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CURRENT RESEARCH ON VACCINATION AGAINST POLIOMYELITIS*†

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I am highly honored by your invitation to participate in this symposium on virus diseases and to tell you something about current research on vaccination against poliomyelitis. Since I have only a limited knowledge of the French language I had difficulty in deciding whether I should speak in English and take the chance of not being understood by a few or to speak in French and be misunderstood by many. For the sake of those who do not understand English I decided to read my communication in French, and I hope that you will pardon my poor pronunciation and accent.

There are two basic approaches to immunization against any infectious disease. One approach depends on an attempt to stimulate an immune response by inoculation of large amounts of preformed antigen consisting either of the completely inactivated or "killed" infectious agent or a suitable chemical derivative thereof. The other approach depends on the production of a very mild or subclinical infection by means of an antigenically related infectious agent (as in the use of vaccinia to protect against variola) or by means of an avirulent variant of the infectious agent (as in the use of the experimentally developed 17 D strain of virus to protect against yellow fever). The former approach, when it is successful, has the advantage of dealing with dead, non-infectious material; the disadvantages are that large amounts of antigen are needed, that many doses have to be inoculated to obtain an adequate immune response, and, because the immune response is usually transitory, inoculations must be repeated at frequent intervals if immunity

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is to be maintained during a person's lifetime. In the case of poliomyelitis, with viruses widespread among human beings throughout the world and with the clinical manifestations of infection becoming more severe as people grow older, there is a need for protection throughout life. One cannot rely too much on the continuing immunizing effects of spontaneously acquired poliomyelitis infections, because exposure to such infections is constantly diminishing in the very countries which would require vaccination the most. The advantages of the second approach, when a suitable mild infectious agent or avirulent variant can be found, are 1) that only single inoculations of small amounts are required, and 2) that the resulting mild or subclinical infection often produces long-lasting immunity. The disadvantages are that one must deal with a living agent which requires not only the most meticulous stepwise studies before one can be assured of its safety for large-scale use but also careful control of its cultivation to make sure that some new and undesirable mutant does not become dominant.

You are undoubtedly already aware that recent new discoveries have made it possible to investigate both of these approaches in the case of poliomyelitis. As long as the nervous system of certain living animals was the only place in which poliomyelitis viruses could be propagated, the yields of virus were too small to provide the large amounts of preformed antigen required to stimulate an immune response, and the potential danger from postinoculation encephalomyelitis was too great. Another great drawback was that until very recently we did not know whether the number of distinct immunologic types of the virus was small or large. While there were early indications that the virulence of some strains of poliomyelitis virus could be modified by propagation in rodents (1, 2), the most important strains encountered in human epidemics could not be propagated in rodents, and the facilities for the scientific study of virulence and avirulence were limited by the fact that identification of a poliomyelitis virus could be made only on the basis of its virulence in living animals. The following discoveries of the past four years have made it possible to begin a new attack on vaccination against poliomyelitis:

(1) the demonstration that only three main immunologic types of virus cause the human disease, and that the Type 1 virus is responsible for most epidemics (3);

(2) the demonstration that poliomyelitis viruses can be propagated *in vitro*, not only in human embryonic nervous tissue, as was first reported in 1936 (4), but also in non-nervous human embryonic and adult tissues as well as in certain tissues of adult monkeys (5);

(3) the identification of poliomyelitis viruses by an *in vitro* cytopathogenic effect (6), which not only made it possible enormously to increase the scope of work on poliomyelitis but also, for the first time,

to recognize a poliomyelitis virus even if it possessed limited or no virulence for experimental animals; and

(4) the conversion of highly virulent strains of all 3 immunologic types into relatively avirulent variants by special methods of cultivation and segregation in non-nervous tissue (7), as well as the demonstration that such relatively avirulent strains exist in nature and can be recovered from healthy children.

Other factors of importance in orienting the new studies were the growing conviction that the primary site of infection in human poliomyelitis was the alimentary tract (8, 9), and the demonstration that smaller amounts of antibody were required to protect animals against paralysis when virus was given by mouth or other extraneural routes than when infection occurred along the olfactory pathway from the nose or after direct intracerebral injection (10).

I shall first present a personal evaluation of the problems related to the work on "killed-virus" vaccines, and then summarize the results of recent studies in my own laboratory on the experimental production and natural occurrence of relatively avirulent strains of poliomyelitis virus and their immunogenic activity in monkeys and chimpanzees. The problems relating to the development of "killed-virus" vaccines are quantitative rather than qualitative. The tissues in which the poliomyelitis viruses can now be grown for vaccine production are not available in unlimited supply, and the amount of antigen in the killed cultures is unfortunately not sufficiently abundant to provide a wide margin of immunogenic effectiveness. Normal human tissues are excluded, for the present, because of the possible danger of contamination with such viruses as infectious hepatitis for which no practical tests are currently available. Cultures of malignant human cells, which provide an excellent medium for the propagation of the poliomyelitis viruses, are regarded as unsuitable because not enough is known about the long-range potentialities of cell-free fluids from malignant tissues. Only monkey kidneys are suitable at the present time because the level of viral multiplication in other monkey tissues has been too low for practical purposes.

Detailed quantitative studies are indicated because of Salk's demonstration that an arbitrarily selected dose of 1 ml. of formalinized vaccine repeated 3 times has produced antibody for all 3 types of virus in a small number of children whose sera, in a final dilution of 1:8, had no demonstrable antibody of any type prior to inoculation (11). There are as yet no data to indicate whether the antibody engendered by this dose of vaccine can persist for 6 to 8 months during a period of the year when reenforcement by spontaneous infection can be excluded. The effectiveness of a given dose of "killed-virus" vaccine in human beings is influenced by the individual's previous exposure to infection with any of

the 3 types of virus. Several investigators (11, 12, 13) have now found that much less vaccine is required for a booster effect than for the production of antibody *de novo*. Because of a certain degree of antigenic relationship among the 3 types of virus, infection with one type can produce a transitory low-grade antibody response to another (14). For these reasons, the most reliable measure of antigenic potency in human beings is obtained in children whose undiluted serum is devoid of antibody for any of the 3 types. Since there are indications of minor antigenic differences among strains of the same immunologic type (3, 15, 16), it is also important to determine whether or not low-grade antibody responses to the strains of virus contained in the vaccine are also effective against a number of other homotypic strains.

A systematic approach to the elucidation of the important quantitative aspects in the development of "killed-virus" vaccines cannot be made without a standardized, simple yet quantitative, laboratory method of assay by which the antigenic potency of different preparations can be measured and compared. By means of such a method one could obtain data to indicate the best strains of virus to use and the best technique for complete inactivation with the least loss in antigenic potency and optimum stability on storage. Having obtained such information, one would then be able to establish the relationship between the assay value of a given preparation and the minimal amount required for the regular production of sufficient antibody to persist for at least 6 months in children whose undiluted sera possessed no antibody for any of the 3 types prior to inoculation. Such a method of assay is also essential for the establishment of standards by which it would be possible to evaluate or reproduce the results that might be obtained in extremely laborious experiments on hundreds of thousands of children —because the effectiveness of any poliomyelitis vaccine must ultimately be established by its capacity to prevent the naturally occurring paralytic disease of human beings. This type of systematic investigation takes time and much of it is now in progress. Humanitarian considerations, however, have prompted a more rapid test of the effectiveness of an empirically selected dose of formalinized vaccine in the prevention of the paralytic disease in large numbers of human beings. If the results of the current human tests in the U. S. prove to be conclusive or encouraging, further progress can be made by relating the observed effects to the antigenic potency of the preparations that were used. If the results are not good, it will not mean that "killed-virus" vaccines cannot impart temporary immunity to paralytic poliomyelitis, because there is no doubt that better "killed-virus" vaccines than the ones used in the current tests can be prepared by improved methods of cultivation and inactivation and by the use of strains which produce almost a hundred times more virus.

The basic questions of ultimate safety of "killed-virus" vaccines are concerned with the control of the complete inactivation of the virulent viruses incorporated in the vaccine and the side reactions which might be associated with the repeated injections of formalinized extracts of kidney tissue. Poliomyelitis viruses have proved to be somewhat more difficult to inactivate by formalin than other neurotropic viruses. This merely means that it takes longer to get rid of the last residual minute amounts of virus. For practical purposes, however, it also means that strains of poliomyelitis virus, such as the virulent Type 1 Mahoney strain, which produce paralysis in the minutest amounts as readily after intramuscular as after intracerebral injection should not be used. This unnecessary extra risk can be avoided by using other virulent or relatively avirulent strains which do not possess this objectionable property. Vaccines prepared from monkey kidney cultures contain very minute amounts of protein, and only careful observations on tens of thousands of individuals receiving repeated doses and booster inoculations will indicate the extent, if any, of the risk of nephrotoxic or anaphylactic reactions. The question of the possible presence of soluble Rh antigen derived from the erythrocytes in the monkey kidneys also needs to be investigated to eliminate the possibility that repeated injection of vaccine may sensitize Rh negative girls.

I should now like to present some of the data on the experimentally segregated and naturally occurring strains of modified virulence for monkeys which have been studied in my laboratory during the past year. Doctors Johan Winsser, Walter A. Hennessen, M. Ramos Alvarez and Hisayo Nakai collaborated in different phases of these studies. We attempted to "convert" highly virulent strains of the 3 immunologic types of poliomyelitis virus—"Mahoney", "Y-SK", and "Leon"—into avirulent variants for cynomolgus monkeys by cultivation in cynomolgus kidney tissue cultures. Fig. 1 is shown for the benefit of those who have not previously seen the *in vitro* cytopathogenic effect of poliomyelitis virus. At the top is a picture of unstained epithelial cells which grow out of pieces of monkey kidney on the walls of ordinary glass test tubes. In the middle is shown beginning viral change in the form of a plaque which consists of rounded-up, degenerated epithelial cells, and in the bottom of the picture all the epithelial cells have undergone the viral cytopathogenic change. The specificity of this change is proved by the fact that it can be prevented by homotypic but not by heterotypic antiserum. After the cellular debris is removed by low-speed centrifugation, examination of the water-clear culture fluid with the electron microscope reveals a practically pure suspension of virus particles which are shown in Fig. 2. When single or small numbers of virus particles were used as seed for serial passages in monkey kidney tissue cultures, there was no change in the virulence of these viruses

FIG. 1.—*In vitro cytopathogenic effect of poliomyelitis virus*

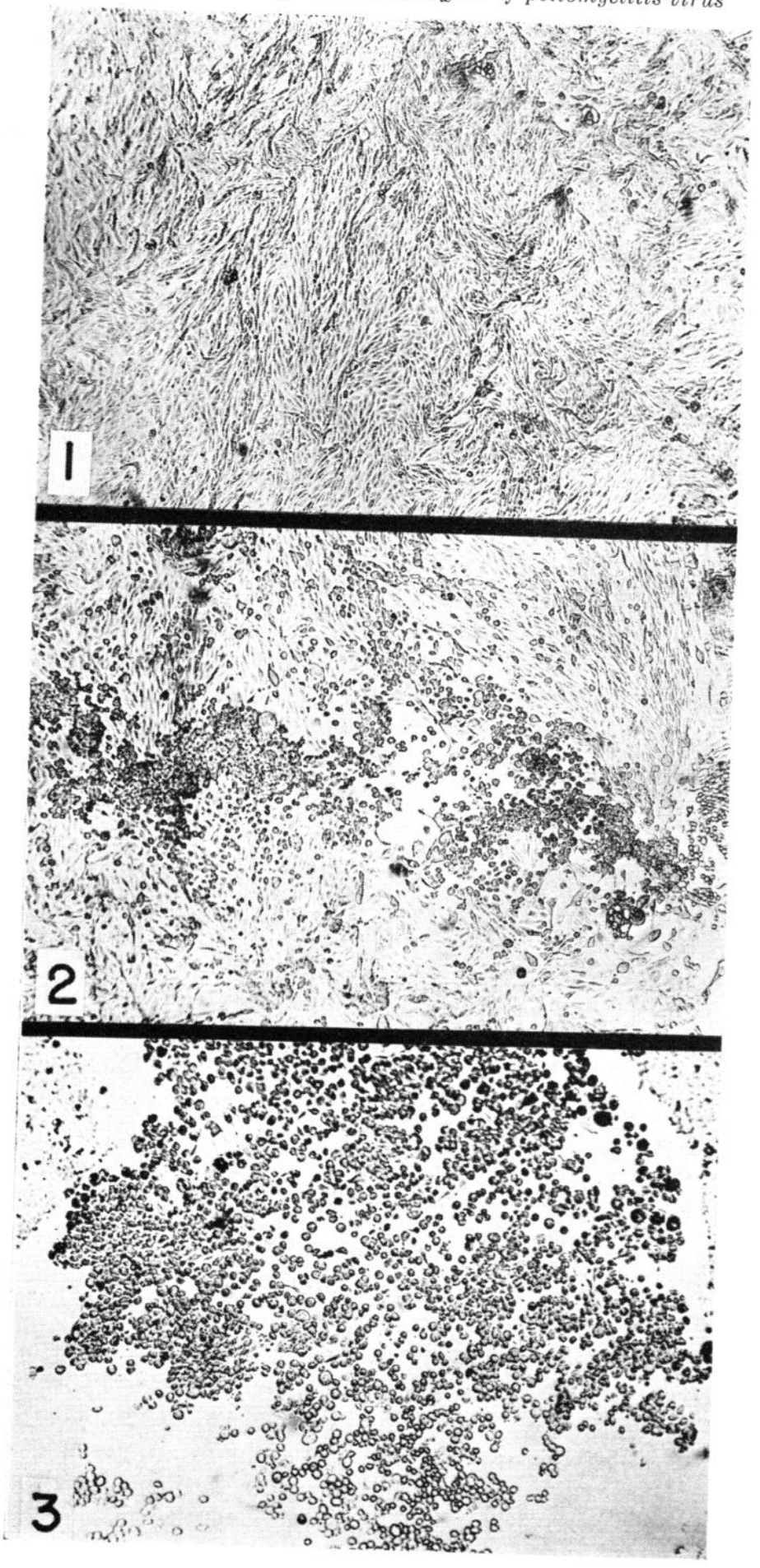

FIG. 2.—*Examination of the water-clear culture fluid with the electron microscope reveals a practically pure suspension of virus particles.*

for monkeys. On the other hand, the use of inocula containing approximately a million or more virus particles combined with rapid passages at 24 hour or shorter intervals—a procedure designed to favor the overgrowth of virus particles capable of most rapid reproduction in non-nervous tissue—gave rise to culture fluids with diminished virulence and unusual patterns of reaction in cynomolgus monkeys. The results suggested that these culture fluids might contain a mixed population of virulent and avirulent virus particles. After an adequate number of such rapid passages had been made, it proved possible by means of the terminal dilution technique to segregate variants of each of the three types which proved to be avirulent after administration by the intracerebral, intramuscular or oral routes in cynomolgus monkeys.

The data shown in Table 1 indicate that the Type 1 (Mahoney) virus in the tenth kidney culture fluid resulting from passages with minimal inocula was of such virulence that even a single tissue culture cytopathogenic dose was capable of producing prostrating paralytic infection in intracerebrally inoculated monkeys; on the other hand, the thirty-third kidney passage culture fluid, propagated and purified by the described procedure, contained about thirty times more virus but none of the twenty-eight monkeys inoculated intracerebrally with amounts varying from sixteen to sixteen million fifty per cent tissue culture cytopathogenic doses (TCD_{50}) exhibited either paralysis or lesions in the nervous system. Essentially the same results are shown in Table 2 for the Type 2 (Y-SK) virus, in which the fifty-first kidney passage culture was found to be avirulent for monkeys by the intracerebral route. The unmodified Type 3 (Leon) virus produced pros-

TABLE 1.—*Intracerebral virulence in Cynomolgus monkeys of type 1 poliomyelitis virus (Mahoney strain) propagated in different ways in Cynomolgus kidney tissue cultures*

Kidney passage 10 Serial passages with minimal inocula		Kidney passage 33 Rapid passages with large inocula followed by purification by terminal dilution technique		
No. of TCD 50* inoculated	No. of monkeys paralyzed	No. of TCD 50 inoculated	No. of monkeys paralyzed	No. showing CNS lesions
500,000	5/5	16,000,000	0/4	0/4
50,000	5/5	1,600,000	0/4	0/4
5,000	5/5	160,000	0/4	0/4
500	5/5	16,000	0/4	0/4
50	3/5	1,600	0/4	0/4
5	4/5	160	0/4	0/4
0.5	1/5	16	0/4	0/4

* TCD 50 = 50 per cent tissue culture cytopathogenic dose.

trating paralytic poliomyelitis in all intracerebrally inoculated cynomolgus monkeys. The data shown in Table 3 indicate that after propagation and purification by the described procedure the thirty-fourth passage of the Type 3 virus produced neither paralysis nor lesions in the seventy cynomolgus monkeys inoculated intracerebrally or intramuscularly with amounts varying from one to ten million TCD_{50}. The absence of antibody formation in all the intracerebrally inoculated monkeys which received less than ten million TCD_{50} strongly suggests that the virus failed to multiply after intracerebral injection. In the intramuscularly inoculated monkeys, there was considerable variation in individual response, although an occasional animal developed antibody after a single injection of one-one hundred thousandth of a ml. of tissue culture fluid.

TABLE 2.—*Intracerebral virulence in Cynomolgus monkeys of type 2 poliomyelitis virus (Y-SK strain) propagated in different ways in Cynomolgus kidney tissue cultures*

Kidney passage 10 Serial passages with minimal inocula		Kidney passage 51 Rapid passages with large inocula followed by purification by terminal dilution technique		
No. of TCD 50* inoculated	No. of monkeys paralyzed	No. of TCD 50 inoculated	No. of monkeys paralyzed	No. showing CNS lesions
1,600,000	4/4	8,000,000	0/4	0/4
160,000	4/4	800,000	0/4	0/4
16,000	4/4	80,000	0/4	0/4
1,600	3/4	8,000	0/4	0/4
160	4/4	800	0/4	0/4
16	2/3	80	0/4	0/4
1.6	0/3	8	0/4	0/4

* TCD 50 = 50 per cent tissue culture cytopathogenic dose.

TABLE 3.—*Intracerebral and intramuscular virulence and immunogenic activity in Cynomolgus monkeys of 34th passage of type 3 poliomyelitis virus (Leon strain) in Cynomolgus kidney tissue culture*

Virus segregated by terminal dilution technique after 30 rapid passages with large inocula

No. of TCD 50* inoculated	Intracerebral group			Intramuscular group		
	Paralysis	CNS lesions	Antibody**	Paralysis	CNS lesions	Antibody**
10,000,000	0/6	0/6	2/6	0/5	0/5	5/5
1,000,000	0/4	0/4	0/4	0/5	0/5	4/5
100,000	0/4	0/4	0/4	0/5	0/5	2/5
10,000	0/4	0/4	0/4	0/5	0/5	2/5
1,000	0/4	0/4	0/4	0/5	0/5	0/5
100	0/4	0/4	0/4	0/5	0/5	1/5
10	0/4	0/4	0/4	0/5	0/5	0/5
1	—	—	—	0/3	0/3	0/3

* TCD 50 = 50 per cent monkey kidney tissue culture cytopathogenic dose.
** Antibody = effect of 0.1 ml of undiluted serum vs. 100 TCD 50 of virus.

These types 1 and 2 "monkey-intracerebral-avirulent" strains produced paralysis in mice after intraspinal but not after intracerebral inoculation, while the Type 3 virus was without effect in mice inoculated by either route. Although no lesions were found in large numbers of sections of the nervous system in any of the eighty-six intracerebrally inoculated monkeys, recorded in Tables 1, 2, and 3, focal neuronal and infiltrative lesions were found in the spinal cord of five of sixty-eight monkeys which were inoculated intramuscularly with the Type 1 or 2 viruses, but in none of forty similarly inoculated with various amounts of the Type 3 virus. This led to the suspicion that the viruses which were avirulent for monkeys by the intracerebral route might prove to be paralytogenic after direct inoculation into the gray matter of the spinal cord. The data shown in Table 4 indicate that this was indeed found to be the case. It is noteworthy, however, that the quantitative tests revealed that the 3 types of virus differed in the regularity with which amounts of less than one million TCD_{50} could produce paralysis after direct spinal injection in monkeys. The quantitative data in both monkeys and mice indicate that even after direct spinal injection only a small proportion of the virus particles find susceptible cells. In view of the behavior of these variants in the spinal cord of monkeys and mice, it is of utmost interest that the eleven chimpanzees inoculated intraspinally with about two million TCD_{50} of these three strains exhibited neither paralysis nor lesions.

As increasing numbers of monkeys and mice were inoculated intracerebrally with large doses of the "monkey-spinal-variant" viruses, we observed a certain number of animals which developed paralysis with

TABLE 4.—*Intraspinal paralytogenic activity of monkey intracerebral avirulent variants in monkeys, mice and chimpanzees*

No. of TCD 50* inoculated	Type 1. Mahoney, KP 33			Type 2. Y-SK KP 51			Type 3. Leon, KP 34		
	Monkey	Mouse	Chimpanzee	Monkey	Mouse	Chimpanzee	Monkey	Mouse	Chimpanzee
1,000,000	3/4	—	0/5**	3/4	—	0/3**	3/4	—	0/3**
100,000	4/4	28/30		2/4	10/10		1/3	0/10	
10,000	3/3	4/10	0/3	2/4	10/10		1/4	0/10	
1,000	3/3	5/10		1/4	8/10		0/4	0/10	
100	1/4	2/10		0/4	1/10		0/4	0/10	
10	0/4	0/10		0/4	0/9		0/3	0/10	

* TCD 50 = 50 per cent monkey kidney tissue culture cytopathogenic dose. Same amounts of virus inoculated intracerebrally in cynomolgus monkeys produced neither paralysis nor lesions.

** All chimpanzees were inoculated with twice the dose given to monkeys, i.e. about 2 million TCD 50.

typical lesions, but, despite the frequent presence of large amounts of virus in the spinal cord as measured by tissue culture, further intracerebral passage was, as a rule, negative. The most likely explanation here is that, as a result of trauma or chance, a few of the large number of the intracerebrally inoculated "spinal-variant" particles found their way to susceptible cells. However, still another phenomenon was encountered in monkeys which for the present I can interpret only as a manifestation of an incomplete or altered reproduction of the modified virus in the spinal cord. The characteristic of this phenomenon is that despite the presence of typical neuronal lesions in the spinal cord no cytopathogenic virus can be demonstrated in tissue cultures and both intracerebral and intraspinal passages in other monkeys are negative.

The only poliomyelitis viruses which Dr. Ramos Alvarez and I have recovered thus far in monkey kidney tissue cultures from rectal swabs of healthy Cincinnati children who had no contact with known cases of poliomyelitis proved to be "spinal variants" on inoculation in monkeys (Table 5). The progeny test on the few monkeys, which developed paralysis after intracerebral injection, gave the pattern of the "spinal variant" in one instance and of the incomplete multiplication phenomenon in two instances. It is clear from this finding that the "monkey-spinal-variant" type of avirulent poliomyelitis virus is not merely a laboratory artefact but also occurs in nature.

The modified viruses which we produced experimentally by selective propagation in cultures of monkey kidney tissue produced no obvious pathologic visceral changes *in vivo* in cynomolgus monkeys. It is also noteworthy that despite the fact that these modified viruses were cultivated in cynomolgus non-nervous tissue, they were less effective than the virulent parent strains in infecting cynomolgus monkeys by

TABLE 5.—*Monkey "spinal variant" poliomyelitis viruses recovered in tissue culture from rectal swabs of healthy children*

Immunologic type	Strain	TCD 50/ml. of culture fluid	Paralytic effect of culture fluid in monkeys inoculated	
			Intracerebrally 0.5 ml.	Intraspinally 0.1 ml.
2	FAF-117	$10^{6.2}$	0/4	4/4
	FAF-116	$10^{6.7}$	1*/4	4/4
	SAC-266	$10^{5.5}$	2**/5	4/4
3	GLE.	$10^{5.3}$	0/4	3/4

* Paralysis left lower 9th day—spinal cord—no virus in tissue culture; negative i. cer. passage in monkeys.

** Paralysis left foot and leg 7th day—spinal cord—$10^{4.2}$ TCD 50/gm.; negative i. cer. passage in monkeys. Paralysis right lower 13th day—spinal cord—no virus culture; negative i. cer. passage in monkeys.

the intramuscular and oral routes. The results shown in Table 6 indicate that, while ten TCD_{50} of the original Mahoney virus produced antibody in all monkeys (and paralysis in most), one hundred thousand TCD_{50} of the modified variant were required to produce antibody in all monkeys, although in an occasional animal even ten TCD_{50} sufficed. The same phenomenon may be seen in the data for the "Y-SK" virus shown in Table 7. Feeding of the "Mahoney" variant strain to cynomolgus monkeys produced antibody without paralysis, but approximately ten thousand times more of the avirulent than of the virulent parent virus had to be fed to infect a similar proportion of animals. The monkeys which developed antibody even in very low titer were resistant to the paralytic consequences of an oral challenge with large

TABLE 6.—*Comparative paralytogenic and immunogenic activity in intramuscularly inoculated Cynomolgus monkeys of original type 1 poliomyelitis virus (Mahoney strain) and variant obtained by special cultivation in Cynomolgus kidney tissue culture*

No. of TCD 50* inoculated	Cynomolgus CNS suspension		Kidney passage 33	
	Paralysis	Antibody	Paralysis	Antibody
10,000,000	—	—	0/10	10/10
1,000,000	—	—	0/5	5/5
100,000	4/5	5/5	0/5	5/5
10,000	5/5	5/5	0/9	2/9
1,000	4/5	5/5	0/5	2/5
100	4/5	5/5	0/4	0/4
10	3/4	4/4	0/5	1/5
1	0/4	1/4	0/5	0/5

* TCD 50 = 50 per cent tissue culture cytopathogenic dose.

TABLE 7.—*Comparative paralytogenic and immunogenic activity in intramuscularly inoculated Cynomolgus monkeys of type 2 poliomyelitis virus (Y-SK strain) at two different stages of cultivation in Cynomolgus kidney tissue cultures*

No. of TCD 50* inoculated	Kidney passage 34 Passages every 48 hours and then "purified"		Kidney passage 51 Passages every 24 hours and then "purified"	
	Paralysis	Antibody	Paralysis	Antibody
80,000,000	4/5	5/5	—	—
8,000,000	4/5	5/5	0/5	5/5
800,000	2/5	5/5	0/5	5/5
80,000	2/5	5/5	0/5	4/5
8,000	0/5	5/5	0/5	3/5
800	0/5	3/5	0/5	1/5
80	0/5	2/5	0/5	0/5
8	0/5	1/5	0/5	0/5

* TCD 50 = 50 per cent tissue culture cytopathogenic dose.

doses of the virulent parent strain, while no such immunity was found in the monkeys which failed to develop demonstrable antibody (7).

An even more marked diminution in the intramuscular and oral infective capacity for cynomolgus monkeys was observed in our laboratory (17) with the Brunhilde virus modified by cultivation in human non-nervous tissues by Enders and his associates (18). The data shown in Table 8 indicate, however, that even smaller doses of the same culture fluid were regularly immunogenic in chimpanzees, although intracerebral injection of the virus in this species produced neither paralysis nor lesions. Only one of the 11 chimpanzees excreted virus, and this virus did not produce paralysis after intracerebral injection in monkeys.

The fact that chimpanzees develop immunogenic, nonparalytic infections with small doses of virus, which are ineffective in most cynomolgus

TABLE 8.—*Immunogenic activity of modified Brunhilde virus in Cynomolgus monkeys and chimpanzees*

Route	Inoculum		Antibody	
	Culture fluid, ml.	TCD 50 log	Cynomolgus Monkeys	Chimpanzees
Oral	15	6.9	2/20	—
	5	6.4	—	4/4
Intramuscular	1	5.7	3/9	—
	0.1	4.7	3/10	4/4
Intracerebral	1	5.7	3/5	3/3
	0.1	4.7	0/4	—

monkeys, is in line with other data indicating that chimpanzees are more susceptible to extraneural infection but more resistant to the paralytic effects of poliomyelitis virus than are cynomolgus monkeys. We have also tested the mouse-adapted Mahoney strain of Li and Schaeffer (19) and found that it also has the properties of a "spinal variant" in monkeys. At this time, therefore, there is as yet no strain of poliomyelitis virus that does not possess to some extent the properties of the "spinal variant" in monkeys. While we are at present engaged in experiments to determine whether or not strains may be produced or found, which are completely devoid of paralytogenic activity in the spinal cord of monkeys, the currently available strains of each of the three immunologic types which produce neither paralysis nor lesions after injection into the spinal cord of chimpanzees deserve study as potential candidates for human immunization. In my opinion, viruses which are completely avirulent for chimpanzees even after direct spinal injection may be regarded as safe for orienting studies on human beings. Such studies should also be quantitative. They should establish the minimal amounts of virus which are immunogenic by the oral and by the intramuscular or intracutaneous routes, the frequency and concentration of viral excretion as well as the pathogenic properties of the excreted virus. Studies must also be carried out on the question of interference when all 3 living viruses are administered simultaneously. Living avirulent poliomyelitis viruses may perhaps best be used for the active immunization of infants during the first few months of life while they still have some placentally transmitted antibody from their mothers, and careful studies of this question must also be undertaken.

I hope that my remarks have left the impression that while the prospects for immunization against poliomyelitis are very good, there is still a great deal of fundamental work that remains to be done.

ADDENDUM TO LECTURE DELIVERED AT BRUSSELS IN JUNE 1954*

Since this lecture was delivered in June 1954 additional information has been gathered on the effect of the 3 types of chimpanzee avirulent poliomyelitis viruses in chimpanzees and human beings. A single feeding of 0.5 ml. to 1 ml. of culture fluid of each of the 3 types of virus produced a rapid immunogenic, clinically inapparent infection in chimpanzees. When a mixture of all 3 viruses was fed to 2 chimpanzees, the Type 1 and Type 2 antibodies developed rapidly while the response to the Type 3 virus was suppressed. When the same mixture of all 3 viruses was injected intramuscularly in $\frac{1}{10}$ the dose into 2 chimpanzees both developed antibodies to all 3 types but the response to the Type 1 and Type 3 viruses was minimal or negligible. Tests on human adult volun-

* Received for publication October, 1954.

teers indicated that the Type 2 and Type 3 chimpanzee avirulent strains, after being administered by mouth, can multiply in the human alimentary tract even in individuals who possess low titers of naturally acquired homotypic antibody, and that such clinically inapparent infection is followed by a rise in antibody titer.

No viremia was detected in 21 chimpanzees which received the various modified viruses by mouth or intramuscularly. Poliomyelitis virus was found in the stools at 7 to 28 days in 9 of 15 orally infected chimpanzees but in none of the 6 which received the viruses intramuscularly. The characteristics of the viruses excreted in the stools by the chimpanzees and the human volunteers were tested by intracerebral inoculation in monkeys and, with one possible exception which needs further study, there is no evidence that intracerebrally virulent mutants arose in the alimentary tract of chimpanzees or human beings. However, much more work needs to be done along these lines before a definitive answer can be given to this important question. The data mentioned in this addendum were reported at the Third International Poliomyelitis Conference in Rome, September 1954, and will appear in the Proceedings of this Conference.

SUMMARY

Current research on vaccination against poliomyelitis is based on the following discoveries of the past 4 years: 1) demonstration that only 3 main immunologic types of virus cause the human disease, and that the Type 1 virus is responsible for most epidemics; 2) cultivation of virus in non-nervous tissue *in vitro*, and demonstration that exceptionally high titers of virus can be achieved in kidney tissue cultures; 3) identification of poliomyelitis viruses by *in vitro* cytopathogenic effect; and 4) conversion of highly virulent viruses into avirulent variants by cultivation and segregation in non-nervous tissue.

Work on "killed-virus" vaccines, consisting of viruses completely inactivated by formalin or ultraviolet light is still in the early empiric stages, but it has been demonstrated that when sufficiently large amounts are given in repeated doses to children antibodies against all 3 types can be produced. The quantitative aspects relative to potency of vaccines produced in different ways and on a large scale, their stability on storage, the optimum dosage required to yield persistence of antibody for at least 8 months, as well as other important practical questions are still in the process of investigation. The best "killed-virus" vaccines that may ultimately be produced would probably have to be administered in repeated doses at frequent periods throughout a person's lifetime, because exposure to spontaneous infection is constantly diminishing in the very countries which would require vaccination the most.

The objective of immunization by means of avirulent viruses is to imitate what nature does for the vast majority of human beings without incurring the unpredictable and varying risk of paralysis which accompanies the natural acquisition of immunity. Toward that end there are now available experimentally produced strains of each of the 3 immunologic types of sufficiently modified virulence that even direct intraspinal injection in chimpanzees produces neither paralysis nor lesions. These viruses can immunize cynomolgus monkeys and chimpanzees when small amounts are given by mouth or intramuscularly. These viruses must next be studied in a careful stepwise manner in human beings.

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ESTUDIO DE LA VACUNACION CONTRA LA POLIOMIELITIS (*Resumen*)

Los estudios actuales de la vacunación contra la poliomielitis se basan en los siguientes descubrimientos hechos durante los últimos 4 años: (1) que sólo 3 principales tipos inmunológicos de virus causan la enfermedad humana, y que el virus de Tipo 1 es el causante de la mayoría de las epidemias; (2) que el virus puede cultivarse en un tejido no nervioso *in vitro* y lograr títulos de virus excepcionalmente altos en los cultivos en tejido del riñón; (3) identificación de los virus de la poliomielitis por su efecto citopatogénico *in vitro*, y, (4) transforma-

ción de los virus altamente infecciosos en variantes avirulentas por medio del cultivo y separación en tejido no nervioso.

Los estudios de vacunas de "virus inactivado," compuestas de virus completamente inactivado con formalina o con rayos ultravioleta, se encuentran aún en las primeras fases empíricas, pero se ha comprobado que administradas a niños en cantidad suficiente y en dosis repetidas se pueden producir anticuerpos contra los tres tipos. Los aspectos cuantitativos relativos a la potencia de las vacunas producidas de diferentes maneras y en gran escala, su estabilidad durante la conservación, la dosis óptima necesaria para producir anticuerpos cuya acción dure 8 meses por lo menos, así como otras importantes cuestiones prácticas, se encuentran todavía en proceso de investigación. Las mejores vacunas de "virus inactivado" que en definitiva pueden producirse, tendrán probablemente que ser administradas en dosis repetidas a cortos intervalos durante toda la vida de una persona, debido a que la exposición a la infección espontánea es cada vez menor en los mismos países en que más necesaria sería la vacuna.

El objetivo de la inmunización por medio de los virus avirulentos es imitar lo que hace la naturaleza con la mayoría de los seres humanos sin incurrir en los peligros imprevistos y diversos de la parálisis que acompaña a la adquisición natural de la inmunidad. Con este objetivo se dispone ahora de cepas producidas experimentalmente de cada uno de los tres tipos inmunológicos, de virulencia suficientemente modificada para que incluso la inyección intraespinal directa en chimpancés no produzca parálisis ni lesiones. Estos virus pueden inmunizar monos cinomologos y chimpancés si se les administran pequeñas cantidades por vía oral o intramuscularmente. Estos virus deben ser estudiados cuidadosamente en los seres humanos.