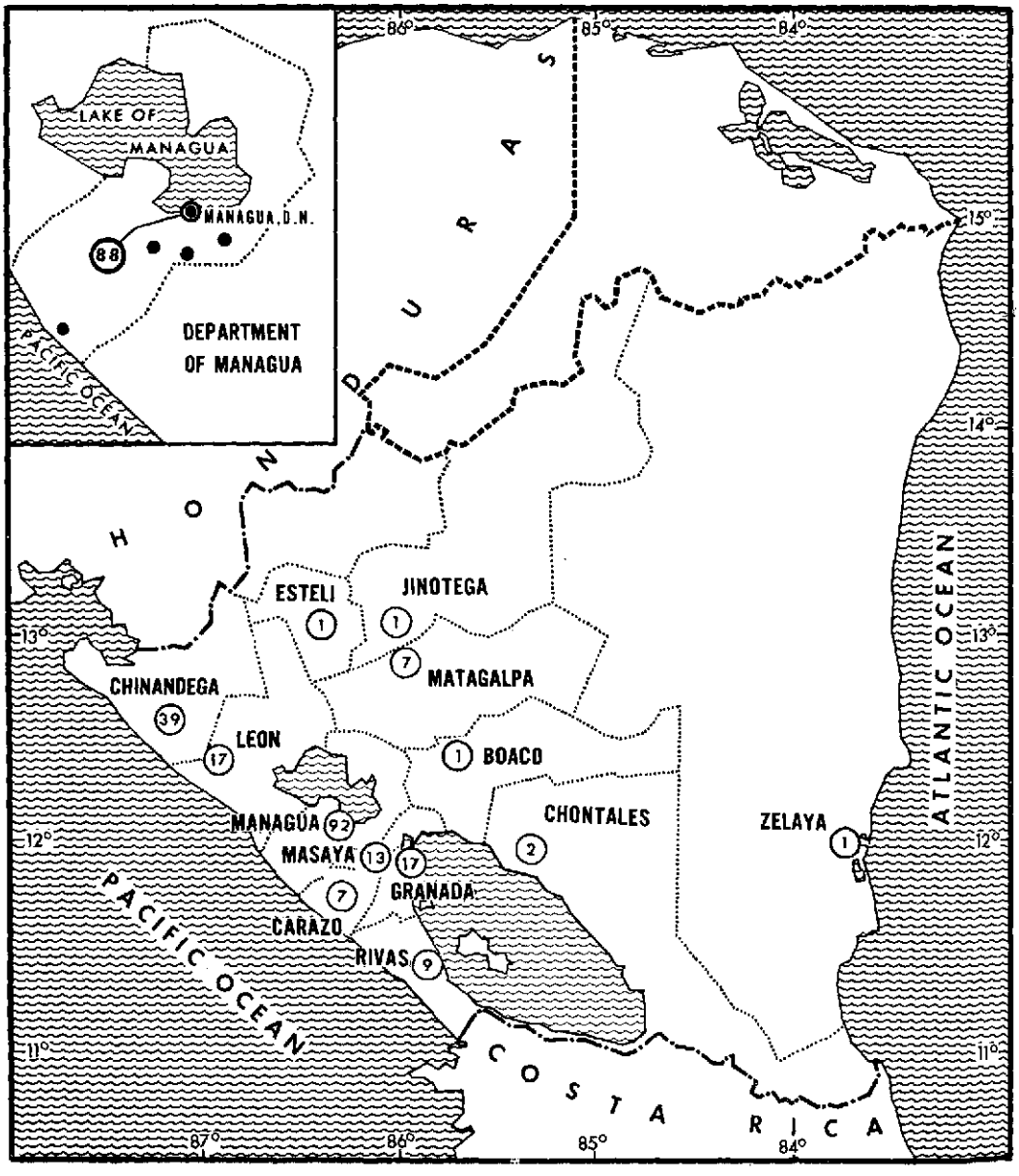
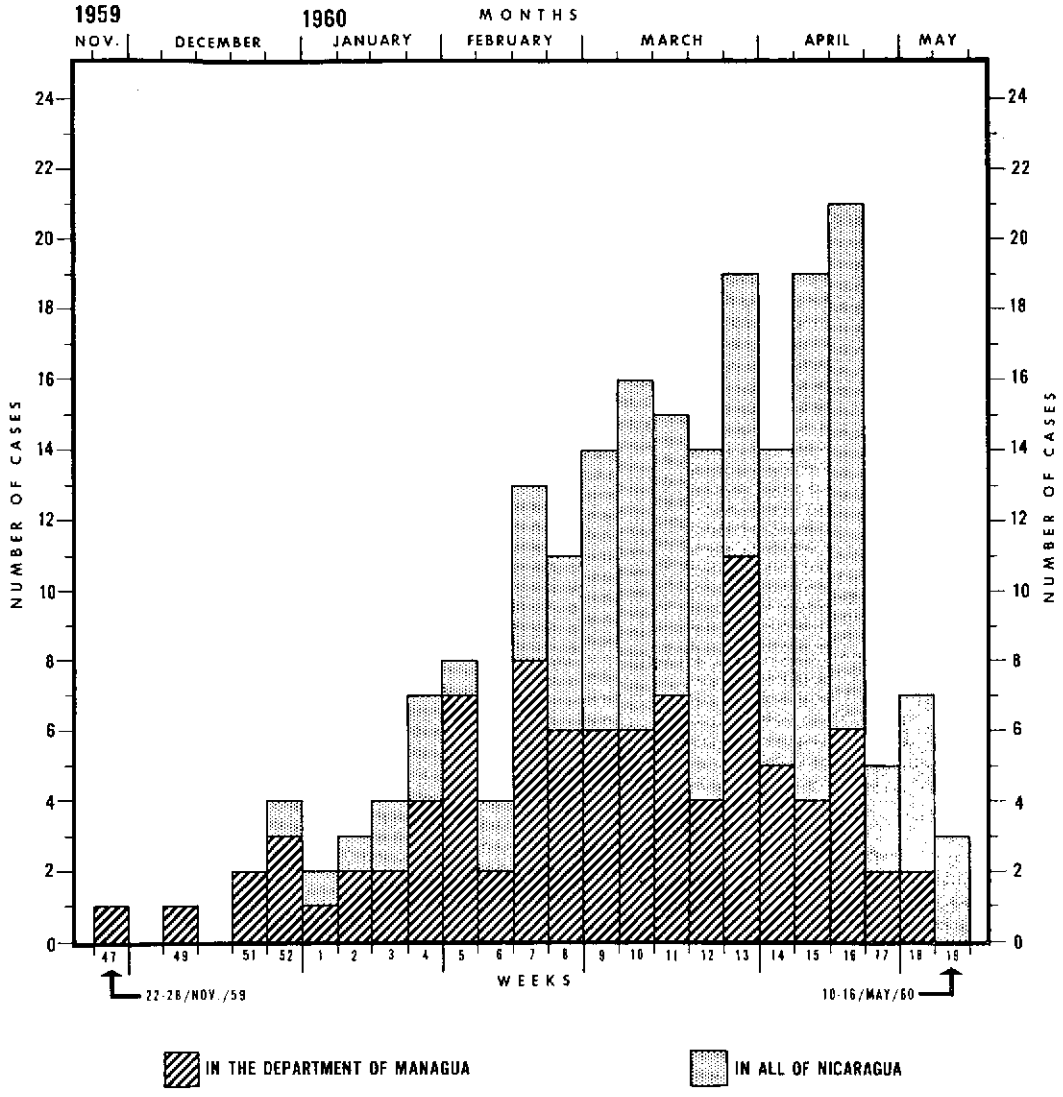


FIGURE 3

NOTIFIED CASES OF PARALYTIC POLIOMYELITIS, BY WEEK OF ONSET, IN THE DEPARTMENT OF MANAGUA AND ALL OF NICARAGUA DURING EPIDEMIC PERIOD 1959-1960



1, 3, and 4). Four deaths were recorded, giving a case fatality rate of 1.8 per cent versus 7.1 per cent in 1958.

Thirteen of the 16 departments of Nicaragua were involved in the epidemic. Of the 92 cases in Managua, 88 were recorded in the City of Managua and four in other communities of the Department (Fig. 2). As in 1958, the highest incidence of poliomyelitis in the rest of the country was observed in the Departments of Chinandega, León, and Masaya.

If we consider collectively the 225 cases reported in the country from 1 January 1959 to 14 May 1960 (Table 3), it can be noted that as in 1958, 75 per cent of these cases were in children less than two years of age. However, the proportion of cases in this age group in Managua in 1959-1960 was 74 per cent versus

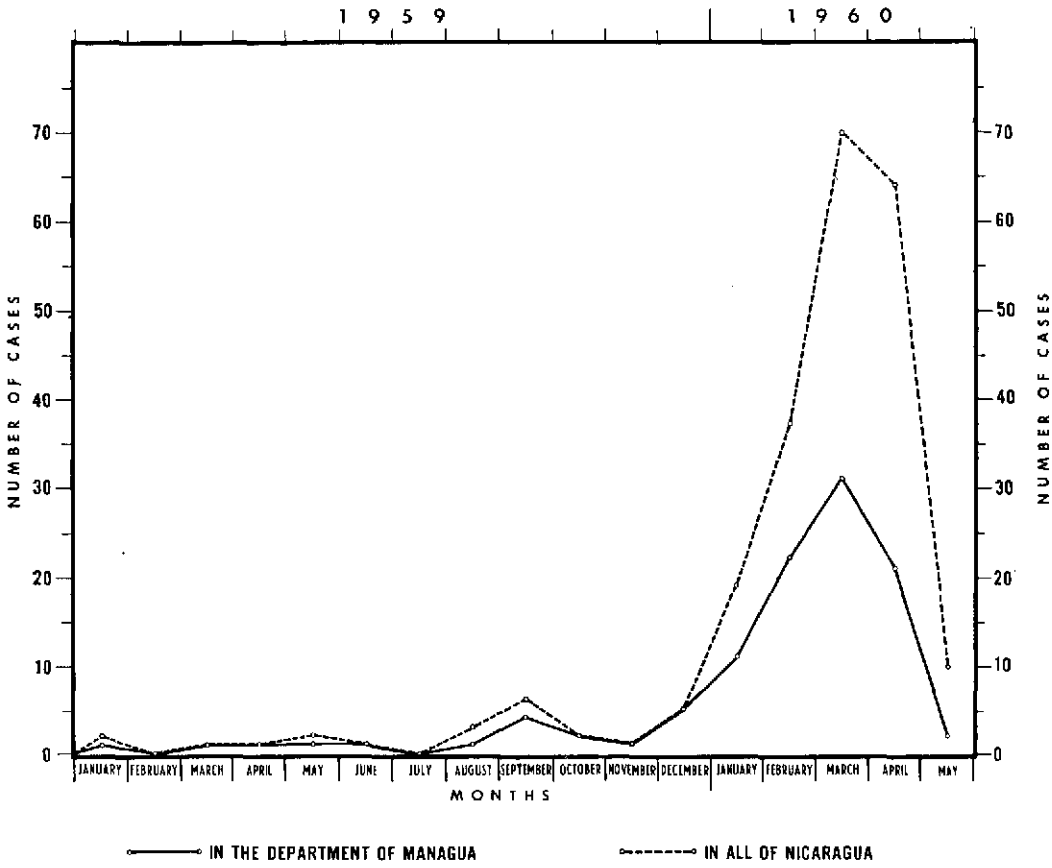


FIG. 4. Notified cases of paralytic poliomyelitis in the department of Managua and all of Nicaragua by months, 1959-1960

TABLE 3. AGE DISTRIBUTION OF REPORTED CASES OF PARALYTIC POLIOMYELITIS IN NICARAGUA, MANAGUA, AND THE REST OF THE COUNTRY, 1 JANUARY 1959 TO 14 MAY 1960

AGE IN YEARS	TOTAL		MANAGUA		REST OF THE DEPARTMENTS	
	NO.	PER CENT	NO.	PER CENT	NO.	PER CENT
Total	225	100	103	100	122	100
<1	53	24	30	29	23	19
1	115	51	46	45	69	57
2	24	11	10	9	14	11
3	13	6	8	8	5	4
4	10	4	5	5	5	4
5–9	8	3	3	3	5	5
10 and over	2	1	1	1	1	1

85 per cent in 1958. Only two cases in the country occurred in persons over ten years of age and one of these was in Managua—an adult European who died.

In Table 4, the attack rates for poliomyelitis are shown for Nicaragua and Managua since 1951. During this period the rates for 1959, both in Nicaragua, as a whole, and in Managua, were the lowest experienced in the 10-year period.

TABLE 4. REPORTED CASES OF PARALYTIC POLIOMYELITIS AND ATTACK RATES PER 100,000 POPULATION IN NICARAGUA AND THE DEPARTMENT OF MANAGUA 1951-1960*

YEARS	NICARAGUA		MANAGUA	
	CASES	RATES	CASES	RATES
1951	32	2.9	22	11.4
1952	24	2.1	20	10.1
1953	191	16.4	99	48.3
1954	45	3.7	28	13.2
1955	113	9.1	69	31.5
1956	48	3.7	36	15.9
1957	68	5.1	34	14.5
1958	256	18.6	107	44.1
1959	23	1.7	15	6.4
1960	202	13.6	98	33.5

* Up to 14 May.

POLIOMYELITIS IN VACCINEES

Among the 225 cases reported, 27 (or 12 per cent) occurred in persons who received one or more virus types by oral vaccination. Twenty-one of these cases were reported from Managua and the remaining from the Department of Chinandega (four cases), Masaya (one case), and Rivas (one case) (Fig. 1 and Table 5). No deaths were recorded among these 27 cases. Table 6 shows the type of vaccine virus received and the intervals between vaccination and the probable date of onset of symptoms. It will be noted that the longer intervals are associated with the monovalent vaccine, the use of which was discontinued in February 1959. There were three cases which developed in children one to nine days after emergency vaccination was started in communities where no oral vaccine had previously been used.

VIROLOGICAL STUDIES

In February 1960 arrangements were made with the Middle America Research Unit in Panama and the Viral and Rickettsial Research Section of the American Cyanamid Company in Pearl River, New York, to conduct virological studies on stool samples from suspected cases of poliomyelitis. An effort was made to obtain specimens and clinical histories from all cases as promptly as possible after the onset of symptoms. However, in many instances the cooperation of patients and families could not be se-

TABLE 5. REPORTED CASES OF PARALYTIC POLIOMYELITIS BY VACCINE STATUS AND AGE GROUPS IN NICARAGUA 1959-1960*

AGE IN YEARS	NICARAGUA		DEPARTMENT OF MANAGUA	
	VACCINATED	NOT VACCINATED	VACCINATED	NOT VACCINATED
Total	27	198	21	82
<1	8	45	3	27
1	10	105	9	37
2	3	21	3	7
3	2	11	2	6
4	2	8	2	3
5–9	2	6	2	1
10 and more	—	2	—	1

* Up to 14 May.

TABLE 6. REPORTED CASES OF PARALYTIC POLIOMYELITIS IN NICARAGUA AMONG VACCINEES: BY VIRUS TYPE RECEIVED AND INTERVALS BETWEEN VACCINE ADMINISTRATION AND PROBABLE DATE OF ONSET OF SYMPTOMS

ORAL VACCINE VIRUS TYPE	TOTAL	NUMBER OF PARALYTIC CASES				
		1–9 DAYS	10–30 DAYS	1–4 MONTHS	5–20 MONTHS*	UNSPECIFIED
TOTAL	27	4	1	3	12	7
2, 3, and 1	8	—	—	1	7	—
2 and 3	3	—	—	—	2	1
2	4	—	—	—	1	3
Trivalent	12	4	1	2	2	3

* Up to 14 May.

cured; in other cases the information obtained was incomplete.

Before February 1960, blood and fecal material (stool samples or rectal swabs) were obtained from suspected cases admitted to the poliomyelitis unit of the general hospital in Managua and sent to Pearl River. Type 3 poliovirus was isolated from three of these cases. Beginning in February and up to 14 May 1960, duplicates of 96 stool specimens were sent both to Pearl River and to Panama. Fecal material was collected from 55 per cent of all cases notified in the country up to 14 May, and from 70 per cent of those reported from Managua. The majority of fecal material samples were obtained soon after onset of paralysis. From three patients, swabs were obtained three, six, and eight weeks after onset, respectively. Up to 20 May 90 specimens were examined in one or both laboratories. All of the samples collected were obtained from cases which occurred in 1960, with the exception of one which had onset in 1959, and was seen six months after the appearance of symptoms. Poliovirus Type 1 was isolated from 47 cases and Type 3 virus from two patients. No poliovirus was isolated from four specimens and retests of these samples are currently in progress. Nine specimens were negative for cytopathogenic agents. From four specimens, cytopathogenic agents were isolated that were not neutralized by Types 1 and/or 2, and/or 3 poliovirus antisera. The examination of 16 recently received specimens is incomplete.

From the 27 cases with a history of vaccine administration, specimens for virus study were obtained from 20 of them: blood and fecal material from three cases, blood from only four cases, and fecal material from only 13 patients. Up to 31 May results were available for 11 of these cases: Type 1 poliovirus was isolated from six of the cases, from one case Type 3 poliovirus was recovered, from two cases non-poliovirus was isolated, and from two cases no agents were recovered. The isolation of poliovirus Type 1, even in absence of serologic confirmation, probably indicates, under the present circumstances, that the paralytic disease observed was due to the isolated type of virus.

POLIOMYELITIS SURVEILLANCE IN NICARAGUA

For 15 of the 27 cases reported as having been fed the live poliovirus vaccine, their vaccination history was documented. Records for the remaining 12 were either incomplete or absent, and as a consequence, it cannot be established whether or not these individuals received all three types of poliovirus vaccine. Since Type 1 virus was the last strain fed in the 1958 campaign and approximately 17 per cent of those who started the monovalent feedings did not receive Type 1 vaccine, a corresponding proportion would not have been vaccinated against the epidemic type of 1959-1960 although they had a history of vaccination.

Table 7 summarizes the information available for the 15 paralytic cases whose vaccination his-

TABLE 7. PARALYTIC POLIOMYELITIS CASES REPORTED IN NICARAGUA SUBSEQUENT TO ORAL POLIOVIRUS VACCINATION; WITH COMPLETE INFORMATION ON ORAL VACCINATION, NICARAGUA, 1959-1960*

CASE NO.	AGE (IN MONTHS)	ORAL VACCINATION				PROBABLE ONSET OF ILLNESS	LABORATORY DATA						
							FECAL MATERIAL				BLOOD		
		DATE OF ADMINISTRATION	POLIOVIRUS TYPE				DATE OF COLLECTION	RESULTS			DATE OF COLLECTION	ANTIBODY TITERS TO POLIOVIRUS TYPE	
			1	2	3			NEG.	POLIO	OTHER		1	2	3
1 LMS	36	Sept.–Oct. 1958	x	x	x	Jan.–1959	NO				27/I/60	128	>256	>256
2 JCM	36	Sept.–Oct. 1958	x	x	x	6/VI/59	NO				NO			
3 IMM	60	Sept.–Oct. 1958	x	x	x	9/IV/59	NO				5/I/60	128	128	128
4 APR	84	Sept.–Oct. 1958	x	x	x	8/X/59	NO				5/I/60	>256	32	>256
5 MLAM	32	11/XII/59	Trivalent			24/XII/59	7/I/60		T3		7/I/60	128	128	32
6 ABM	17	Sept.–Oct. 1958	x	x	x	28/XII/59	NO				7/I/60	>256	128	256
7 JCOV	20	Sept.–Oct. 1958	x	x	x	15/I/60	11/III/60			x	NO			
8 WBZO	24	Sept.–Oct. 1958	x	x	x	27/II/60	3/III/60	x			NO			
9 IMR	7	14/III/60	Trivalent			23/III/60	25/III/60		T1		12/V/60	>256	<4	>256

TABLE 7. *Continued*

Case No.	Age (in Months)	Oral Vaccination		Probable Onset of Illness	Laboratory Data							
					Fecal Material				Blood			
		Date of Administration	Poliovirus Type		Date of Collection	Results			Date of Collection	Antibody Titers to Poliovirus Type		
			1 2 3			Neg.	Polio	Other		1	2	3
10 JRL	9	10/II/60	Trivalent	29/III/60	5/IV/60	Pending			4/IV/60	Pending		
11 RJMM	10	8/IV/60	Trivalent	10/IV/60	19/IV/60	Pending			NO			
12 LMGN	5	9/IV/60	Trivalent	10/IV/60	NO				NO			
13 MVZ	7	6/III/60	Trivalent	20/IV/60	NO				NO			
14 RSG	12	25/III/59	Trivalent	6/IV/60	20/IV/60	Pending						
15 ALR	48	Sept.–Oct. 1958	x x x	30/IV/60	NO				NO			

* Up to 14 May.

tory was confirmed. It may be noted in the table that eight of these patients were vaccinated during the mass vaccination program in Managua in 1958, and they received all three types of poliovirus as monovalent vaccines. The other seven patients had received liquid trivalent vaccine. These two groups will be considered separately.

Cases among monovalent-vaccine-fed children. Cases Nos. 3 and 4, from whom fecal material was not collected, recovered completely within four months after the onset of paralysis. This could suggest that the transient paralysis observed was not due to poliovirus infection.

From the stool sample of case No. 7, a non-poliovirus agent was recovered in monkey-kidney tissue culture (MKTC). The sample was collected 54 days after onset.

In case No. 8, from whom a stool specimen was collected five days after onset, no agent was recovered by any of the laboratories. This fact may suggest an etiology unrelated to poliovirus. Unfortunately, stool specimens were not available from cases Nos. 1, 2, and 6. Serologic results on two of these cases do not make it possible to rule out poliovirus infection, and these cases, therefore, could be considered as vaccine failures.

Cases among trivalent-vaccine-fed children. The onset of paralysis in cases Nos. 11 and 12 occurred within 48 hours after vaccine administration. The onset of paralysis in cases Nos. 10 and 13, occurred 49 and 44 days, respectively, after vaccine administration, and thus represent onsets well beyond the accepted incubation period.[8] They will be regarded as instances of vaccine failure if the stool and blood studies which are in progress confirm the clinical diagnosis of poliomyelitis.

Cases Nos. 5 and 9 had onsets nine to 13 days after feeding, and in spite of the fact that they occurred within the period of the outbreak under consideration, they deserve comment since both might be suspected of vaccine strain etiology.

Case No. 5. Although this 32-month-old child had had four injections of Salk vaccine, the last one being eight months previously, she was fed the trivalent vaccine on 11 December 1959. Her illness had onset 13 days later and after four days with a temperature of 38.5° C. she developed paralysis in both legs and then quadriplegia at which time she was hospitalized. One week later she was sufficiently improved to be taken home. Shortly thereafter her parents took her to Mexico City for rehabilitation treatment. After two and a half months she returned almost fully recovered. She was seen in mid-May at which time there was still some weakness in the right deltoid and right leg, but without any suggestion of muscular atrophy in either legs or arms. The prognosis of the pediatrician who treated her in Mexico City was for complete recovery in 30 to 60 days. A stool specimen from this patient, obtained 27 days after vaccination and 15 days after onset, yielded Type 3 poliovirus. Neutralizing antibodies to all three types of poliovirus were demonstrated in a serum specimen which was collected on the same date as the stool specimen. Available laboratory data do not rule out poliovirus infection, but the clinical evolution of this patient's disease is rather suggestive of a non-poliovirus etiology.

Case No. 9. Poliovirus Type 1 has been isolated from a stool specimen taken 11 days after vaccination and two days after onset. Laboratory studies are not yet complete and the case is still under observation. The interval between vaccine administration and onset, as well as the fact that the virus isolated corresponds in type to the one responsible for the epidemic, requires that both natural infection and vaccine implication be carefully studied. Investigations designed to provide additional data for consideration are still in progress.

In Table 7a the data are summarized for 12 paralytic cases for which the records for the dates and types of vaccine administered are incomplete or missing, or the patients failed to receive all three types of virus. Cases Nos. 16, 17, and 18 received only vaccines for Types 2 and 3 during the mass vaccination program in 1958. Stools from cases Nos. 16 and 18 were collected 25 and nine days, respectively, after onset, and, when examined in MKTC one yielded a non-poliovirus and the other no virus. Type 1 poliovirus was isolated from case No. 17. Because none of these three patients had received Type 1 vaccine they may be dismissed from further consideration.

Cases Nos. 21, 23, 25, and 27 were apparently vaccinated as infants and are assumed to have received one dose of monovalent vaccine, presumably Type 2, since trivalent vaccine did not come into use in Managua until sometime in

TABLE 7A. PARALYTIC POLIOMYELITIS CASES REPORTED IN CHILDREN FED ORAL POLIOVIRUS VACCINE; WITH INCOMPLETE INFORMATION ON ORAL VACCINATION, NICARAGUA 1959-1960*

CASE NO.	AGE (IN MONTHS)	ORAL VACCINATION				PROBABLE ONSET OF ILLNESS	LABORATORY DATA			
							FECAL MATERIAL DATA			
		DATE OF ADMINISTRATION	POLIOVIRUS TYPE				DATE OF COLLECTION	RESULTS		
			1	2	3			NEG.	POLIO	OTHER
16 ECP	21	Unspecified		x	x	22/I/60	17/II/60			x
17 AJR	48	?/XI/1958		x	x	25/I/60	7/III/60		T1	
18 MEC	7	?/IX/1958		x	x	6/II/60	17/II/60	x		
19 VRB	18	?/X/1959	Trivalent			25/II/60	7/III/60		T1	
20 RAP	18	Unspecified	Trivalent			6/III/60	7/III/60		T1	
21 JFRC	14	Unspecified		x		13/III/60	22/III/60		T1	
22 JATO	10	Unspecified	Trivalent			20/II/60	24/II/60		T1	
23 ATM	7	Unspecified		x		2/III/60	NO			
24 MSL	9	28/III/1960	Trivalent (?)			6/IV/60	19/IV/60		Pending	
25 MASV	18	Unspecified		x		6/IV/60	NO			
26 RASD	18	Unspecified	Trivalent			17/IV/60	25/IV/60		Pending	
27 MTTG	18	?/?/1959		x (?)		22/IV/60	NO			

* Up to 14 May.
(?) Unverified.

March, 1959. The incomplete record for case No. 23 carries the notation "apparently received one dose of vaccine. Card not found." These five cases may all have received only Type 2 vaccine. Cases Nos. 19, 20, 22, and 26, if it be assumed that they received trivalent vaccine in late 1959, may be regarded as failures to respond to the vaccine.

The onset of illness in case No. 24 occurred nine days after vaccine trivalent (?) administration. A stool specimen collected 15 days after onset is being investigated.

DISCUSSION

The poliomyelitis outbreak in Managua has provided a unique opportunity for the study of the disease in a population about which we had recent and detailed data regarding its type specific poliomyelitis susceptibility. Moreover, the outbreak came as a direct natural challenge in a group approximately one year after the oral vaccination of somewhat more than one half of the children under 10 years of age.

Official population estimates give the number of children under 10 years of age in Managua in 1959 at 88,983. Of this number, 49,585 received Type 1 oral poliovirus vaccine. Serologic data obtained from prevaccination sampling showed that 21.8 per cent of the children under 10 years of age were seronegative for Type 1 poliovirus. It may be calculated, therefore, that there were 8,589 Type 1 seronegatives among the unvaccinated children and 10,809 among those vaccinated. However, the seronegative conversion rate in Type 1 vaccinated children was 74 per cent. Thus, 2,810 Type 1 negatives remained among the vaccinated children. If these 2,810 be added to the 8,589 Type 1 negatives not vaccinated, it may be assumed that this pool of 11,399 seronegatives produced the 92 cases of paralytic poliomyelitis reported in Managua.

From this it appears that it required 123.9 Type 1 seronegatives to yield one paralytic case. On the basis of these calculations, the 2,810 residual Type 1 seronegatives among vaccinated children would be expected to give rise to 23 paralytic cases. It has already been observed that 21 such cases with a history of oral poliovirus vaccination have been reported in Managua and of these only 15 had a confirmed history of having received Type 1 vaccine virus.

This analysis of the Managua experience involves several assumptions: (a) the adequacy of serologic sampling in 1958; (b) the unchanged susceptibility status of children between the time of the serologic survey and the natural challenge; and (c) the completeness of reporting. There exist, however, specific data to support and justify the analysis. The results of the study, we believe, clearly indicate that the mass vaccination program with oral poliovirus vaccine resulted in the protection of that proportion of vaccinated children who had been shown by serologic test to have responded to the Type 1 vaccine strain. For reasons which we cannot fully explain, the proportion of Type 1 susceptibles responding in Managua was less than that found in many other trials with the same vaccine strain. However, it was a very significant proportion and one which successfully withstood the challenge of natural exposure a year later.

There was nothing about the 1959-60 poliomyelitis epidemic in Nicaragua which suggested that its evolution was different from similar outbreaks in the past.

The distribution of cases by age, time and geographic location resembled previous epidemics in the country. In Managua the proportion of cases under two years of age was lower in 1960 than in the 1958 Type 2 epidemic. Unknowingly, while attempting to defend ourselves against the further inroads of a Type 2 epidemic in 1958, we were gathering the facts with which to interpret a Type 1 epidemic in 1960. Equally, without intention, we were preparing a challenge trial for the Type 1 vaccine strain—the one which must provide protection against the most common cause of paralytic poliomyelitis throughout the world. In our opinion that vaccine has successfully withstood the challenge.

REFERENCES

1. Martins da Silva, M., Berrios, M. L., and Alcocer, J. J.: The use of attenuated poliovirus in an epidemic area—Managua, Nicaragua, 1958. *Am. J. Hyg.* **71**(2)179-192 (March) 1960.
2. Nicaragua, Ministry of Economy, Department of Biostatistics: Population groups by age and sex in rural and urban areas of Nicaragua. (Official communication)
3. Erickson, G. M., Flipse, M. E., Menzin, A. W.,

Clayton, L. B., Markush, R. E., and Hardy, A. V.: Preliminary report of epidemiological surveillance in a mass field trial with oral polio vaccine (See this volume pp. 445-456).

26. VACCINATION WITH ATTENUATED POLIOVIRUSES IN COSTA RICA. SECOND PROGRESS REPORT

Section I. Vaccination Program

J. NÚÑEZ, O. VARGAS-MÉNDEZ, E. C. GUEVARA, J. M. QUIRCE, J. A. MONTOYA, H. DOANY, AND M. MARTINS DA SILVA

Section II: Surveillance Program

J. M. QUIRCE, J. NÚÑEZ, E. C. GUEVARA, J. A. MONTOYA, H. DOANY, AND A. SHELOKOV

Dr. VARGAS-MÉNDEZ (*presenting Section I of the paper*): Poliomyelitis is a problem of increasing importance in Costa Rica. The number of reported cases of the disease has varied from year to year, ranging from as few as four in 1949 to more than 1,000 cases in the major epidemic in 1954, during which approximately 80 per cent were children under five years of age. Costa Rica's poliomyelitis experience of the past few years is illustrated in Table 1. As you can see from the table, the high percentage of the cases fell within the age group up to five years of age.

Since attempts to control the disease with Salk vaccine have involved relatively high costs for vaccine, syringes, personnel, etc., the public health authorities welcomed the opportunity to employ live virus oral vaccine, which held the promise of longer immunity and greater ease of application—advantages of prime importance in public health administration.

The preliminary plans for mass application of the oral vaccine were described last year at the First Conference. This communication is a progress report on the nationwide program begun on 17 March 1959. The surveillance aspects of this program will be discussed separately, in Section II.

ORGANIZATION OF THE PROGRAM

1. *Monovalent vaccine.* Originally the plan was to vaccinate all children under the age of 11, throughout the country, with monovalent vaccine. The campaign was initiated in the national capital and extended to five of the six provincial capitals and 10 of the larger cities. In San José, a house-to-house campaign was conducted by teams of public health and auxiliary nurses. Records were kept by name and location for all vaccinated children, and from some of them, randomly selected, pre-vaccination blood samples were collected. In the larger cities, similar personnel vaccinated the children at local health centers.

TABLE 1. COSTA RICA—POLIOMYELITIS BY AGE 1954-1960

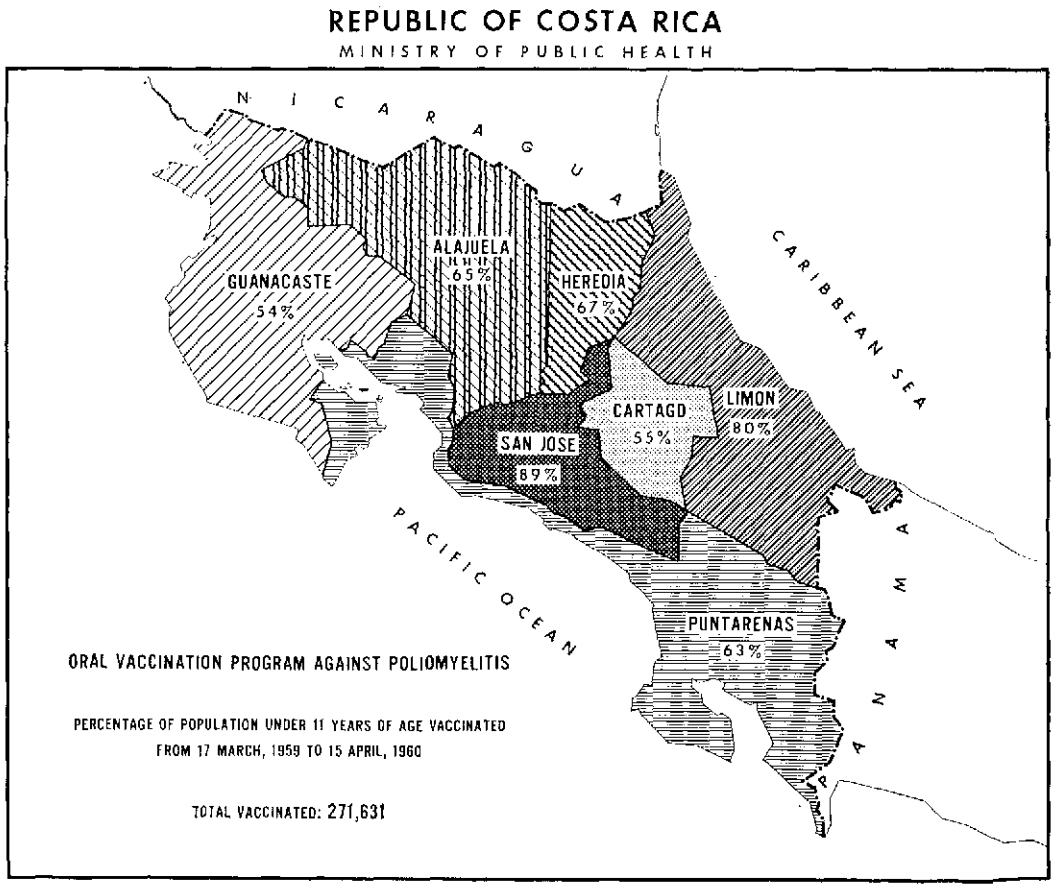
YEARS	CASES	AGE IN YEARS				
		<1	1–4	5–14	> 15	UNK.
1954	1,081	198	673	101	37	5
1955	45	4	28	7	6	0
1956	170	31	112	16	10	1
1957	51	8	29	8	4	2
1958	62	14	34	7	4	3
1959	41*	7	29	5	0	0
1960†	31	12	14	4	1	0

* Includes cases occurring before initiation of program.
† Up to April.

2. *Trivalent vaccine.* As the monovalent program was progressing, the trivalent vaccine became available. This new vaccine had the obvious advantage of greater speed of application and saving of labor. In addition, it showed evidence of greater opportunity for intra- and inter-familial spread of the vaccine viruses. Because it was becoming apparent that about 15 per cent of the children in the monovalent program failed to complete the series of feedings, the Ministry of Health decided to continue the program with the alternative vaccine. Beginning in October 1959, the trivalent vaccine was administered in health centers. It was the only vaccine used in one of the provinces. In smaller communities and rural areas the trivalent vaccine was used extensively to complete the national program. However, vaccination stations were also established in areas previously vaccinated, for children who had not completed monovalent feedings or had been missed in the first campaign. Great care was taken in the transportation of the imported vaccine by timed flights and timed arrivals previously agreed upon, and in storage in proper refrigeration while the vaccine was kept in San José. Also, the vaccine taken to the field was kept in small sized iceboxes and maintained at the appropriate temperatures all the time.

3. *Newborn vaccination.* In a limited number of hospitals a program was initiated 15 April 1959, to vaccinate newborn infants within the first 48 hours of life with a bivalent vaccine containing poliovirus Types 2 and 3, following a month later with monovalent Type 1 vaccine. Under this bivalent-monovalent scheme 2,225 infants were vaccinated. Another 234 infants were vaccinated with two doses of trivalent vaccine given 12 to 14 weeks apart.

The vaccines used in the Costa Rican national



program were supplied by the Lederle Laboratories, American Cyanamid - Company, Pearl River, N. Y., U.S.A. The monovalent vaccine of poliovirus Types 1 and 3 contained approximately $10^{6.1}$ TCD_{50} and the Type 2 from $10^{5.8}$ to $10^{6.2}$ TCD_{50} per 0.7 ml. dose. The trivalent vaccine had a concentration of $10^{6.2}$ TCD_{50} of each poliovirus type per 2 ml. dose. The bivalent Type 2 and 3 vaccine and the monovalent Type 1 used for the newborn infants contained $10^{6.0}$ TCD_{50} per 1.0 ml. dose.

Distribution of the vaccinees geographically

TABLE 2. ORAL VACCINATION AGAINST POLIOMYELITIS IN COSTA RICA—17 MARCH 1959 to 15 APRIL 1960

LOCALITY	ESTIMATED POPULATION <11 YEARS AS OF 31-12-59	CHILDREN VACCINATED			
		VACCINE			
		MONOVALENT*	TRIVALENT	TOTAL	%
TOTAL POP.	382,905	122,552	149,079	271,631†	71
PROVINCES:					
SAN JOSE	122,792	68,875	42,067	110,942	89
(METROP. AREA)	(74,286)	(66,336)	(6,798)	(73,134)‡	(98)
ALAJUELA	75,670	25,123	24,446	49,569	66
CARTAGO	49,818	12,814	14,600	27,414	55
HEREDIA	22,248	3,752	11,088	14,840	67
GUANACASTE	50,123	—	26,854	26,854	54
PUNTARENAS	44,745	7,724	20,335	28,059	63
LIMON	17,509	4,264	9,689	13,953	80

* Each type one month apart.
† Including 2,225 newborns who received bivalent Types 2 and 3 vaccine, followed by Type 1 one month later.
‡ Figures revised by Dept. Biostatistics, already included in the province of San José.

TABLE 3. DISTRIBUTION OF COSTA RICAN CHILDREN UNDER 11 YEARS BY AGE, IMMUNITY, AND VACCINATION STATUS—1959-60

AGE (YRS.)	ESTIMATED NUMBER IN POPULATION AS OF 31-XII-1959	% VACCINATED	NO. TESTED BEFORE VACC.	% SERONEGATIVES BY TYPE		
				1	2	3
<1	44,001	66	29	100	96	69
1	35,142	59	65	95	57	58
2	38,013	60	92	80	30	40
3	37,784	64	118	40	8	21
4	35,831	65	134	26	8	10
5-10	192,134	79	358	6	5	6
Total	382,905	71	794	32	16	19

and by age is shown in Fig. 1 and Tables 2 and 3. Fig. 1 gives the calculated percentage of children reached by the program, by province. In certain places the percentage may seem low, and transportation facilities and other factors influence the number of children vaccinated. Table 3 summarizes the results of a prevaccination serologic survey in children under 11 years of age, whose accelerating rate of infection in relation to age is strikingly illustrated in Figure 2. Table 3 also shows how the percentage of seronegatives by type diminished rapidly as age increased. In Table 4, the number of serologically susceptible children who received the vaccine is estimated by applying the percentage of type-specific seronegatives

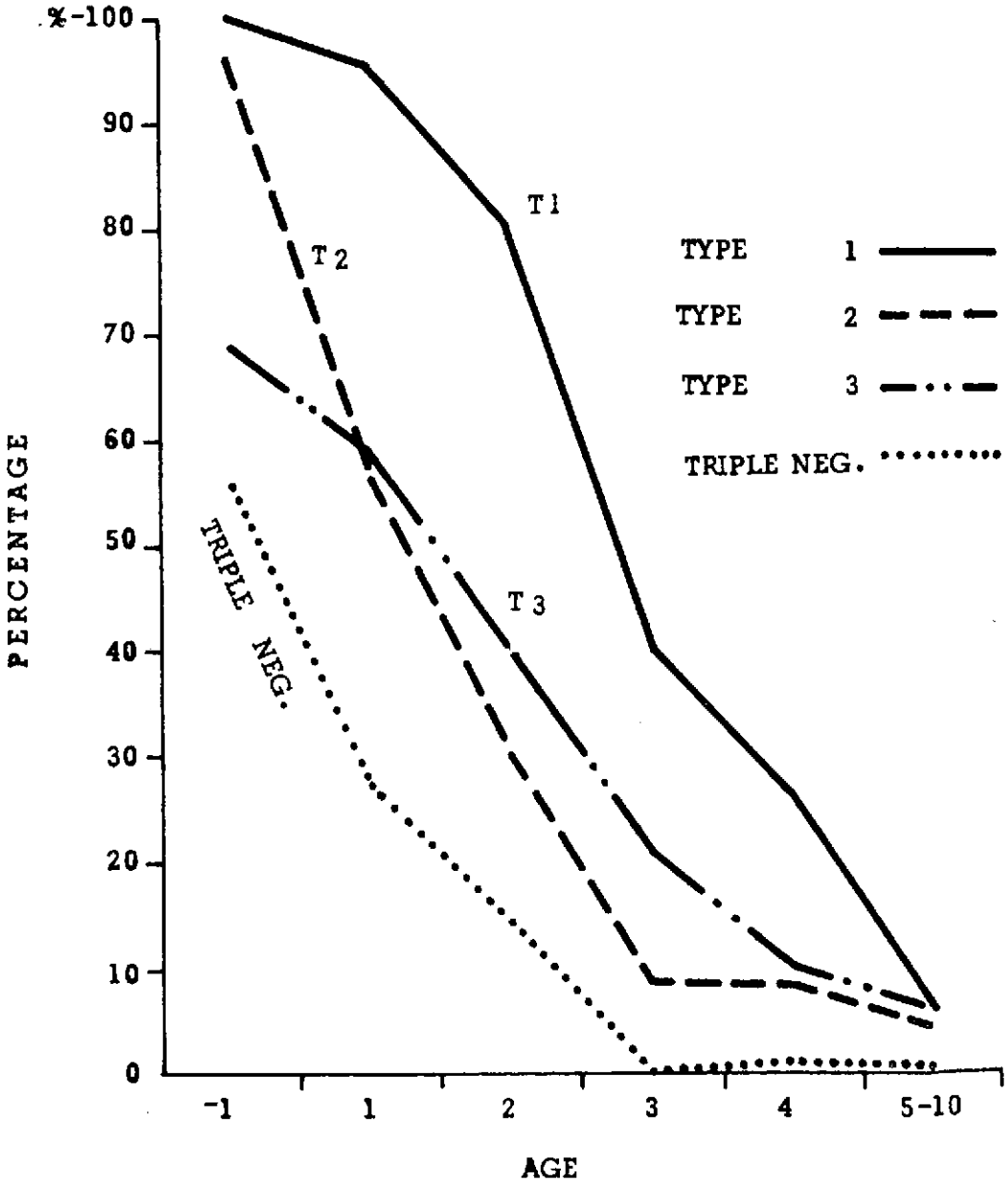


FIG. 2. Type specific seronegatives by age—prevaccination survey of 794 sera in Costa Rica.

TABLE 4. DISTRIBUTION OF MONOVALENT AND TRIVALENT-VACCINATED CHILDREN BY AGE AND ESTIMATED PREVACCINATION SERONEGATIVES BY VIRUS TYPE

AGE	NUMBER CHILDERN VACCINATED		TOTAL NUMBER CHILDERN VACCINATED	PER CENT NEGATIVE IN SURVEYS			ESTIMATED NUMBER SERONEGATIVES FED		
	MONOVALENT	TRIVALENT		1	2	3	1	2	3
<1	12,103	16,792	28,895	100	96	69	28,845	27,835	19,874
1	9,405	11,246	20,651	95	57	58	19,701	11,750	12,080
2	10,389	12,586	22,975	80	30	40	18,472	6,984	9,236
3	10,691	13,392	24,083	40	8	21	9,585	2,047	5,105
4	10,574	12,710	23,284	26	8	10	6,077	1,909	2,421
5–10	69,390	82,353	151,743	6	5	6	9,256	7,132	9,256
Total	122,552	149,079	271,631				91,936	57,657	57,972

established in the prevaccination survey to the total number of children vaccinated with monovalent and trivalent vaccines. Distribution of these seronegatives as single, double, and triple negatives, and of the triple positives is shown by age in Table 5.

Results of serologic assays of the responses of children of various ages to the monovalent and trivalent vaccines and of infants to vaccination in the first week of life are summarized in Table 6 below and Fig. 3. The post-vaccination blood samples from vaccinated newborns were collected from five to eight months after vaccination and the frequencies of seronegatives among them were compared with those found among unvaccinated infants under one year of age in the prevaccination survey. Table 7 summarizes the responses to monovalent vaccine by age for each type and the percentages for all three.

DISCUSSION

An ample system for the reporting of untoward reactions following vaccine ingestion was established at the start of the vaccination campaign. Reports were received of many instances of gastro-intestinal disturbances, skin reactions, respiratory distress, and miscellaneous complaints which were duly investigated and treated when necessary. There was no evidence of etiological relationship of any of these complaints to the vaccine. The number of such reports steadily declined as the program progressed and its novelty wore off.

TABLE 5. PREVACCINATION ANTIBODY STATUS OF 664 INDIVIDUALS BY AGE

No Antibodies to	Age in Years						
	<1	1	2	3	4	5–10	Adult*
3 Types	13	13	10	0	1	1	0
2 Types	9	20	15	14	7	1	7
1 Type	0	8	24	32	22	27	20
0 Types	1	6	10	33	55	194	121

* Cord blood.

TABLE 6. COSTA RICA—ORAL POLIOVIRUS VACCINE 1959-1960. TYPE SPECIFIC SERONEGATIVES BEFORE AND AFTER VACCINATION, AND CONVERSION RATES

Program	No. Sera Tested	No. Seronegatives Pre/Post Vaccination			% Seronegative Conversion Rate		
		1	2	3	1	2	3
Monovalent	726	243/44	98/62	137/12	82	37	92
Trivalent	102	85/12	45/23	28/3	86	49	89
(*Triple Negs*)	59	59/18	59/40	59/10	70	32	83
					Seronegative Rate (%)		
Unvaccinated*	29	29/–	28/–	20/–	100	96	69
Vaccinated	125	23/–	83/–	10/–	18	66	8
Difference					82	30	61

* Survey data for children <1 year old—Table 3.

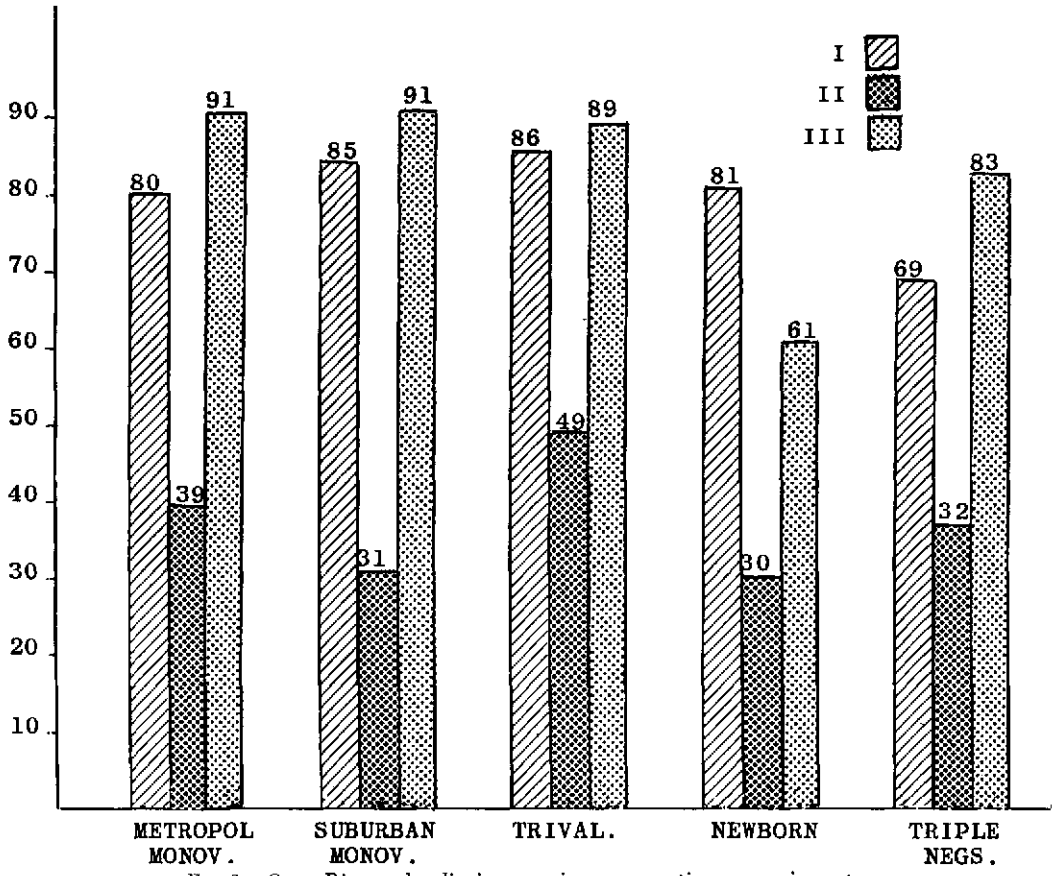


FIG. 3. Costa Rica oral poliovirus vaccine seronegative conversion rates.

TABLE 7. SAN JOSÉ, COSTA RICA—CONVERSION RATES BY AGE FOLLOWING MONOVALENT VACCINATION IN 447 CHILDREN

AGE	TESTED	1		2		3		TOTAL		CONVERSION RATE (%)
		PRE	POST	PRE	POST	PRE	POST	PRE	POST	
<1	20	20	7	19	13	13	2	52	22	58
1	33	31	9	16	10	19	1	66	20	70
2	52	44	4	15	11	21	2	80	17	79
3	67	26	4	6	2	14	2	46	8	83
4	76	22	4	5	3	9	1	36	8	78
5-9	176	9	2	8	3	12	0	29	5	83
10+	23					1	0	1	0	
Total	447	152	30	69	42	89	8	310	80	74

Costa Rica's vaccination program was organized to protect the greatest number of children against poliomyelitis as rapidly as possible. For this reason, the more speedily applied trivalent vaccine was quickly adopted, and thereafter used for operations in new areas and for clean-up operations where monovalent vaccine had previously been used. The purpose of the clean-up operations was to reach children previously missed and to vaccinate those who had not completed the three monovalent doses.

A basis for estimating the state of immunity of that part of the population primarily at risk was provided by the pre-vaccination blood samples collected in the serologic survey undertaken in the earlier stage of the vaccination program. Triple negative sera were confined almost exclusively to children under the age of three years, and double negatives after this age were uncommon. Almost 90 per cent of the children were triple positive by the time they reached the five- to 10-year age group. In conjunction with the known age-incidence of the disease, the information provided by the survey focused attention on that segment of the childhood population in which to concentrate the greatest control effort.

In addition to providing a substantial basis for judging the relative efficacy of vaccine strains and of methods of administration, the sera collected pre- and post-vaccination in different areas and in different vaccination programs served to confirm the safety of the vaccines, since the data indicate the frequency of infection by the vaccine strains. The serologic studies show that conversion rates with the monovalent and trivalent vaccines are comparable, although with the trivalent, Type 2 conversions were slightly better. The significance of this difference is difficult to assess, but coupled with the other advantages of trivalent vaccine, even a slight advantage in this strain, apparently the weakest of the three, is worthwhile. Although they are based on relatively small numbers, the conversion rates among triple negatives appear to be essentially like those of homotypic-negative children in general.

The true response of infants vaccinated during the first week of life is difficult to assess on the basis of titer alone, since the infants were bled from five to eight months after vaccination. However, sufficient instances of seronegatives were found at different intervals after birth to justify the assumption that without the intervention of vaccination or natural infection, all but seven of the 125 infants would have been triple negatives at the time of bleeding. It therefore seemed justifiable to compare the incidence of seronegatives of these vaccinated infants with that of unvaccinated infants under one year of age as indicated by the general prevaccination survey. On this basis, the conversion rates among newborn infants have considerable interest. Meager though the data are, it is believed that they indicate clearly that newborn infants can and do respond to vaccination. It might, therefore, be advisable to establish vaccination services in maternity hospitals. In Costa Rica, however, relatively few of the babies in the rural areas are born in hospitals, so that it obviously is impossible to rely on such a program to reach more than a small part of the population requiring protection.

In our opinion, based on experience in Costa Rica, a thoroughly organized and conducted mass vaccination campaign followed by a maintenance program operating through well-baby and pre-school clinics in community health centers and through mobile health units in rural areas should provide adequate protection in keeping with population growth.

SUMMARY

1. From a Public Health point of view, the attenuated poliovirus oral vaccine offers marked advantages over the injected Salk-type vaccine.

2. It was noted that by the use of separate monovalent vaccine, the number of children receiving each subsequent dose decreased and that this disadvantage alone justified the use of the trivalent vaccine. Conversion rates for both types of vaccines were comparable.

3. The prevaccination serologic survey shows that under the conditions prevailing in 1959, most of the child population progressively changes from triple negative at eight to 12 months of age to triple positive by five to 10 years of age.

Section II. Surveillance Program

DR. QUIRCE *(presenting Section II of the paper, through an interpreter):* When the decision was made to undertake the mass vaccination campaign with oral poliovirus vaccine, plans were developed for the two major aspects of the project. The organization for the administration of the vaccine has been discussed in the preceding section; this discussion deals with the organization and findings of the surveillance program.

Information regarding the technical details of the vaccination campaign were distributed by various means to medical societies and practicing physicians and discussed with the medical staffs of the hospitals and health centers throughout the country. The purpose of the program and the manner of its operation were explained to the lay public by means of the press, radio, and local announcements over mobile loudspeaker units. At suitable times, further details were supplied to the churches and schools. In these ways a systematic effort was made to create a maximum of interest and understanding at all levels of the public.

As a result of the severe 1954 epidemic, the medical profession specifically and the public in general had been made painfully aware of poliomyelitis and this had served to increase the efficiency of the reporting of poliomyelitis and similar clinical diseases. We believe that the reporting of paralytic poliomyelitis after 1954 accurately reflects the incidence of the disease in Costa Rica, and that as a result of this and the preparatory measures taken early in 1959 we were in a position to carry out an effective surveillance program. Other factors operated as a part of the national health service to supply us with information about the occurrence of paralytic disease. These were the activities of the Crippled Children's Program of the Ministry of Health which organized visits of the director of the Rehabilitation Center, physiotherapists and appliance experts to local health centers in various parts of the nation. This staff, as well as the nurses, physicians and welfare workers throughout the country were the principal sources of information regarding cases or suspected cases of poliomyelitis during the vaccination campaign.

A central office was set up in San José to receive and investigate reports from local sources as well as those from the seven provinces. The chief physician of the office and his assistant were aided during part of the program by a representative from the Pan American Health Organization and two representatives from the Communicable Disease Center of the U.S. Public Health Service. Cases for investigation were usually screened by the physicians of the local health centers and hospitals, but periodic visits were made by the staff of the Surveillance Service to the Rehabilitation Center and general hospital in San José and elsewhere in search of unreported cases. When the clinical characteristics of the case warranted, blood and stool specimens were collected and dispatched to the Middle America Research Unit Laboratory in Panama and to the Pan American Sanitary Bureau tissue-culture laboratory in Cali, Colombia for diagnostic service. Many of the specimens were also examined at the Viral and Rickettsial Research Section of Lederle Laboratories in Pearl River. Periodically, a panel of personnel from the diagnostic laboratories and the Surveillance Service met and reviewed the clinical, epidemiological and laboratory evidence assembled, and decided the status of each of the reported cases of suspected poliomyelitis. Conferences for this purpose were held twice in San José and once in Panama. Except for those cases which have accumulated since the last panel meeting in mid-May, the judgment of this conference group has been accepted in classifying the cases now to be reviewed.

From the initiation of the oral vaccination program in Costa Rica in March of 1959 to mid-May 1960, 326 suspected cases of poliomyelitis were reported to or uncovered by the Surveillance Service. The first 51 of these were reviewed by the conference group in January 1960 and 25 were rejected as being without neurologic manifestations. Twenty of the remaining cases were segregated as non-poliomyelitis cases with nervous manifestations due to various causes. Six confirmed polio cases were accepted and all of them were without a history of oral vaccination. A report reviewing these cases was issued and circulated among the various cooperating agencies involved in the Costa Rican program.

Two subsequent panel conferences reviewed the 275 cases reported after the first meeting. Of this total, all but 72 were rejected as being without neurological content. The 72 cases were segregated into non-polio cases with neurologic signs and symptoms due to such diverse causes as facial paralysis of unknown etiology, a number of which had been seen before the vaccination campaign started, polyneuritis, birth trauma, tuberculous meningitis. There were also cases of post-infectious encephalitis, meningitis, and herpetic infection. Among these 72 cases were 53 which were accepted as paralytic poliomyelitis. Forty-five of these were non-vaccinated individuals and 14 had a history of oral vaccination. The age distribution of these 59 cases is shown in Table 1. During the course of the investigation of the neurologic cases, fecal or blood or both types of specimens were examined by the laboratories cooperating in the program. In addition, stools and blood samples from 62 household contacts of suspected cases were investigated. Twenty-eight of the contact specimens failed to yield any virus and from the remainder, Types 1 and 3 poliovirus were isolated once each, and Type 2 eight times. Non-polioviruses were isolated 17 times and results of seven contact stool examinations are still pending.

The results of the virological studies on the stool specimens from the vaccinated and non-vaccinated cases reported with confirmed neurologic symptoms are summarized in Table 2. Here it may be seen that polioviruses were isolated from eight of the 49 non-paralytic cases manifesting neurologic signs. Five of the eight isolations were made from vaccinated individuals. Three of the isolates were Type 1 poliovirus, three were Type 2 and two were Type 3. Other non-polio enteroviruses were isolated from nine patients in the non-paralytic group.

Confirmed Poliomyelitis Cases. Of the 326 reported instances of suspected poliomyelitis reported to the Surveillance Service and

TABLE 1. PARALYTIC POLIOMYELITIS INCIDENCE BY AGE IN COSTA RICA SINCE THE BEGINNING OF THE ORAL VACCINATION PROGRAM (17 MARCH 1959 TO 17 APRIL 1960)

AGE IN YEARS	CASES: VACCINATED	CASES: NOT VACCINATED	TOTAL
<1	5	11	16
1	5	10	15
2	2	11	13
3	1	5	6
4	0	1	1
5–9	0	3	3
>10	1	4	5
Totals	14	45	59

TABLE 2. CASES WITH NEUROLOGICAL SYMPTOMS—RESULTS OF STOOL EXAMINATIONS

ISOLATES	NON-PARALYTIC: VACCINATED	NON-PARALYTIC: NOT VACCINATED	PARALYTIC: VACCINATED	PARALYTIC: NOT VACCINATED	TOTAL
Poliovirus Type 1	2*	1	0	1	4
Poliovirus Type 2	2	1	5†	12	20
Poliovirus Type 3	1	1	1†	2	5
Other Enteric Virus	3	6	2	4	15
Negative	1	26	1	5	33
Pending	2	3	2	3	10
Total	11	38	11	27	87

* One case who received trivalent vaccine 16 days prior to stool collection.
† Includes one patient who received trivalent vaccine nine days before stool collection.

screened by the conference panels, 59 were accepted as paralytic poliomyelitis. Forty-five of these occurred in non-vaccinated persons. Results of virological studies on the 27 of the 45 from whom specimens were obtained are summarized in Table 2. Poliovirus was isolated from 15 of these stool samples. Type 2 poliovirus was isolated from 12 patients, Type 3 from two, and Type 1 from one. Non-polio enteroviruses were isolated from four, no virus was obtained from five stools, and the results in three instances are pending. Of the 59 polio cases, 26 were in the metropolitan area and 13 of these were in non-vaccinated individuals. The remaining 33 cases were scattered among all of the seven provinces and 32 of them were in non-vaccinated persons. It should be noted that two of the cases in non-vaccinates, which were clinically accepted as paralytic poliomyelitis, were seronegative for all three types of poliovirus and in one of these patients the serologic finding was confirmed by a second blood specimen obtained one month after the first.

The remaining 14 of the 59 confirmed paralytic poliomyelitis cases occurred in individuals who had received oral poliovirus vaccine. Thirteen of the 14 cases were reported from the metropolitan area and the other came from the province of San José. The virological findings in these cases are summarized in Table 2, where it may be seen that six poliovirus isolates were recovered. Types 2 and 3 were both recovered from one patient, and four of the other five isolates in cases were Type 2 poliovirus. Type 1 was not recovered. Non-polio enteroviruses were isolated from two specimens and one failed to yield any virus. Stool examinations are incomplete in two instances.

Table 3 presents a summary of accumulated data pertaining to the 14 paralytic cases which had a history of oral poliovirus vaccination. Stool and blood specimens were collected from 13 of them, and from one case serum only was obtained. Up to this time, complete laboratory results are available for seven of the 14 patients. For three others, serologic findings are available, and in two of these stool examinations are incomplete. Of the remaining four recently reported cases, neither stool nor blood specimens have yet been examined.

With the exception of case No. 286 which will be discussed separately, all of the patients received oral poliovaccine more than seven weeks before the onset of illness.

Case No. 53. Isolation of ECHO virus from the fecal material obtained more than one month after onset is not very significant for the implication of this agent as the cause of the illness; however, the isolation of ECHO virus from four of its six household contacts gives reason to entertain this possibility. The absence of Type 2 antibodies more than a month after the illness started shows clearly that the symptoms were not caused by Type 2 virus, either wild or of vaccine origin, although it does indicate a lack of response to the Type 2 vaccine. Type 1 vaccine was not fed to this patient and the high titer of Type 1 antibodies suggests the possibility that a Type 1 infection may have been the cause of the disease.

Cases Nos. 269, 270, 277, and 283. Two of the patients received the full course of oral vaccination with monovalent strains, and one received trivalent vaccine. The last one (283) received only Type 2 vaccine. The lapse of time between vaccination and onset exculpates the vaccine as the cause of symptoms in all four of these cases. The isolation of Type 2 poliovirus from the fecal specimens of these cases plus the presence of Type 2 specific antibodies in their sera indicate a lack of response to the Type 2 vaccine. Also the serologic results of Cases Nos. 270 and 277 indicate a lack of response to Type 1 vaccine.

Case No. 228. No stools were collected. Serology establishes Type 1 infection and lack of response to Type 2 vaccine administered at six months of age.

Case No. 282. The last monovalent vaccine dose was administered five months before illness occurred. No enteric viruses were found in the stool collected nine days after onset. The serology indicates the possibility of Type 1 infection and consequently a Type 1 vaccine failure.

Case No. 286. This patient received trivalent vaccine ten days before the onset of illness. Preliminary examination of the stool collected nine days after onset, indicated the presence of Types 2 and 3 poliovirus. Also, the presence of a Coxsackie virus is suggested by mouse inoculation results. A serum taken on the same day as the stool sample showed an antibody titer of 1:128 against Types 1 and 2, and a titer of 1:4 against Type 3. The stool examination is not

TABLE 3. PARALYTIC POLIOMYELITIS CASES WITH HISTORY OF ORAL POLIOVIRUS VACCINATION REPORTED IN COSTA RICA—RESULTS OF LABORATORY INVESTIGATION

CASE NO.	AGE (IN MOS.)	SEX	DATES PROVIDED						LABORATORY FINDINGS (CALI OR LED.)				
			VACCINATION			ILLNESS ONSET	SAMPLING		ANTIBODY TITERS TO POLIOVIRUS TYPE			VIRUS ISOLATED	
			POLIOVIRUS TYPE										
			1	2	3		STOOL	BLOOD	1	2	3	POLIO	OTHER
53	6	?	—	4/1/59	?	7/24/59	8/31	8/29	512	<4	64		ECHO
228	12	F	5/6/59	4/6	—	10/5/59	—	10/10 11/11	8 >1024	<4 8	16 16		
269	15	M	Trivalent 10/23/59			2/5/60	2/29	2/29	512	256	32	T.2	
270	28	M	April–July, 1959			2/4/60	2/25	2/25 3/30	<4 <4	8 256	64 16	T.2	
277	16	M	—	4/16/59	—	2/17/60	2/25	2/25	<4	256	>1024	T.2	
282	11 yrs.	M	5/29/59	4/15	7/21	2/21/60	3/1	3/1 3/28	16 256	64 256	64 64	NONE	
283	10	M	—	4/28/59	—	2/22/60	2/29	2/29	<4	1024	<4	T.2	
286	9	F	Trivalent 2/19/60			2/29/60	3/9	3/9	128	128	4	T.2 & 3 & other enteric	
303	14	M	5/17/59	4/20	7/5	3/21/60	3/29	3/29	<4	<4	16	PENDING	
304	36	F	6/2/59	5/6	7/11	3/26/60	3/29	3/29	<4	16	>1024	PENDING	
307	5	M	—	11/2/59	11/2	3/31/60	4/6	4/6	PENDING				
310	24	F	5/6/59	4/3	6/24	4/5/60	4/12	4/12	PENDING				
320	10	F	Trivalent 10/28/59 Trivalent 1/22/60			3/15/60	4/26	4/26	PENDING				
321	4	M	Trivalent 12/29/59			4/13/60	4/29	4/29	PENDING				

yet completed. A convalescent serum will be necessary for a complete analysis of the case.

Cases Nos. 303 and 304. The stool data are not yet available, and results on the acute serum only are not sufficient to establish a diagnosis. However, the serologic data indicate lack of response in Case No. 303 to Types 1 and 2 vaccine, and in Case No. 304 to Type 1 vaccine. The vaccines were given six months before the onset of illness.

Cases Nos. 307, 310, 320 and 321. Vaccination was more than seven weeks before the symptoms appeared, so that the vaccine is not implicated as the etiologic agent. Since the stool and blood examinations are incomplete, the analyses of these cases will be reported later.

COMMENT

Of the 59 cases diagnosed as paralytic poliomyelitis which have occurred in Costa Rica since the start of the oral vaccination program on 17 March 1959, 14 occurred among vaccinated individuals. The onset of symptoms was at least seven weeks after vaccination in 13 of the 14. In the 14th case the onset was 10 days after the administration of the trivalent vaccine. Laboratory studies of sera and rectal swabs are as yet incomplete in this case.

The attack rate in the outbreak in the metropolitan area in 1960 was 128 per 100,000 among the unvaccinated, and 14 per 100,000 among the vaccinated individuals.

DISCUSSION

CHAIRMAN STUART-HARRIS: Are there any questions or discussion on the last few papers presented?

DR. KITAOKA: I should like to ask a question. For the past few days several speakers have pointed out the advantage of a trivalent vaccine.

There is no doubt that high conversion rate was followed by oral vaccination of trivalent vaccine, notwithstanding the interference phenomenon which has been observed among different poliovirus types or with other enteroviruses.

But now the question is whether or not the genetic stability of attenuated poliovirus might be influenced by a combination of different poliovirus types or with other viruses.

The reason for my question is that when the monovalent vaccine was used no untoward reaction was observed, but when trivalent vaccine was administered some reaction was recognized.

DR. DULBECCO: I do not think the question can be answered. It can only be said that one cause of possible genetic instability would be the occurrence of a recombination between the two types, but this has never been proved. Certainly we do not have any evidence.

DR. PAUL: I think we should be most grateful to Dr. Vargas-Méndez and Dr. Quirce for giving us this detailed report. I can certainly testify personally to the work and effort put into this campaign.

What we have seen and heard this afternoon particularly concerns poliomyelitis in patients who received the oral vaccine. You might call them failures to immunize. I believe that anyone interested in the subject obviously must pay a great deal of attention to these failures. Why did they happen?

We have assumed, and it has been said many times here this week, that antibodies might be interpreted as a measure of effectiveness.

What was probably meant—and this again has been pointed out—is: Are we really in line with what an antibody response is and what is its relation to its capacity to infect?

We have said several times that some of the laboratories here have different techniques, and I think this was brought out by the fact that our study, reported earlier this week, brought out the fact that we were using slightly different criteria, and that we arrived at a slightly different though not appreciably different result.

I note that 13 out of the 14 cases in Costa Rica were in the very young children in whom the response was perhaps not as good as in the whole age group taken from 0 to 12 years.

Just for the record, with regard to the report from Nicaragua, I note that it contains a statement that "21.8 per cent of the children under 10 years of age were seronegative for Type 1 poliovirus." I want to be sure this is not a misprint.

We have had little occasion to do serological studies in adjacent countries (one in Guatemala) and we saw the results Dr. Alcocer obtained from a country adjacent to Costa Rica. That 21.8 per cent of the children under 10 should be seronegative Type 1 was certainly not the finding in the adjacent countries. In fact, it was almost zero per cent at the time the child reached six years.

If this observation is true, that this country was ripe for an epidemic, this should be taken into account as the reason why they perhaps had one, since this occurs also in the presence of a vaccination.

CHAIRMAN STUART-HARRIS: Dr. Alcocer, do you wish to answer Dr. Paul's question?

DR. ALCOCER: I should like to have Dr. Martins da Silva answer that question. He is more acquainted with the statistical data used in the paper presented to the Conference in 1959.

DR. MARTINS DA SILVA: The 21.8 per cent is correct. It was given last year at this Conference. It represents the percentage of negatives to Type 1 prior to the vaccination program in 1958.

DR. MURRAY: I believe that the last few reports given are important in the sense that they seem to have more of a different flavor than the corresponding reports presented at the Confer-

ence last year. The information now tends to be in better perspective. We can see more of what has happened; we tend to be more conservative in the type of opinions we derived from these results. I cannot help being impressed with the figures that were given in the case of Nicaragua. Even if we calculate the number of cases in terms of the Type 1 seronegatives, as was presented in the paper, a rough calculation comes out at a rate of somewhere near 700 per 100,000, which represents a really tremendous occurrence of poliomyelitis. One would hesitate to guess what would have happened if the rest of the population had not been vaccinated. Even so, one wonders just why and how the disease can be propagated at such a tremendous rate.

DR. QUIRCE: (*through an interpreter*): To confirm Dr. Paul's statements, I should like to say that the cases we had in Costa Rica were 86 per cent in children under five years of age.

An interesting point, which is worthwhile mentioning, is that during the most serious epidemic we had, in 1954, the attack rate was 118 per 100,000 inhabitants. In the outbreak we had in the first three months of the year in the metropolitan area, the attack rate was 128 per 100,000 inhabitants, among the non-vaccinated groups.

DR. VARGAS-MÉNDEZ: I wish to add something with regard to this problem of the enteroviruses. You saw by the chart that there were seronegatives to polioviruses up to the age of four years. We have also taken a large number of rectal swabs during the program. Since we have no virus laboratory (although we hope to have one in the near future, with the help of the Pan American Sanitary Bureau), we have to send the samples to Cali, Panama, and Pearl River. We have now received reports from Dr. Shelokov, Dr. Doany, and from Dr. Cox on findings which include: Coxsackie B-3, Coxsackie A-4, ECHO-8, ECHO-7, ECHO-14, ECHO-12, adenovirus, enterovirus of unknown origin, and so on. We hope to have more information about this point in the future. We have to investigate this and relate it in some way or other to the possibilities of interference.

DR. LANGMUIR: As I listened to the reports this afternoon I take some solace in the fact that our colleagues below the border have been experiencing some of the same problems as we have. It seems to me that all of us have been over-optimistic.

The attack rates in Nicaragua, and even in Costa Rica, seem to be higher than one would have anticipated had nothing been done at all. We have certainly observed that in this country, in Des Moines, Kansas City, Little Rock, Seattle, and New Haven. We have an attack rate in selected unvaccinated residual minority groups in our population which are excessive, higher than any recorded before, to my knowledge.

I believe that this is a very serious situation, the full import of which I am not yet prepared to judge, although I have gone on record as believing that this is due to selection out of viruses of greater virulence than were the average in prior times.

I believe the lesson, however, is quite clear. Regardless of whether or not we choose to follow an inactivated vaccine program or a live virus vaccine program, we shall have to strive, and somehow, we shall have to reach a much higher proportion of our population adequately and fully vaccinated than has been achieved as yet in most parts of the world.

In Dr. Voroshilova's report presented yesterday, I was deeply impressed with the amount of time and attention she took early in her presentation to emphasize the necessity of giving not just one triple vaccine once to the population, but I believe the plan calls for intensive effort at three months of age, four months of age, six months of age, the first birthday, the second birthday, and the third birthday—six doses are planned. This to me represents the realities of the situation: to fight the polioviruses will require a great deal of effort and a degree of organization which has not yet been achieved.

CHAIRMAN STUART-HARRIS: We shall now proceed with Dr. Zhdanov's paper on "Large-Scale Practical Trials and Use of Live Poliovirus Vaccine in the USSR."

27. LARGE-SCALE PRACTICAL TRIALS AND USE OF LIVE POLIOVIRUS VACCINE IN THE USSR

PROFESSOR V. M. ZHDANOV
with the participation of
PROFESSOR M. P. CHUMAKOV AND PROFESSOR A. A. SMORODINTSEV
USSR Academy of Medical Sciences, Moscow, USSR

DR. ZHDANOV *(presenting the paper)*: Since the text of my report* was distributed among the participants of the Conference, there is no need for me to read it. I shall therefore only try briefly to convey its content, and then mention some additional considerations on the problem. I have been encouraged to do so both by the interesting remarks made by Dr. Stuart-Harris and by Dr. Paul's profound and well-presented report.

The problem of mass immunization against poliomyelitis—a comparatively rare but severe disease—has suggested some specific requirements for the use of the vaccine, namely:

1. Complete safety of the preparations;
2. The practically full irreactivity of vaccination;
3. Sufficiently full efficacy of the vaccine, in the sense of prevention of the disease;
4. The possibility of a sharp reduction of poliovirus circulation;
5. Low cost of preparation, facility of production, and availability to the population;
6. A suitable and convenient method of administration.

The Salk vaccine meets the first three requirements mentioned above. Therefore, once its production was organized, an intensive offensive against poliomyelitis was started. However, soon thereafter, some deficiencies in this generally good preparation became evident.

Although it produces in individuals a specific defense against the paralytic disease, immunization with the Salk vaccine does not prevent the circulation of wild poliovirus strains because the intestinal-tract cells remain susceptible to them. Moreover, about 20 to 30 per cent of vaccinees remain unprotected against the paralytic disease, and the immunity decreases at a significant rate during subsequent years.

These deficiencies became dramatically known soon after the first triumphant years of the Salk vaccine, when severe epidemics of poliomyelitis appeared in Israel, Hungary, and some localities of the USA, despite the large percentage of vaccinated persons in those countries.

As a result of research work done by Sabin, Koprowski, Cox, Smorodintsev, Chumakov, and others, a basis for production and field trials of live poliomyelitis vaccine derived from attenuated virus strains was established, and this vaccine appeared to satisfy the six principal requirements mentioned above. Its particularly important property is the capacity to produce in vaccinees a degree of immunity which not only ensures the individual defense of the vaccinees but also renders the intestinal-tract cells insusceptible to the virus, thus sharply reducing the circulation of wild polioviruses among the population.

Other important advantages of this vaccine are its low cost, its availability to the population, and its simple method of administration, i.e., by the oral route, as compared to injections of the Salk vaccine.

The various stages of research and practical use of the live poliomyelitis vaccine in the USSR are well known to you. They constitute a good example of cooperation between scientists of the USA and USSR, namely, A. Sabin, M. Chumakov, A. Smorodintsev, and their associates. These stages were:

Research work done by A. Smorodintsev (1957-1958) and M. Chumakov (1958) and their associates on the safety of Sabin's attenuated strains, and carefully carried out mass experiments in children;

First field trials (1958) in some tens of thousands of the population;

Mass immunization of the population early in 1959 in the Baltic republics of Esthonia, Latvia, and Lithuania;

* For complete text, see pp. 578-587.

Immunization, in 1959, of 15 million persons in different parts of the USSR; and finally, the mass immunization campaign against poliomyelitis in 1960, with the aim of vaccinating about 75-80 million, that is, practically all of the population in the country susceptible to poliomyelitis.

Some figures on the subject are shown in the text of the paper, and the results of the field trial in the Baltic republics were mentioned in the report by Professor Chumakov.

I merely wish to add that the epidemiological situation in the Baltic republics, established as favorable in 1959, remained the same during the first four months of 1960. During this period, only two cases of polio were recorded in Esthonia, four cases in Lithuania, and seven cases in Latvia, as compared with tens and hundreds of cases in previous years in some other areas where the population had not been vaccinated. Dr. Sabin, who is acquainted with these data, considers that even these isolated cases may be considered as doubtful because of hyper diagnosis of the disease.

On the basis of research and field observations carried out in the USSR, we can summarize our conclusions as follows:

1. The safety of the live vaccine, prepared from Dr. Sabin's attenuated strains, has been proved and it has been shown that no reversion of vaccine strains took place during the entire vaccination campaign.

I should like to stress this point particularly in connection with vaccination carried out in Moscow and Leningrad, and in other large cities, where the public health service is fully adequate and where even isolated cases of the disease could not be overlooked.

2. It was established that the vaccine had a high degree of immunological effectiveness, and during this work optimal schemes of immunization were elaborated.

3. Virus secretion and its spread among the contacts appeared to be not only harmless but even favorable as a complementary useful property of the vaccine.

4. A convenient method of administration and use of the live polio vaccine was elaborated as dragée-candy.

5. It was shown that the vaccination can produce a satisfactory epidemiological effect in an inter-epidemic period as well as during an epidemic.

All this permits us not only to hope but also to be certain that the live polio vaccine is a very effective means of preventing infection. Moreover, large-scale use of the live polio vaccine has put forward as a theoretically possible and a practicable task the eradication of poliomyelitis.

This problem—the problem of eradicating poliomyelitis—needs, however, further thorough study.

Eradication of a disease which affects only man and has no reservoirs in nature (and poliomyelitis is an example of such a disease) can be achieved in two principal ways: by destroying the agent or by correcting the reactions of the host organism.

A classic example of the first way is the eradication of smallpox. The acute course of the infection and a highly effective post-infectious immunity, in the presence of a good vaccine which closely imitates natural postinfectious immunity, make theoretically possible and practically feasible the task of eradicating the disease; in other words, the complete eradication of smallpox throughout the world can be achieved by means of vaccinating the entire population of the globe.

This task is practically completed in the majority of the economically developed countries and now, on the initiative of the WHO, the problem is being solved in all parts of the world. I am optimistic and believe that during the next decade the smallpox virus will be eliminated throughout the world, remaining only in laboratories, like certain animals which die out and remain only in zoos.

An example of the second way is the eradication of Escherichia coli-dysentery, or, as we call it in Russia, coli-enteritis. There is little doubt that enteritis in infants, or at least an important part of this disease, is the pathological reaction of a certain portion of infants to the settlement of coli-bacteria in their intestines, which inevitably takes place when infants, at the age of three to six months, begin to be fed food in addition to their mother's milk. When the children reach the age of one or one and a half years, they are ready to bear these normal inhabitants of their intestines without any sequelae, but severe illnesses and even deaths may occur in

some of them. How is coli-enteritis to be prevented? Scarcely by destroying coli-bacteria, a very unrealistic task. A more realistic approach would be to seek means of correcting the pathological reaction of the child organism.

Turning back to poliomyelitis, or better, to the problem of eradicating poliomyelitis, one must clearly ask: In what way can and should we solve this task—by destroying the agent or by correcting the pathological reactions of the organism?

Poliomyelitis infection may be compared with an iceberg: most of it is under water and only the top, above. The part below the water is like asymptomatic infection and the top, a paralytic disease. What should be done to prevent collision with the iceberg—sinking or destroying it?

It seems to me that Salk vaccine acted only by correcting the pathological reaction of the human organism, whereas live vaccine makes practically possible the destruction of the poliovirus, that is, complete destruction of the iceberg. The live polio vaccine puts forward the problem—principally and practically—of eradication of poliomyelitis up to the complete destruction of the agent as a biological species.

However, this task is not so simple as it may seem, and therefore in beginning the offensive against poliomyelitis one must draw up a good strategy and a flexible tactic.

There are in human organisms two large cavities which communicate with the external world: the respiratory and the intestinal tracts. Numerous inhabitants populate them. Some appear in the host's early infancy and leave when the child's organism ripens immunologically and becomes an inconvenient place of habitation. Others come periodically for short visits. And some come to stay as long as the host lives. This parasite population varies from host to host, and not always is there a place for a newcomer like a vaccine strain of poliovirus.

Therefore, there are many specific problems connected with the necessity of increasing the efficacy of vaccination with the live poliomyelitis vaccine.

Mass vaccination is only the first step toward solving the problem of an offensive against poliomyelitis. Thereafter must follow a careful study of the conditions necessary for the successful immunization of different groups of the population—in cities and villages, in families and kindergartens, among different socio-economic groups, in different climatic zones, and at different seasons. It is necessary to determine the best and surest ways to suppress virus interference, remembering that it takes place not on a neutral soil but within the human organism, which helps itself with its immunological reactions.

Finally, one must elaborate different schedules of immunization under different conditions and in different countries.

And yet, in spite of all the difficulties which we already know and those which will confront us, the problem of eradication of poliomyelitis will be solved in the next years.

I should like to conclude by mentioning again the newly developing theoretical epidemiology, based on research done in the field of poliomyelitis. Papers given here show how far we have come from Topley's mouse towns.

I think that the further development of ecological epidemiology or pattern epidemiology will help us not only win the fight against poliomyelitis, but also intervene actively in the life of the inhabitants of the large cavities of the human organism—to destroy weeds and to seed cultured plants.

(*The complete text of the paper is as follows:*)

THE PROBLEM OF MASS VACCINATION AGAINST POLIOMYELITIS

The usual steps taken to combat epidemics—early detection and isolation of the sources of infection, quarantine, and disinfection of the foci of the disease—are palliative measures in the case of poliomyelitis because of the widespread occurrence of symptomless virus carriage and the impossibility of diagnosing all the inapparent infectious forms of the disease.

It is therefore generally accepted that successful control of the epidemic spread of poliomyelitis can be achieved only by really large-scale immunization of the whole susceptible population and, in the first instance, of the age groups from 0 to 15-20 years of age.

In view of this, the task of vaccinating many millions of persons against poliomyelitis, which is a comparatively rare disease, makes it necessary that the vaccine should fulfill certain par-

ticular, specific requirements, of which the following are essential:

(1) the vaccine must be completely *harmless;*

(2) there should be a complete absence in practice of untoward reactions to vaccination;

(3) the vaccine should be highly effective (it is desirable that immunity should develop and be maintained for several years in the overwhelming majority of those vaccinated);

(4) there should be as a result of mass vaccination *a sharp reduction in the circulation* among the population of "wild" epidemic strains *of the causative agent of poliomyelitis;*

(5) it should be *easy to supply* high-quality, harmless vaccine for carrying out mass vaccination of the population against poliomyelitis (on any scale) and this means that there must be an inexpensive and highly productive method of preparing the vaccine and reliable procedures for assessing its quality;

(6) there should be *a simple and convenient method* of *administering the vaccine* which would enable mass vaccination to be speedily carried out.

Only if all these conditions are fulfilled will it be possible to carry out the task of complete immunization of the whole susceptible population against poliomyelitis in the shortest possible time.

The use of the prophylactic vaccine against poliomyelitis, made by the method of Jonas Salk (1951-1954) from formalin-inactivated virus, was the first great achievement in the large-scale specific prophylaxis of poliomyelitis. The Salk vaccine to a considerable degree satisfies the first three of the six requirements for poliomyelitis vaccine listed above: it is harmless, causes no reactions in practice, makes it possible to reduce the incidence of paralytic forms of the disease by 70-80 per cent, and sharply reduces mortality. The technique of mass-producing this vaccine was mastered in the Soviet Union in 1956-1957 and during the last four years about 24 million ml. of the vaccine have been produced and used in the Soviet Union (about 7 million persons have been inoculated). The Salk vaccine made in the Soviet Union fully satisfied the high standards demanded.

Nevertheless, the Salk vaccine is not sufficiently effective. It leaves as many as 20-30 per cent of vaccinated persons unprotected against paralysis. The immunity it establishes is not complete and solid. In persons vaccinated with the Salk vaccine the cells of the digestive tract, the main "portals of entry" of the poliomyelitis infection remain susceptible to infection with the poliovirus, which is thus able to multiply and to be transmitted to others. In view of the further fact that it is in practice impossible to ensure 100 per cent inoculation of all children by injections of Salk vaccine (because of the many reasons for exemption based on contra-indications and the growing up of new age groups, etc.), the polioviruses continuing to circulate among the population indisputably constitute a threat of new poliomyelitis epidemics. During recent years several examples of the occurrence of epidemics have accumulated (in Israel, 1958, in a number of regions of the United States of America, and in Hungary, 1959, etc.), despite the extensive campaigns for large-scale injection of Salk vaccine previously undertaken.

The production of a sufficiently immunogenic inactivated vaccine and the laboratory testing of its harmlessness require great efforts and considerable expenditure; this slows down the rate of development of production and makes it impossible to ensure the timely immunization of all who need it.

The very technique of intramuscular or subcutaneous injection of the killed vaccine is too cumbersome for giving repeated injections (a minimum of four is needed) to many tens of millions of people.

All this gave impetus to the search for new methods of specific prophylaxis against poliomyelitis. The most promising method proved to be the use of live vaccines.

Experimental research and epidemiological and clinical investigations (A. B. Sabin (1951-1959), Koprowski *et al.*, Cox, Cabasso *et al.*, A. A. Smorodintsev, M. P. Chumakov *et al.* (1959)) laid a good theoretical foundation for the elaboration of suitable methods and for large-scale testing of a live vaccine made from attenuated poliovirus strains completely harmless for man.

The main advantages of immunization with live vaccine over immunization with inactivated vaccine are as follows:

(a) the convenience of oral administration compared with injections;

(b) a saving of roughly one hundred-fold in inoculation material compared with the killed

vaccine which makes it possible to reduce the use of monkeys considerably and to cut down other production expenditures;

(c) the possibility of establishing more complete immunity by developing an insusceptibility in the cells directly situated in the "portals of entry" of the infection, i.e., in the walls of the alimentary tract (including the tonsillar ring) which could not be ensured by injections of the killed vaccine. The poliovirus is capable of multiplication in those vaccinated with the Salk vaccine, but does not gain a hold in the intestines of persons immunized with the live vaccine.

This circumstance is very important for reducing the extent to which dangerous paralytogenetic "wild" strains of epidemic poliovirus circulate among the population and eliminating the risk of poliomyelitis epidemics.

Dr. A. B. Sabin (Cincinnati, Ohio, USA), using the isolated colonies technique (the plaque method), successfully selected and purified attenuated vaccinal strains of poliovirus of three types which satisfied the special requirements of the WHO Expert Committee on Poliomyelitis (1957).

A. B. Sabin's live vaccine was successfully tested in 1954-1958 on small groups of people in the United States of America, the Netherlands, and Mexico (under 4,000 persons to begin with), then in Singapore (200,000 persons in 1958), in Czechoslovakia (143,000 children in 1958-1959), and in the USSR (over 30,000 persons in 1957-1958).

The Sabin vaccinal strains were subjected to careful tests in Leningrad and Moscow in 1957-1958. Professor A. A. Smorodintsev and his colleagues in Leningrad demonstrated the absence of any appreciable reactions to the live vaccine made from the A. B. Sabin strains when over 1,200 small children were orally immunized. In 1957-1958, in the same institute, the stability of the main characteristics of the attenuated Sabin strains was established, since, despite a series of consecutive passages through the intestinal canals of completely susceptible children (children possessing no antibodies), no appreciable intensification in neurovirulence for monkeys occurred in the vaccinal strains. Observations in 1958 at the Institute for the Study of Poliomyelitis in Moscow also confirmed the main laboratory characteristics of the attenuated strains and the harmlessness for man of peroral immunization with the Sabin vaccine.

In September 1958 the Poliomyelitis Vaccination Committee of the USSR Ministry of Health (Chairman, Professor V. M. Zhdanov) raised the question in the USSR Ministry of Health and before the Presidium of the Academy of Medical Sciences of the USSR of proceeding with trials of the live antipoliomyelitis vaccine in epidemic conditions on a larger scale. In October 1958 the Presidium of the USSR Academy of Medical Sciences adopted a resolution approving the beginning of practical trials of the method of peroral immunization of the population with a live vaccine made from the attenuated strains, harmless for man, of Types 1, 2, and 3 selected by A. B. Sabin.

In November 1958 the USSR Ministry of Health, at the insistence of the Presidium of the Academy of Medical Sciences, authorized Professor A. A. Smorodintsev and Professor M. P. Chumakov to carry out practical tests of the live vaccine for the immunization of 40,000 children against poliomyelitis. In November 1958 the Vaccines and Sera Committee of the USSR Ministry of Health issued temporary instructions for the production and control of live poliomyelitis vaccine made from the Sabin strains and regulations governing its use for human immunization.

In 1958 and 1959 Professor A. A. Smorodintsev and his colleagues in the Virology Department of the Leningrad Institute of Experimental Medicine prepared about 2,000,000 doses of live vaccine of Types 1, 2, and 3 from the Sabin strains.

At the end of 1958 and the beginning of 1959 the Institute for the Study of Poliomyelitis of the USSR Academy of Medical Sciences used Sabin strains of Types 1, 2, and 3 to prepare large batches of live vaccine made from Sabin strains.

This vaccine passed extensive control tests with good results and in addition was subjected by A. B. Sabin himself in the United States to comparative tests on monkeys with other samples of live vaccine; his findings were favorable.

From January-April 1959, in compliance with a decision of the Collegium of the USSR Ministry of Health, the Institute for the Study of Poliomyelitis carried out the oral immunization of 27,000 persons in the Estonian and Lithuanian SSR with the original live vaccine received

from A. B. Sabin in the United States. At the same time, under the guidance of Professor A. A. Smorodintsev, more than 12,000 persons in the Latvian SSR were immunized with a live vaccine prepared in Leningrad from Sabin strains which had undergone a number of passages in liver-cell cultures.

The extremely favorable results obtained from this first mass immunization with live vaccine in the USSR, which demonstrated that the vaccine was completely safe, encouraged a number of Ministries of Health in the Union Republics to decide to turn to mass immunization with live antipoliomyelitis vaccine of Soviet manufacture. These decisions were approved and supported by the Poliomyelitis Vaccination Committee, attached to the USSR Ministry of Health, and by officials of the Ministry.

In the period from March to July 1959 in the Esthonian, Lithuanian, Kazakh, Latvian, Byelorussian, and Moldavian SSR, and also partly in the territory of the RSFSR, large-scale oral vaccination with live vaccine was successfully carried out with a coverage of over 3,500,000 persons, mainly in the age group from two months to 20 years. In Esthonia and the city of Alma-Ata, age groups up to 40-50 years were immunized.

The first results of this large-scale vaccination campaign were discussed at a meeting of the Presidium of the USSR Academy of Medical Sciences on 13 May 1959 and at an All-Union Scientific Conference on Live Vaccine held from 22-25 May 1959 and attended by research workers from the United States of America, Czechoslovakia, Hungary, Bulgaria, and China. They were subsequently discussed at the First International Conference on Live Poliovirus Vaccines held in Washington under the auspices of the Pan American Health Organization/World Health Organization from 22-26 June 1959.

In May 1959 the Presidium of the USSR Academy of Medical Sciences noted the great scientific and practical importance of the work carried out by the two poliomyelitis institutes of the Academy in studying live poliovirus vaccine, and pointed to the need for a further extension of vaccination with a view to determining the epidemiological effectiveness of oral vaccination under various conditions. The question of studying the stability of vaccinal strains and their possible interference with other enteroviruses was also put forward as an urgent task for research on live vaccine.

Between May 1959 and November 1959, at the request of Ministries of Health and various Oblast Departments of Health, extensive work was continued on peroral immunization with live vaccine produced in the Institute for the Study of Poliomyelitis of the USSR Academy of Medical Sciences. The total number of persons vaccinated with the live vaccine by 30 December 1959 exceeded 15,200,000. Immunization was carried out in all republics except Turkmenia (see Table 1). In addition,

TABLE 1. PROVISIONAL DATA ON THE NUMBER OF PERSONS IN THE USSR VACCINATED WITH THE LIVE ANTI-POLIOMYELITIS VACCINE BY 30 DECEMBER 1959 (TO THE NEAREST THOUSAND)

RSFSR	3,310,000
Ukraine	2,378,000
Kazakhstan	1,590,000
Uzbekistan	2,200,000
Byelorussia	540,000
Georgia	730,000
Azerbaidzhan	770,000
Moldavia	380,000
Lithuania	547,000
Latvia	480,000
Kirghizia	500,000
Tadzhikistan	380,000
Armenia	700,000
Turkmenia	—
Esthonia	695,000
Total	15,200,000

Note: The total population in the areas covered by the inoculation campaign is about 65,000,000 persons.

more than 2,300,000 children were inoculated with Type 1 of the vaccine in Hungary, about 40,000 children in Bulgaria, 3,000 children in Pekin, and about 50,000 children in Hanoi, People's Republic of Viet Nam.

In a number of cities and oblasts live poliovirus vaccine was successfully used in 1959 in the period when morbidity rises (July, August and September) with a view to possibly influencing outbreaks and reducing the incidence of the disease.

On 25 November 1959 the Presidium of the USSR Academy of Medical Sciences again discussed the results of study and use of the live anti-poliomyelitis vaccine made from the Sabin strains and approved the large-scale utilization of the vaccine for specific prophylaxis in the USSR. The USSR Ministry of Health issued a decree concerning the oral immunization with live vaccine of the whole population of the Soviet Union between two months and 20 years of age in 1960. The vaccination program envisages the immunization of 75,000,000 persons during 1960, 52,000,000 of them before July.

The Poliomyelitis Institute of the USSR Academy of Medical Sciences (Director M. P. Chumakov) was entrusted the task of supplying the whole country with live vaccine and giving guidance on methods of use. In fulfillment of this task, the Institute drew up instructions on methods of organizing vaccination with the live vaccine; these instructions were approved by the Ministry.

Preliminary results of the implementation in 1960 of the mass immunization program in the Soviet Union, covering persons up to 20 years of age, showed the following features.

In the first three months of 1960, 104,283,000 vaccinal doses of live vaccine prepared in Moscow from the A. B. Sabin strains were distributed throughout the country. They included 64,791,000 doses of monovaccine of Type 1, 19,558,000 doses of monovaccine of Type 3, 5,297,000 doses of monovaccine of Type 2, and 14.637,000 doses of a trivalent mixture of Types 1, 2, and 3 used in the re-vaccination of persons to whom single types of the live vaccine had been administered earlier (2,704,000 doses of the trivalent vaccine were used for primary vaccination) (see Table 2).

In the RSFSR, the Ukrainian SSR, Latvia, and Armenia, three-stage vaccination with monovaccines will be carried out. In some oblasts of the Ukraine a trivalent mixture has been administered twice, while in the remaining oblasts monovaccines have been administered separately.

Taking into account the usual wastage which occurs (15-20 per cent) in the distribution of vaccine during a campaign, it can be estimated that in the first quarter of 1960 Type 1 of the live poliovirus vaccine was administered to about 55 million persons in the Soviet Union (roughly 70 per cent of the population under 20 years of age). Of these, about 15 million have also been given in 1960 a monovaccine of Type 3 and about 4 million persons have been given all three doses. Over 14 million persons to whom a live vaccine was administered in 1959 have been revaccinated under the 1960 program. Vaccinations continued successfully in the second quarter of 1960. The results of this work are set out in Tables 1 and 2.

In addition to ensuring the implementation of the internal program of large-scale vaccination against poliomyelitis with the live vaccine made from the Sabin strains, the Soviet Union, as part of its friendly assistance to those countries, has sent vaccines of Type 1 and 3 separately for 2,500,000 children in Hungary, of Type 3 and Type 2 for between 2,500,000 and 2,000,000 persons in Czechoslovakia, for 1,500,-000 persons in Viet Nam, and 2,200,000 in Bulgaria. In addition, small quantities have been sent to Albania and China.

We do not doubt but what the extensive program of live vaccine administration we have outlined will be fulfilled. Of course, such a scale and rate of anti-poliomyelitis vaccination would be quite impossible with the killed-virus Salk vaccine. Only oral immunization with live poliovirus vaccine makes it possible to carry out mass vaccination of the susceptible population within a short period. This is a guarantee of radical prophylaxis against poliomyelitis epidemics.

The organization of the campaign and observation of those vaccinated have been carried out under the direct guidance, so far as method is concerned, of members of the staff of the Poliomyelitis Institute and the Virology Department of the Leningrad Institute of Experimental Medicine of the USSR Academy of Medical Sciences. The vaccines were administered by district

TABLE 2. DATA ON THE DISTRIBUTION OF THE LIVE POLIOVIRUS VACCINE MADE FROM A. B. SABIN STRAINS THROUGHOUT THE REPUBLICS OF THE SOVIET UNION IN THE FIRST QUARTER OF 1960

REPUBLIC	NUMBER OF VACCINAL DOSES ISSUED, IN THOUSANDS					
	MONOVACCINE OF			TRIVALENT MIXTURE OF TYPES 1, 2, AND 3	TOTAL	INCLUDING NUMBER OF DOSES USED IN PRIMARY IMMUNIZATION
	TYPE 1	TYPE 2	TYPE 3			
RSFSR	36,535	2,242	13,674	2,050	54,501	36,535
Ukraine	11,753	2,123	2,076	5,117	21,059	12,023
Byelorussia	3,000	—	2,910	—	5,910	3,000
Uzbekistan	2,976	—	—	4,406	7,382	3,500
Kazakhstan	2,294	—	120	353	2,767	2,100
Georgia	1,500	—	—	—	1,500	1,500
Azerbaidzhan	1,404	30	—	—	1,434	1,400
Moldavia	600	—	—	900	1,500	1,100
Lithuania	750	—	—	—	750	750
Latvia	300	—	—	400	700	700
Kirghizia	800	—	—	—	800	800
Tadzhikistan	750	—	—	1,101	1,851	750
Armenia (in December 1959–900,000 doses)	—	902	900	—	1,802	900
Esthonia	850	—	—	—	850	800
Turkmenia	1,478	—	—	—	1,478	1,100
Total	64,990	5,297	19,680	14,327	104,284	66,958

Note: In view of the large amount of immunization with live poliovirus vaccine done in a number of republics in 1959, the whole population of those republics (Esthonian SSR, Lithuanian SSR, Georgian SSR, Azerbaidzhanian SSR, Moldavian SSR, Uzbek SSR, Tadzhik SSR, Kirghiz SSR, and Turkmenian SSR) will receive two doses in 1960, the first consisting of a monovaccine of Type 1 and the second of a trivalent mixture of Types 1, 2, and 3.

medical establishments on a strictly voluntary basis. The public was drawn into the work to the greatest possible extent by means of extensive health education and propaganda, use being made of the press, radio, television, newsreels, talks, leaflets, etc.

In the areas covered by the campaign, consultative teams of experienced physicians were set up for a thorough investigation of all cases of disease of the central nervous system which might give rise to a suspicion of poliomyelitis. In the Esthonian and Lithuanian SSR Epidemiological Bureaux were established for detecting and investigating all cases of poliomyelitis. In addition, travelling groups of consultants were organized in Esthonia, Lithuania, the Moscow Oblast, the city of Karaganda, the Sverdlovsk and Orenburg Oblasts, etc. More than 1,500 vaccinated persons and persons in contact with them were given serological and virological examinations. The laboratory diagnosis of cases of poliomyelitis in areas covered by the campaign was considerably extended and consolidated in the Baltic republics, Tashkent, Alma-Ata, Karaganda, Moscow Oblast, etc. All this has made it possible considerably to improve the organization of mass vaccination, to provide for careful observation to be kept over the vaccinated, to intensify the diagnosis of poliomyelitis, and to ensure the fullest possible registration of all cases of illness during and after administration of the vaccine. In children's establishments in a number of places long-term observations of the temperature and state of health of the vaccinated children and children in contact with them were carried out.

We laid down as our *main principle* in organizing oral administration of live anti-poliovirus vaccine that it should be on a *mass scale* and *simultaneously carried out* throughout the territory of a whole rayon, city or oblast in order to establish extensive immunity among the population in the shortest possible time and to reduce to a minimum the possibility of lengthy circulation among susceptible persons of any strains of poliovirus and to eliminate the possibility of an increase in their virulence through serial passage in children.

The first results of the study and mass use of live poliovirus vaccine made from Sabin strains in the USSR in 1959 were summed up in Report No. 2 of the Institute for the Study of Poliomyelitis of the USSR Academy of Medical Sciences and of many Republican, Oblast, and City Sanitational and Epidemiological Centers.*

On a basis of the extensive research carried out in the Soviet Union by several teams of scientists and of practical trials, the following conclusions can be drawn concerning the most important questions connected with live poliovirus vaccine:

1. *Harmlessness of the Live Vaccine.* We may consider as completely settled the question of whether the live vaccine made from the Albert Sabin strains and in the manufacture of which certain conditions have been fulfilled, are completely harmless and will cause no reactions. The practical trials of the vaccine on a very large scale have merely confirmed the fact. The safety tests for the vaccine during manufacture perhaps still need simplification and improvement, but even in their present form they ensure reproducibility of results.

The problem of reversion of the pathogenic properties in the strains of live poliovirus vaccine was eliminated in practice by the simultaneous coverage of the whole susceptible population in an oblast by a large-scale immunization campaign, since immunity in the population developed more quickly than could changes in the poliovirus strains, which were thus not given an opportunity of continuous passage. No cases of poliomyelitis caused by the actual administration of live vaccine made from Sabin strains have been recorded in the Soviet Union.

The reaction-causing properties of the live poliovirus vaccine were studied by many physicians in the vaccination areas on the basis of the material provided by records of complaints from those vaccinated. In the opinion of the specialists, under conditions of mass vaccination, cases of the coincidence of vaccination with symptoms of various kinds caused by other diseases may be observed. It proved impossible to establish beyond doubt a connection between any complaint or symptom and the vaccine itself. The total incidence of so-called vaccinal reactions was very low (not more than 3 per 100,000). The question of the reaction-causing properties of the live vaccine evidently still re-

* *On Mass Oral Immunization of Population in the Soviet Union against Poliomyelitis with Live Vaccine from A. B. Sabin's Attenuated Strains*, Moscow, 1960.

quires more thorough study but it is not of great practical importance.

2. *The Immunological Activity of the Live Poliovirus Vaccine.* Extensive serological research has been carried out in the Soviet Union on the basis of several thousand paired blood specimens taken from persons to whom the live vaccine had been administered under various epidemiological conditions or in connection with various vaccination schedules.

This research proved that as a rule the immunological activity of attenuated Sabin strains is fully comparable with the best standards of the inactivated Salk vaccine and even in general surpasses them in respect of the earliness of appearance and duration of humoral immunity.

In the Soviet Union, the immunization schedule most often used was the administration of separate monovaccines of Types 1, 2, and 3 followed by revaccination with a trivalent mixture. At the same time, large-scale trials of a single- or two-stage administration of the trivalent mixture were undertaken. According to serological data and epidemiological observations, these schedules also produced good results. Obviously, it is advisable to leave a choice of several schedules of live vaccine administration in accordance with the epidemiological situation and local conditions. The results of serological examinations show the influence of the time factor on the gradual intensification of the immunological response of the vaccinated to vaccination. The results of antibody titration three months after immunization with Type 1 were definitely better than one month after.

During the mass immunization and re-immunization campaign it was established that the live poliomyelitis vaccine, in contrast to the killed-virus Salk vaccine, creates not only a humoral but also a local immunity-resistance of the cells of the alimentary tract, including the tonsillar ring, leading to the gradual restriction of the circulation of poliovirus strains among the public.

Questions of the dynamics of development of local immunological resistance against poliovirus still require further study and more detailed research.

3. *Virus Carriage and Contact Transmission.* Oral immunization with live poliovirus vaccines is followed by excretion of vaccinal strains in the feces for a more or less lengthy period. Around those vaccinated all persons susceptible to poliomyelitis become infected with the vaccinal strains in a very short time and undergo latent immunization. No undesirable phenomena were noted with regard to virus carriage and transmission of the vaccinal virus. On the contrary, it can be considered as established that virus carriage and contact transmission under conditions of immunization with the live vaccine are exclusively favorable factors, which make possible a more solid and speedy immunization of families and communities.

4. *Interference.* Under conditions of oral immunization with live poliovirus vaccine, special importance is attached to phenomena of interference between street and vaccinal strains of poliovirus, between the non-poliomyelitic enteroviruses (of the ECHO and Coxsackie groups) and the live vaccine, and internal group interference between the individual types of virus in the live vaccine itself.

As a result of extensive research, it has been established that interference between vaccinal and other enteroviruses takes place with varying frequency under different epidemiological conditions and in different months of the year. The winter months are evidently the most favorable season for vaccination. The data obtained made it possible to draw the temporary conclusion that the majority of cases of failure of oral immunization can be attributed to the influence of interference on the vaccinal process. Interference can probably be overcome by means of vaccination on an especially large scale (covering not less than 50 per cent of the susceptible population) and as a result of repeated vaccinations over one or two years.

The laws governing interference between enteroviruses and the vaccinal strains of poliovirus require further, more complete investigation.

5. *The Use of Dragées Containing Live Poliovirus Vaccine.* A series of investigations in the Institute for the Study of Poliomyelitis of the USSR Academy of Medical Sciences have shown that the vaccinal strains are preserved inside the dragée and have demonstrated the possibility of enclosing the virus in dragées for use in mass immunization campaigns. The "take rate" of the vaccinal virus administered in the dragée form was just as satisfactory as that of the liquid product. The serological results of

immunization with vaccine in dragée form were fully comparable to the response to immunization with liquid live vaccine.

This has provided a basis, in 1960, for turning to the large-scale use of dragées containing live anti-poliomyelitis vaccine in carrying out the program for mass vaccination of the population up to 20 years of age.

The advantages of immunization with dragées containing vaccine under conditions of mass vaccination in the Soviet Union, lay in the fact that the vaccine was received in ready-for-use dragée form and there was no need to dilute the vaccine at the vaccination center. Vaccination by this means is also more attractive for children and is easy to carry out not only for medical workers in the medium and junior grades but also for teachers in schools and children's establishments. Vaccine in dragée form can be distributed in a short period and on a very large scale. Some further difficulties in this matter, such as the need for the vaccine dragées to be used quickly, to be stored at refrigerator temperature and to be transported in containers cooled to 10° C. proved fairly easy to overcome. Vaccine in the form of dragées is more convenient in certain conditions than the liquid live vaccine.

6. *The Epidemiological Effectiveness of Mass Immunization with Live Poliovirus Vaccine in the Soviet Union According to the Data for 1959.* In six republics of the Soviet Union; Esthonia, Lithuania, Kazakhstan, Byelorussia, Moldavia, and Latvia, and also in a number of Oblasts in the RSFSR, three-stage immunization with the live vaccine was carried out in 1959 before the beginning of the summer poliomyelitis season, or in conditions favorable for comparison of the morbidity rate among vaccinated and unvaccinated persons. These observa-

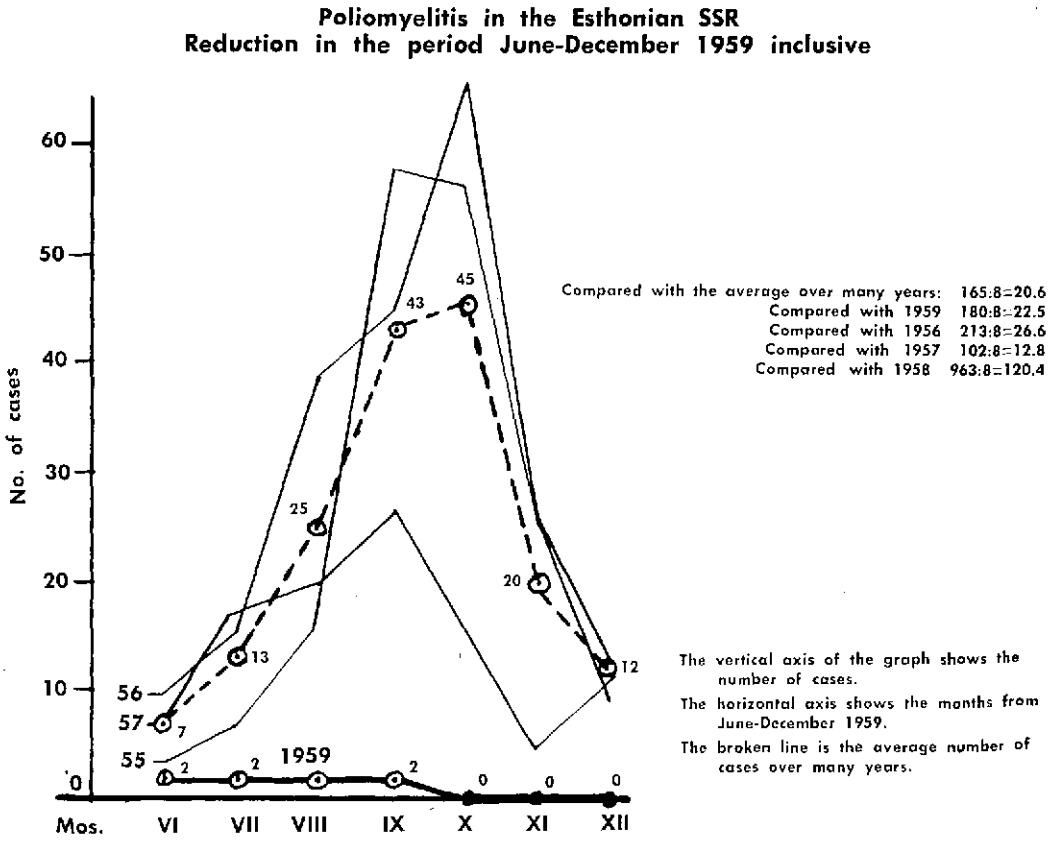


DIAGRAM 1. Poliomyelitis in the Esthonian SSR. Reduction in the number of cases in 1959 after immunization with the live vaccine.

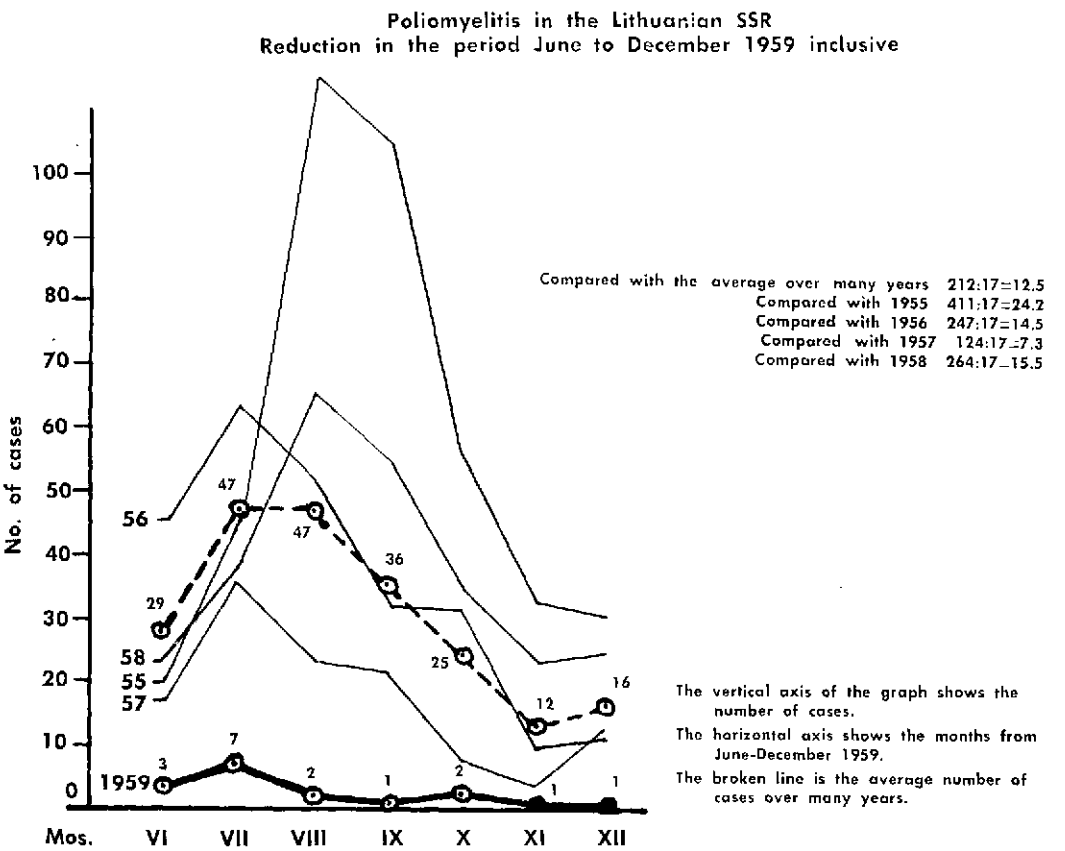


DIAGRAM 2. Poliomyelitis in the Lithuanian SSR. Reduction in the number of cases in 1959 after immunization with the live vaccine.

tions, carried out in carefully controlled surveys, established that mass immunization with live poliovirus vaccine is highly effective from an epidemiological point of view.

In the Esthonian, Lithuanian (see Diagrams 1 and 2), and Latvian Republics, where mass vaccination had been finished in the main before 1 June 1959, a sharp reduction in morbidity (down to single cases) was achieved in the second half of the year. This can be considered as a proof of the effectiveness of the live vaccine.

In the Moscow Oblast, the City of Karaganda and some other regions, where oral administration of trivalent vaccine was begun at the height of the seasonal rise in morbidity, a considerable reduction was nevertheless noted in the number of cases among vaccinated persons compared with the number among the unvaccinated (between 9.5 and 10.8-fold).

It is important to note that single-stage mass vaccination with trivalent live vaccine carried out during a poliomyelitis epidemic in Tashkent in 1959 fully confirmed the possibility of seriously reducing the epidemic incidence of poliomyelitis in five weeks as a result of the interference of the vaccinal virus with the epidemic process and the increasing level of immunization of susceptible groups.

In conclusion, a feature worthy of comment is the fruitful international cooperation on the problem of the live poliovirus vaccine which has grown up between scientists in the USA, the Soviet Union, Czechoslovakia, Hungary, the German Democratic Republic, the People's Republic of China, Bulgaria, etc. on the basis of a wide exchange of scientific information, personal meetings, the exchange of the results of research work and mutual support.

What has been done so far is undoubtedly only a very promising beginning to regular inter-

national cooperation between medical research workers in the future with a view to the possible elimination of some infectious diseases or a sharp reduction in their number.

BIBLIOGRAPHY

Chumakov, M. P., *et al.:* "The mass oral immunization of the population in the Soviet Union against poliomyelitis with live vaccine made from attenuated Sabin strains." Moscow, 1960.

————: *Vopr. virus.* **5**:520-529, 529-533, 1959.

Proceedings of the Fourth Session of the Institute for the Study of Poliomyelitis of the USSR Academy of Medical Sciences and of the International Symposium on Live Poliovirus Vaccine, Moscow, 17-20 May, 1960.

Smorodintsev, A. A., *et al.:* Results of a study of the reactogenic and immunogenic properties of live antipoliomyelitis vaccine. *Bull. World Health Org.* **20**:1053-1074, 1959.

NINTH SESSION

FRIDAY, 10 JUNE 1960, 2:00 P.M.

Chairman

DR. OSCAR VARGAS-MÉNDEZ
Director General of Health
San José, Costa Rica

(DISCUSSION)

SUMMARY OF THE CONFERENCE

APPENDIX

DISCUSSION

CHAIRMAN VARGAS-MÉNDEZ: The meeting is now called to order. The first part will be devoted to reports by those who may be interested in giving them, or to reports on programs now in progress.

DR. SABIN: I do not know whether this is the best time for this report; perhaps it should have been presented at an earlier session of this Conference.

The purpose in giving it is to describe a mass vaccination with live poliovirus vaccine, which has been carried out in Cincinnati on 175,000 children from about three months to approximately 18 years of age, including the vast majority of the school children.

This program was begun on 24 April, on the initiative of the Cincinnati Board of Health. The chief reason for this undertaking was that, as in the rest of the country, many cases of paralytic poliomyelitis were still occurring, particularly in the unvaccinated preschool-age group, and in the poorer areas of Cincinnati.

In 1959, 26 cases of poliomyelitis occurred in Cincinnati, 13 of them preschool-age children living in the poorer sections of the city.

At first the Board of Health decided to give the vaccine only to the preschool-age children. The private physicians cooperated in this program. People could go either to their own physicians and receive the vaccine or to clinics established by the health department and many hospitals. There was no charge either for the physicians' services or for the vaccine.

The program was under the direction of the health department and myself, and we wanted to have all of the vaccine administered within a period of one week. This is in line with the concept of procedures used in eradication programs, which I personally advocate.

Approximately 300 private physicians cooperated, and within a period of one week they themselves administered the vaccine to about 50,000 children, and the health department and hospital clinics, to about 25,000 children—a total of 75,000.

I should tell you that Cincinnati itself has a population of about 480,000, and together with the surrounding county, the total is about 900,000.

The program was started only for Cincinnati children, but very soon the county also wished to participate. What proportion of the 75,000 children represents the population under six I do not know, for our census is not yet complete, but it does represent the major proportion.

The purpose of this program was not only to provide additional immunization, but also to determine whether or not it might be possible by this mass vaccination to break the chain of transmission of polioviruses.

We also wanted to know what the immunization effectiveness would be in a population such as that in a city like Cincinnati, with different socio-economic groups. Therefore, we collected rectal swabs from over 1,000 preschool-age children of two different socio-economic groups, about half from those attending the clinics and another half from those being attended by their private physicians.

By this time, only about 600 of these 1,000 specimens have been fully tested, but I think it is extremely interesting that even though the temperature has already been high for 10 days in Cincinnati—by high I mean that it reached 29° to 30° C.—by 24 April the incidence of enteric viruses isolated by the same techniques we used in Toluca was only about 4 per cent from the children in the poor areas of the city in contradistinction to 72 per cent that I showed you for Toluca; and none out of 204 specimens tested were from children attended by private physicians.

Now, this is a very striking difference. Those people live in crowded areas, in rather poor houses, but have plenty of running water and other facilities which apparently were not available in Toluca.

We also obtained blood specimens from about 750 children, also from different socio-economic groups, for two purposes; one, to find out what the immune status was in the children who had

no Salk vaccine at all, as well as those who had three or more doses; and two, to measure conversion rates subsequently.

Among the children in the poor areas of the city we bled at least 54 children who had had no Salk vaccine at all and we found one interesting thing: Type 1 poliovirus must have been spreading among them during the past six months because about 50 per cent in each group from seven to 11 months, one to two years and two to three years already had antibodies for Type 1.

It is therefore of interest that the only poliovirus that we isolated among the small number of agents recovered from the rectal swabs was Type 1 poliovirus, which was present in the community before we started the vaccination program.

If we assume that rectal swabs are efficient only to the extent of about 30 per cent, we might estimate that just before feeding of the vaccine during the last week in April, roughly 1 per cent of the children in the poor areas of the city was already disseminating Type 1 poliovirus.

I should like to elaborate further on the soil on which the Type 1 vaccine was seeded. We obtained records on every child not only regarding his own extent of Salk vaccination but also that of his parents and siblings.

The situation in Cincinnati is somewhat like this in preschool children: among the preschool children who had their own private physicians, 73 per cent had had three or more Salk vaccinations; on the other hand, the children who came to all the clinics, representing generally a mixed group, only 54 per cent. But when we limited our investigation to the children from the poorest parts of the city, only 34 per cent had had three or more doses of Salk vaccine.

Furthermore, it is also interesting to note that among the children aged one to two years, who had three doses of Salk vaccine, and who were attended by private physicians, 34 per cent had no demonstrable Type 1 antibody.

I limited this analysis to one- and two-year-old children because (a) it would represent the more recent vaccine used, and (b) it would also give us only the children who had their last dose not more than one year ago.

It was among this kind of population that, within one week, about 75,000 preschool children received the Type 1 vaccine only. The dose was 200,000 plaque-forming units by actual titration of a portion of the diluted vaccine used.

Two and a half to three weeks went by and then, because New York State decided to carry out a program on school children as well as preschool children, Cincinnati demanded that all the school children be included if enough vaccine was available, and they were.

Within a matter of about four days, 90,000 school children received Type 1 oral poliovirus vaccine, all of it being given in the schools after permission cards had been obtained from the parents. They just had a recess for vaccination, and a whole school was covered in no time at all. So it can be done very simply and quickly. Subsequently, about 10,000 additional school children in adjacent county schools also received the vaccine.

It is now six weeks since the 75,000 preschool children were fed the Type 1 vaccine and only about three weeks since the school age children were fed.

The school-age children had much more Salk vaccinations—well over 80 per cent had three or more doses.

But I think it is of interest also to look into the contacts of the preschool children, with regard to Salk vaccine status. We discovered that 46 per cent of the fathers, mostly young fathers of these preschool children, had had no Salk vaccine at all. Seventy-five per cent of the mothers did have Salk vaccine, but of course, not all had three doses. So there is a rather large contact group among young fathers and mothers without Salk vaccine protection.

We set up a very careful surveillance program, supplying tubes and materials for the collection of specimens on any suspect case of central-nervous-system disease that might come into the hospitals.

I should like to tell you that, prior to the beginning of the program in Cincinnati, there was only one reported case of clinical polio in 1960, but the clinical diagnosis must be questioned. As a clinician, I would not have made the diagnosis of poliomyelitis on the findings in this case. It had no pleocytosis. It had hyperactive reflexes with a partial paralysis in one leg. Fortunately,

a stool specimen was obtained early after onset, so that the negative isolations are of some significance. The stool was sent both to the State Health Department and to our own laboratory. We could not isolate any virus.

The child who had had no Salk vaccine had no Type 1 or Type 3 antibody in both the acute and convalescent phase sera, and only a low level of Type 2 antibody, which did not change by the high-avidity test.

So we have no evidence that there was a single case of polio in Cincinnati in 1960 prior to the oral vaccine program. *And there has not been a single case since that time.*

What we have had to investigate were three instances of mumps, where aseptic meningitis was suspected; but in one it was only meningismus, because there was no pleocytosis.

We had one case of acute transitory encephalitis in a child nine years of age who developed fever, vomiting, convulsions, and rash *one day* after feeding of the vaccine. This lasted three days. The child, who had a pleocytosis of 180 cells per cubic millimeter of cerebrospinal fluid, recovered completely. The child had had only one dose of Salk vaccine, and two successive stools, taken four and five days after feeding of the vaccine (on which the data are already complete), yielded no virus at all. So it was probably a naturally immune child in whom the vaccine virus did not multiply.

We had (on request of the physicians) to investigate one patient suffering from acute tonsilitis, with a temperature of 106, but with no pleocytosis; nothing else developed and it quickly cleared up. There was also a child with fever for two days, with some meningismus, but without pleocytosis. No virus was recovered from the stools. It was a child who previously had had five doses of Salk vaccine, and this meningismus was 25 days after the feeding of Type 1 vaccine.

Our program at the present time obviously calls for continued surveillance. The objective is to see whether this extensive coverage will result in the absence of poliomyelitis in Cincinnati for a year.

However, I should point out that the Type 3 vaccine was given to the preschool children during the week of 20 May, and Type 2 will be given during the week of 20 June. The school children will not receive that Type 2 and Type 3 vaccine until November and December.

So if Type 2 and Type 3 polioviruses are circulating in the community, the only barrier they may be expected to encounter will be in the large number of the vaccinated preschool children but not in the school-age children, except that the school-age children may be secondarily infected by their preschool siblings.

It is also our intention to follow through, as we did in Toluca, on the types of poliovirus that will be circulating during the peak period in the summertime, after this massive introduction of the attenuated strains in the community, and to see whether or not, with the amount of Type 1 virus already in the community before vaccination and with what we have introduced, we will find Type 1 virus during September, which is the peak month for polio in Cincinnati.

This, then, is the preliminary report on the mass vaccination experiment in Cincinnati, the kind of observations that have already been made, and what we are looking for.

CHAIRMAN VARGAS-MÉNDEZ: Thank you, Dr. Sabin.

Does anyone else wish to present a preliminary report on a program at present under way, or comment on Dr. Sabin's statements or on anything else discussed during this Conference?

DR. GARD: While I have been listening to the reports and discussions this week, I have become more and more convinced that the procedure we are planning to follow in Sweden is both sound and safe.

We have now used inactivated vaccine for four years and the results obtained so far are very good. We have not yet seen a single case of paralytic poliomyelitis in about 2.5 million persons who have received at least two doses of our Swedish vaccine.

However, we do not know very much about the duration of the protection that has been produced and, whereas we feel fairly satisfied with the findings in follow-up immunity studies, as far as Types 2 and 3 are concerned, we should like to reinforce Type 1 immunity. For this purpose, we are planning to use a combined procedure,

that is, initiating immunization with inactivated vaccine, following up with the administration of Type 1 live virus vaccine.

For the time being, we are not very worried about Type 2 and Type 3, although, of course, we may find that we shall have to add one or both of those types to our arsenal.

We shall probably start vaccinations at the age of three to six months, giving combined triple vaccine plus polio, and then wait until the children have reached the age of two to three years before giving the first dose of live virus.

In doing so we feel that the spread of virus will be better controlled than if we fed infants as, according to our experience, the rate of spread from children above the age of two years is greatly reduced.

However, in our preliminary experiments we have found that full resistance to re-inoculation is not established after a single exposure to live attenuated virus and further, that the titers obtained are not stable but tend to fall off with time. Therefore, we intend to add a second inoculation with live virus during the first year of school.

There seem to be certain advantages to such a procedure; it ought to be safe. We are not using live virus except under the cover of a basic serologic immunity produced by inactivated virus, and we may even get around the difficult problem of extraneous simian viruses in the vaccine in this way, because supposedly the inactivated virus vaccines we have been using in the past and are going to use in the future contain an array of simian viruses, and probably produce serological immunity to those agents.

I should like to point out that this program does not offer any particular organizational difficulties, at least in our country. Practically 100 per cent of the children born in Sweden remain under the supervision and control of the children's health centers up to an age of three to four years. And, as Dr. Sabin just pointed out, vaccination with live virus in the schools is easily accomplished.

DR. BODIAN: I should like to ask Dr. Sabin a question. What criteria would be used in Cincinnati to decide that the chain of transmission has been broken by this program?

DR. SABIN: The criteria would be the incidence of poliomyelitis virus that would be found in the population several months after the completion of the vaccination program and also subsequently.

I believe that all mass vaccination programs with live virus vaccine which are to be started from now on, should have not only good clinical surveillance, but also repeated tests for the viruses in the community. This ultimately should indicate the regimen of live virus vaccination that will be needed to bring intestinal resistance in the population to a level where one really cannot detect polioviruses in the community after the initial period of dissemination.

I do not know what that regimen will be, whether or not it will require refeeding or how many times. But this is a beginning, this is a rational beginning, and I think that virologic surveillance will indicate what should be done in the future. The objective is to have a community without polioviruses circulating in it.

DR. BODIAN: Should no Type 1 viruses be found later in the summer, having been present in the spring, will it be assumed that the chain of transmission has been broken by the feedings of the attenuated virus to the population in Cincinnati?

DR. SABIN: It is not only that Type 1 viruses were present in the spring before we started feeding, but in this population, with 175,000 having received Type 1 vaccine strains, we actually have put in a mass of Type 1 virus.

Now, I should say that if we find a great deal of Type 1 virus we would have very good evidence that this chain of transmission was not broken. If, on the other hand, we find none, we can at least say we found none after feeding 175,000 children with Type 1 virus. If we find a little—I hope you will give me time to think things over.

DR. BODIAN: I have one more question. First, I should like to say that I am very appreciative of what a study like this means, and I think that there is nobody else more competent than Dr. Sabin to get at this very difficult problem, because the type of laboratory surveillance that we are talking about represents quite a feat, which Dr. Sabin is fully capable of accomplishing.

If the chain of transmission is broken in a community like Cincinnati, we do have the problem next year, and the following, of new births in the community, which will produce a susceptible population who are no longer receiving the possibility of immunization by means of naturally occurring strains.

I wonder what plans Dr. Sabin has, because this may be a model for other communities, and I think that what is involved here ought to be clear.

DR. SABIN: With the strains with which we are working, I believe that the evidence required for licensure has been accumulated, and before our program was started I had a letter from the Surgeon General's office (contrary to some of the things that might have appeared in the newspapers this morning) which stated—and I have that letter on record—that with all the new data accumulated in 1959 and the additional data that are being accumulated in 1960, the Public Health Service should be ready to consider applications for licensure.

Therefore, I do expect that if specific requirements for the product are put forth by the Public Health Service, the American pharmaceutical manufacturers, having something definite to work with and having already made great progress on the basis of the preliminary recommendations provided by the Public Health Service, should be in the position to make at least enough vaccine for the Cincinnati children.

Now, the plan then would be to vaccinate children beginning at about two to three months of age. As I said before, I am not prepared to give any recommendations at this time on what to do with the newborn.

So the plan would definitely be to continue with vaccination of the oncoming children through their private physicians and through the clinics. I think the private physicians in the United States can play the most important role in reaching the majority of the preschool-age children. Some of the children, however, will have to get their vaccine in clinics as part of special health department programs.

I should like to make a comment at this point. It is not my intention to make any suggestions for programs in Sweden such as those Dr. Gard has announced. To each his own. But I should like to point out that we cannot disregard any type of poliomyelitis virus. Type 3 and Type 2 viruses are important, and I think Type 2 can be, and is becoming, just as important as Type 3.

I should also like to bring to the attention of this group information that at least was new to me and which I obtained while I was at the Moscow conference. I had thought that poliomyelitis was not a problem in China, but Dr. Ku, Fang-Cho, who attended the conference in Moscow, informed me that since 1955 it had become a problem, and that in 1958 they had a predominantly Type 2 poliomyelitis epidemic.

This brought to my mind that in the same area of the world, in Okinawa, there had been a predominantly Type 2 outbreak before. The following year, in 1959, in Hanoi in North Viet Nam, there was an epidemic of about 1,000 cases of predominantly infantile paralysis, and the only 20 viruses they isolated were all Type 2.

You heard last year of the Type 2 epidemic in Nicaragua, I believe. We cannot disregard any type of poliomyelitis virus; in future programs we must take into consideration all three types of poliovirus vaccine.

DR. DICK: This Conference has had a much more rational attitude than last year. Most people are prepared to accept that we still have a number of things to sort out. These problems are going to be solved by the type of study which Dr. Sabin and others are doing.

At the same time, I wonder whether here we have not a great opportunity for an international study. We have two enormous population groups, one highly immunized with live virus vaccines and the other partially immunized with Salk vaccine. If we could really concentrate on the use of inactive vaccine on this side of the world, we might have two comparative population groups in which we would get a real answer on efficacy.

Now, this would perhaps involve two things, I think. It would involve an interchange of the methods of surveillance, from North America, and, from the USSR, the technique of how to get at 100 per cent of the population with vaccines.

In countries with effective Salk vaccination programs, I think there is no immediate hurry to move over to live virus vaccines until we can guarantee that this method of immunization will be

more effective in eliminating paralytic poliomyelitis.

I think at this meeting we have got rid of a great deal of loose talk about single doses of live virus vaccine producing long-lasting immunity, and about epidemics of immunity. Now we hear of multiple doses and we are not quite so certain about the duration of immunity.

I think we must not be motivated by theories of obsolescence, but obviously, as we all are, by what is best.

One thing which I believe is also very clear to most of us is that we have to give very careful consideration as to which of the six strains of live virus vaccines should be used in further studies.

DR. SMADEL: I believe this has been a most interesting and productive colloquy between Dr. Bodian and Dr. Sabin.

My comment is one regarding certain of the legal requirements for licensing, which I am sure have not been made quite clear to many of the people here, certainly not from the scientific discussion that went on between the two.

The Surgeon General of the United States is not empowered to issue a license for any biological until a manufacturer has demonstrated his capacity to produce in a consistent manner a product which is potent, safe, and useful. Thus, licensing is essentially the final act in the research development of a new biological and is accomplished just before the material is ready for general use and for distribution across state borders.

This procedure of licensing is different from those under which our colleagues in England, Russia, and other countries operate.

DR. KITAOKA: During the Conference many results were published on field trials of the three kinds of vaccines which are called by the same name of "live poliovirus vaccine." Each was prepared by using different strains developed by Dr. Sabin, Dr. Cox, and Dr. Koprowski, respectively. I shoud like, frankly, to ask whether one of them is safer than the other two and if the difference is clear enough to prefer it for use in the community. Also, which of the attenuated poliovirus strains used for the vaccines might still be neurovirulent to some extent, according to the observations made during the mass vaccination trials. We had an experience with no untoward reaction following monovalent vaccine but not with trivalent.

DR. GARD: In reply to Dr. Sabin, I shall only say that we have not disregarded Types 2 and 3. The reason why we are concentrating on Type 1 live virus is that we consider the protection obtained with the use of killed virus vaccine against Types 2 and 3 as being adequate and of longer duration, according to our tests, than against Type 1. That is the reason why we intend to supplement the killed virus vaccine with Type 1 live virus vaccine only.

The future may show that we have to include another type. But for the time being we are only worried about Type 1.

DR. SABIN: Dr. Kitaoka has again raised the question of the use of trivalent and monovalent vaccines, and I should like to take this opportunity to indicate what my own guiding principles are.

Under conditions in which little interference from other viruses may be expected, such as we have shown to obtain in Cincinnati, even among slum populations during the spring of the year—and maybe even less during the winter months—I believe that the feeding of the individual types not only provides the best incidence of immunity for each individual type, but, having the maximum opportunity for extensive multiplication in the intestinal tract, does, on the basis of already existing data, give us the best hope of obtaining the maximum resistance of the intestinal tract.

On the other hand, in other parts of the world where we know that there is massive infection with other enteric viruses throughout the year, and where we know there is such extensive dissemination of naturally occurring polioviruses that children become naturally immune to all three types by the time they are four or five years old, under such conditions we have evidence that nature itself can greatly help in the dissemination of the vaccine viruses.

Under such conditions I believe that the experiment that I reported in Toluca provides an indication of how, by two feedings of trivalent vaccine, one may obtain not only a very high inci-

dence of immunization within a few months, but also a very high interference with dissemination of polioviruses.

For the present, these are my guiding principles about the use of monovalent vaccine in some parts of the world and trivalent vaccine in other parts of the world.

DR. TOBIN: There is one thing that worries me just a little about Dr. Sabin's suggestion that live vaccine should be given in the three-to-six-month age group. This is that at that time many infants will be receiving triple vaccine (diphtheria-tetanus-pertussis), which may have a certain amount of provoking effect.

I was wondering if Dr. Sabin thinks there is any danger of poliomyelitis provocation with triple vaccine in children vaccinated with his attenuated strains. However, on immunological grounds, I should have thought that giving live vaccine is better at that age than giving the killed one.

Because data on antibody responses in infants, presented during this meeting, seem to indicate that these responses persist for at least a year, I would disagree with Dr. Gard. I should have thought that in the case he mentioned and after live vaccine has been fed to infants, one would give killed vaccine to boost the antibody response. We have found that there is no difference in the fall of Types 1, 2, and 3 after a third dose, as long as this gives a true secondary response. This depends on people having circulating antibody at the time of this dose.

DR. SABIN: If I understood Dr. Tobin correctly, he suggested the possibility that, in a child who has had killed virus vaccine, Salk vaccine—is that right?—or any kind of vaccine, administration of live virus vaccine may involve a provoking effect.

DR. TOBIN: No.

DR. SABIN: The triple vaccine you refer to is diphtheria, pertussis, and tetanus?

DR. TOBIN: Yes, you are giving the triple vaccine simultaneously, you see.

DR. SABIN: I should say that whatever the mechanism of provocation may be, it depends upon the effect of the very highly virulent virus. Because the past history of provocation has shown that, when it has been observed, it has been observed during particularly severe epidemics; during the course of ordinary dissemination it has not.

Now, with the Type 1 virus which we are administering, and with the evidence of the complete absence of viremia, which has now been confirmed by the excellent studies of the Czech investigators on triple-negative children of this particular age group of three to six months, I believe the question of provocation is a very minor one—and perhaps actually nonexistent.

Furthermore, in Cincinnati, the only thing that we stopped, in order to avoid any possible problems coming up, were tonsillectomies and adenoidectomies. For the present, we did not want to be involved with this particular problem.

But, otherwise, all other vaccinations have gone on during the period of administration of the oral vaccine to these very small babies and, as I have mentioned, six weeks have gone by and we have had absolutely no problems whatever.

DR. LANGMUIR: As an old hand at the eradication business, and one whose hands have been burned, I should like to welcome Dr. Sabin to this fold.

I had hoped earlier that the Salk vaccine would have influenced the spread of virus sufficiently to make this threshold of immunization a realistic one. But, with the data from many people in this room and Dr. John Fox not here, is was obvious that there was a difference, or a far greater continuity of virus in stool after Salk vaccination than I had anticipated.

I should like to say, however, that the epidemiological evidence in this country seems to me to be pointing rather strongly to the conclusion that there is an effect on spread of virus by Salk vaccine. Essentially, the pattern of concentration of cases in these unvaccinated areas and the absence of infection within large areas of upper-class communities impress me as evidence of the influence of Salk vaccine on spread.

It seems to me that if Dr. Sabin is to succeed in his objective in Cincinnati he has some very real problems that are going to continue for some time. He must have a very thorough coverage of the newborn infants in the community to a very

high level; and how he hopes to achieve this in an American community, I certainly would like to learn.

I am reminded of the paper by Dr. Pundit from India, in which he made a careful study of smallpox in Madras. This is a disease that many of us claim has been eradicated quite successfully from many parts of the world. In spite of a program which Dr. Pundit claims is excellent for maintaining a high proportion of vaccination in the newborn children in Madras, smallpox breaks out repeatedly in that city because of the large number of immigrant children, aged one, two, and three years, who move into the city.

Dr. Sabin, I believe, will have to look into this problem also in Cincinnati, if he hopes to really break the spread of polio infection in that city.

But I certainly wish him well. He is one of the best-qualified people in the country, with a laboratory that can support this necessarily large amount of laboratory surveillance. I am also glad to welcome him into continuing activity in the poliomyelitis field, in spite of his declaration of changing to cancer on the first of January of next year.

DR. PAGANO: In reply to Dr. Tobin's question, we did gather specific data concerning whether diphtheria-pertussis-tetanus inoculations given simultaneously with the oral administration of live poliovirus vaccine might have a provoking effect.

In Philadelphia, where 850 children were given CHAT Type 1 virus, 815 W-Fox Type 3, and 335 P-712 Type 2, approximately 90 per cent of the children also received, during the same routine well-baby clinic visit, a DPT inoculation; many also were given smallpox vaccination.

We saw no evidence in this group of a provoking effect, for, as you will recall, there was no case of paralysis in a vaccinated child.

Of course, allowance has to be made for the numbers of children involved, so that no conclusion can be drawn, but here is some evidence on that point.

DR. KITAOKA: I should like to give Dr. Sabin my comment on which type of poliovirus will be prevalent in the future. It is true that poliovirus Type 2 was prevalent in the southwest Pacific areas, but Type 1 was more often recovered in the epidemic in Taiwan and Japan. According to our experiments, it can be foreseen which type of poliovirus will be expected in the next epidemic season on the basis of an estimation of the immune state of the community during the pre-epidemic season. My question is not which is best, monovalent or trivalent. I am very anxious to know which is the safer among the Sabin, Cox, and Koprowski's vaccines.

DR. SOPER: I have been sitting through this with a great deal of interest, and I have not failed to be greatly impressed by the reports given this week.

I feel like adding to Dr. Langmuir's greeting to the other eradicationists, because I, too, have been burned on the application of the eradication concept; although, on the other hand, I have seen some strikingly successful applications.

I am impressed with the size and volume of vaccination reported from the USSR, and the suggestion of the possibility of eradicating poliomyelitis.

I should like to point out, however, that as we get well into eradication programs, after the easy part of eradication has been done, we come to a point of invisibility of the disease, or the insect that we are eradicating, and at that low level we generally encounter the greatest difficulties.

The term "epidemiology of a disappearing disease" has been introduced in recent years, particularly with regard to malaria, to emphasize the need for special methods to reveal and eliminate the final vestiges of infection.

If Dr. Langmuir's observation is backed up by future findings, we may find that the reverse is true for poliomyelitis; its disappearance may come rapidly.

I should like to point out to Dr. Sabin, however, that eradication cannot be successfully undertaken and maintained on a small scale. In a city with its constant daily outside contacts and movement of population in and out, it is very difficult to imagine a virus being completely eliminated.

There is one word I cannot fail to utter in reply to Dr. Dick's implication that the poliomyelitis situation in the world is well under control with the Salk vaccine. This may be true for

the people in a few of the more advanced countries, it is not, for over three quarters of the world's children. Some 80 to 85 million babies are being born each year into a world heavily infected with dangerous paralytic strains of poliovirus. The great mass of these are not being, and will not be, protected by a vaccine which is expensive and has to be injected. The needs of other peoples can be better appreciated if we visualize what our attitude would be towards live poliovirus vaccines were the Salk vaccine nonexistent.

I consider it a privilege, Mr. Chairman, to have had an opportunity to address this Conference, and I wish to thank you for this opportunity.

DR. SABIN: In reply to Dr. Langmuir, I hope that Cincinnati will not long remain an isolated island.

Furthermore, I regard the United States as a very advanced country, and in this advanced country, in 1959, there were about 6,000 cases of paralytic poliomyelitis reported, 1,000 of them among children who had three doses of Salk vaccine.

Now, I am highly gratified that it was not 18,000, which it might have been if it had not been for Salk vaccine; certainly 6,000 is much less. But I maintain that this is 6,000 cases too many, even for an advanced country, if there is something we can do about it.

To be satisfied that there are only 6,000 instead of 18,000 paralytic cases is no stand to take for an advanced country.

DR. SMADEL: I should like to change the subject slightly. In the closing moments of the Conference last year, I complimented the participants saying that they formed a bold and courageous group. Now, a year later, it is of this courageous group that I should like to ask a question, as an individual scientist, not as a member of a government agency.

How long, in this group, shall we continue to be bound by the prejudices among our colleagues who work in the field of virology and in the cancer field, who insist that we must make vaccines from primary cultures of simian tissue?

This is a key question, because the state of the art is such now that one could grow tissue cultures in large tanks using continuous line cells capable of supporting the growth of poliovirus; moreover, this could be done very cheaply.

I speak with some feeling on this matter, because on several occasions, since the time, five or six years ago, when Dr. Hilleman and I proposed using HeLa cells to prepare live adeno-vaccine, I have had my brow bloodied by the cancerologists who have insisted that one or another of the theoretical factors which might exist in a continuous cell line, could possibly induce cancer in man.

This is the sort of an argument which can never be settled by talk. It is a philosophical point which one may or may not believe. My final rebuttal in this argument, which was as useless as most rebuttals, was that if those in the cancer field believed this, then they had the opportunity to do the most important experiment of the century and to prove the point.

But I still have not, as a scientist, been able to convince anybody, or at least a jury of my peers, that I could make a vaccine, live or dead, in a continuous line cell culture and judicially use this in man.

My challenge, gentlemen, is this: among us, are there any bold enough to do this experiment? We shall never settle it philosophically; it will be settled only by acting.

SUMMARY OF THE CONFERENCE

CHAIRMAN VARGAS-MÉNDEZ: I should now like to call on Dr. Payne to read the summary report of the Conference, prepared by the Summary Committee.

DR. A. M. M.-PAYNE *(Chief Medical Officer, Virus Diseases, World Health Organization)*: As last year, the document before you was prepared by an anonymous group which has attempted to summarize the opinions of all participants at the Conference and to give an objective reflection of the different views expressed here. No attempt has been made to draw conclusions, or to make recommendations, since that was not the purpose of this Conference, which was essentially to bring all available data into the open, onto the table, and to elicit the differences of opinion which may exist, in the hope of eventual resolution by further work.

After Dr. Payne read the document, it was reviewed and discussed by the participants and the following text was unanimously approved:

Introduction

In the year which has elapsed since the First Conference on Live Poliovirus Vaccines, intensive studies of the properties and value of these vaccines have been continued or undertaken in many countries of the world.

These studies have included detailed investigation of the characteristics of vaccine virus strains before and after multiplication in man and of wild strains, and of means of differentiating them. Extensive investigations of the effects of the administration of the vaccines to individuals, in families, in institutions, and to particular groups, have also been carried out. They have also been used on an increasingly large and even massive scale under a great variety of geographical and climatic conditions. Much new factual information on their use and in particular on their safety and efficacy has been accumulated.

This Conference has provided an opportunity to review this work. Some of the problems have been more clearly defined and some of the questions answered.

Laboratory Evidence of Attenuation and Safety

Additional evidence has become available concerning the comparative properties of the proposed vaccine strains. The findings regarding neurovirulence for monkeys reported last year have been confirmed and extended although their epidemiological significance remains to be established. In addition, some of the strains have been shown to have neurovirulence for monkeys when injected intramuscularly. A considerable amount of information was presented concerning markers and their possible correlation with virulence. These results were disappointing as they failed to show, in the case of any one marker by itself, satisfactory correlation with neurovirulence. This avenue of endeavor still appears fruitful and evidence concerning some promising new markers was presented. A number of reports confirmed the instability of many of these strains after passage through the human intestinal tract, as determined by markers and changes in monkey neurovirulence. In some instances these changes were demonstrable after tissue-culture passage alone. This instability limited the use of such markers in the positive identification of vaccine strains after multiplication in the human alimentary tract and their differentiation from wild viruses.

A limited amount of information was available concerning viremia in man following the administration of live vaccines. One report indicated that this was not infrequent in the case of one set of strains, while another report which concerned a single type of a second set was negative. This whole problem requires further investigation and standardization of methods.

Many data were available in reports on a variety of subjects concerning laboratory properties of these strains. This information will be useful in the development of the ultimate control criteria for live poliovirus vaccines. One item of special interest was the demonstration of the

presence of what is presumed to be a hitherto unrecognized virus in live vaccine and indeed in some of the such seed strain preparations. This agent, tentatively called the vacuolating agent, can be identified, thus far, only in tissue-culture preparations made from kidneys of monkeys of the species *Cercopithecus aethiops*. The significance of these findings for man is not known, but it does illustrate the problem presented by adventitious agents in the production and control of live virus vaccine prepared on monkey-kidney cell cultures.

Field evidence of safety

A fairly large series of new field trials involving the use of the three different groups of attenuated poliovirus strains, was reported by 24 groups of workers from 13 countries.

In the small trials involving not more than 500 people, close clinical supervision was available on each and every participant. In the larger trials, some of them nation-wide and ranging from several hundred thousands to many millions of participants, various methods of surveillance techniques were followed. These clinical surveys were designed particularly to detect illness of the central nervous system among those who had received the vaccine or among their intimate associates. In almost all of these trials, including the massive experience in the USSR, the opinion was universal that untoward reactions were either absent or insignificant and the so-called major illness of poliomyelitis had not either directly or indirectly been induced by infection with attenuated poliovirus used as a vaccine. Nor had the progeny of the vaccine virus induced harmful effects as it spread in the local community.

It is to be pointed out that some of the trials were carried out during the off season for poliomyelitis, while in others sporadic or even epidemic poliomyelitis was present coincidentally. In the latter, cases of poliomyelitis are bound to occur following, but not necessarily related to, vaccination. For example, in one large trial which was conducted at a time when a small number of local cases of poliomyelitis were expected in any event, a problem was encountered in determining whether a few post-vaccinal infections of the CNS were so related. The evidence concerning these is as yet incomplete and is being further investigated.

Spread of attenuated polioviruses

As was indicated in the report of last year's Conference, the attenuated viruses which were effective in producing a high level of serologic responses spread readily to intimate family and household associates, in somewhat the same manner, but probably to a lesser degree than do some of the wild polioviruses. This intra-familial spread was reported as ranging from 40 to 80 per cent following feeding of infants, but may be much less following feeding of older children, and involved not only the susceptible contacts but to a lesser degree those already possessing homotypic antibodies. The degree to which these attenuated polioviruses spread into the community was measured in only a few instances. Spread of this kind was not extensive.

In a large geographical area such as Czechoslovakia, there was no evidence that the attenuated strains of polioviruses spread beyond the confines of the districts where they had been administered—at least to a degree to indicate that these adjacent populations were immunized. In some of the reports from the Soviet Union, it did appear that within urban groups of contacts (so-called internal controls) whose members were in relatively close contact with those who had been vaccinated, some immunity was acquired from this experience. More information concerning spread is needed.

Immunization of Special Groups

Premature and newborn babies. The studies carried out by several investigators on babies given different oral vaccines have given results which are substantially in agreement. It appears that the vaccines can be given safely to premature or full-term babies at or shortly after birth and that excretion of virus occurs in a high proportion of instances. The period of virus excretion is, however, shorter in newborns than in babies given vaccine at four months of age or later and an antibody response is demonstrable in only about half of the subjects and is often delayed. The influence of high levels of antibody derived from the mother is thought by some to be an important factor in modifying or masking the antibody response of newborn infants.

Pregnant women. Live poliovirus vaccine has been given to several hundred pregnant women without any untoward effects to the mothers.

Nevertheless, the possible effects of the vaccine on the fetus require further study before it can be assumed that feeding of vaccine, particularly during the first trimester of pregnancy, is harmless. The demonstration that viremia may occur after oral administration of some strains of vaccine raises the special problem of hazard in mothers without antibodies at the time of administration of vaccine. No evidence of harmful effects to the fetus has, however, been reported, nor has the phenomenon of immunological tolerance been encountered.

Other adults and those receiving medical or surgical treatment. Enough adults in different communities have now received oral vaccines, or have acquired infection by contact, to establish the general safety of the vaccine in them, as in children. Nevertheless, there exist groups in the community which require special consideration and study because of the possible modification in them of the process of the infection. These include adults who are negative to all three types of poliovirus, and those receiving treatment with steroids or undergoing surgical treatment on the oropharynx or elsewhere. Although no reports have been made of untoward effects of the vaccine in such persons, the number thus far studied is too few for any final opinion to be expressed.

Evidence of efficacy in man

In the summary of last year, the Conference pointed out the consistency with which administration of oral vaccines to man had been followed by development of demonstrable antibody. Differences of opinion persist as to standards for measurement of such increase, for differentiation of homotypic and heterotypic responses, and for measurement of booster effect. There is, however, apparent agreement as to the significance of antibody response in those without demonstrable antibodies to any of the three types of virus as probably the most valuable serological measure of antigenic capacity. But for comparability of results there is a need for agreement as to the significance of appearance of antibody at the lowest demonstrable level. The serological response of children has been almost uniformly greater than that of adults.

Throughout the Conference emphasis has been placed upon the relationship between antibody response and virus excretion by the vaccinee, inasmuch as resistance to reinfection in the absence of very high antibody levels appears to be conditioned by prior intestinal infection. Continued excretion of the virus by the vaccinee in conjunction with antibody rise may therefore be taken as the best measure of development of increased resistance. On the other hand, evidence was presented that intestinal infection as manifested by virus excretion may develop and continue without appearance of demonstrable antibodies.

At the time of last year's Conference considerable attention was given to the possible interference of coexisting enteroviruses in the establishment of the intestinal vaccine-virus infection essential to the development of resistance, and to the role of one type of poliovirus in interfering with the immunizing effect of other types in polyvalent vaccines. During the past year studies have shown that while monovalent vaccines used in suitable sequence have somewhat greater potency, such interference as may exist is not of such magnitude as to contraindicate the use of polyvalent vaccine, but does justify reinforcement of resistance by subsequent refeeding. Some reports indicate that interference by coexisting enteroviruses can in some measure be overcome by repeated mass administration or by larger doses. The full measure of this interference phenomenon must await further studies.

The final evaluation of efficacy of vaccine must, however, be based on the ultimate effect in controlling or preventing both paralytic poliomyelitis and infection with wild viruses. The many carefully-controlled small studies are admittedly too small to yield conclusive data on these problems, but at the same time have failed to show any suggestion that the vaccine may not be clinically effective. Several mass trials have been immediately followed by low rates of disease such as could be anticipated would follow the use of an effective vaccine, but longer periods of observation are essential because the low attack rate of the paralytic disease and the well-known variability of occurrence of poliomyelitis from one period of time to another in the same community preclude the possibility of definitive conclusions based on short periods of observation.

The occurrence of poliomyelitis in certain areas where extensive use of vaccine had been made in previous years has yielded evidence of lower attack rates in the immunized than in the non-immunized segments of the population. But the occurrence of some cases among those receiving vaccine has indicated the need for additional feedings of oral vaccine if an adequate immunity of the population is to be ensured.

The concept of eradication of poliomyelitis

The widespread use of potent inactivated vaccine may well lead to a substantial reduction in the numbers of cases of paralytic poliomyelitis, but it can do little to eliminate the causal virus from the gastrointestinal tract of man. The possibility of eliminating poliovirus as a human pathogen has been brought much nearer by the development and practical application of live poliovirus vaccines. We can indeed look forward to the eventual eradication of poliomyelitis, as we can look forward to the eradication of diphtheria, smallpox, and other preventable infectious diseases by means of specific prophylactics.

The members of the Conference fully realize that the attempted elimination of such a well-established and almost universally prevalent pathogen as poliovirus is a gigantic task, at present impractical in many countries. The Conference therefore notes with the greatest of interest the campaign aimed at the almost complete eradication of poliomyelitis now being conducted in the USSR.

The first step in such a campaign is the mass vaccination, by feeding attenuated viruses within a short period of time, of the whole population, or at least those age groups most susceptible to infection. Following this "blanketing" with viruses, special studies will have to be conducted to determine the most feasible means of maintaining the resistance of the population. These studies would have to take account of differences in the ecological behavior of polioviruses under various climatic conditions, in various socio-economic groups, and in communities with differing opportunities for dissemination of virus, such as institutions, rural areas, small towns, and large cities.

This concept of complete eradication of poliomyelitis is indeed bold, and it would be well to prepare for disappointments and for the unexpected. The whole problem of the eradication of poliomyelitis is worthy of the closest study not only in the field, but at the theoretical level.

Appendix

The following letter was read at the closing session of the Conference:

Dr. Abraham Horwitz, Director
Pan American Sanitary Bureau
Regional Office of the World Health Organization
1501 New Hampshire Avenue, N.W.
Washington 6, D. C.

Dear Dr. Horwitz:

It was, of course, a keen personal disappointment that I was unable to attend the Second International Conference on Live Poliovirus Vaccines. You may be sure, however, that I have followed the proceedings with intense interest. As the Conference draws to a close, I wish to express the profound pride and satisfaction it affords the Sister Elizabeth Kenny Foundation to have shared with your organization the honor of sponsoring this significant event.

Even to one observing from a distance, it has been clearly apparent that the true spirit of scientific investigation has prevailed throughout the Conference. Perhaps the most striking indication of this has been the uncompromising willingness to present findings fully and frankly, regardless of where they lead.

It seems to me that both conferences have made a major contribution to science, wholly apart from their significance to medicine and public health. Never before to my knowledge has a vaccine been subjected to such searching, rigorous, thorough, and widespread investigation. The magnitude of the data produced and the scrupulous care with which they have been examined seem to me to be unprecedented. It may well be that the entire experience will establish a new standard for the testing and proving of a concept in preventive medicine. The credit for all this is due, of course, to you, your colleagues in the Pan American Health Organization and the World Health Organization and the distinguished delegates to the conferences.

I hope you will convey my most cordial and sincere respects to all the participants. I feel confident that the work surveyed at the Conference will ultimately lead to the final goal—eradication of poliomyelitis.

Sincerely,

(Signed) E. J. HUENEKENS, M.D.
Medical Director
Sister Eizabeth Kenny Foundation
Minneapolis, Minnesota

INDEX