acterize foci described in Brazil, Venezuela, Central America, and Colombia.

For proper control of the disease, countries with areas in which AVL is endemic should encourage the following activities:

Diagnosis. To the extent possible, the use of the fluorescent antibody technique (FAT) should be adopted as a standard procedure. Other serologic techniques, such as the enzyme-linked immunosorbent assay (ELISA), can be introduced.

Information Analysis. There must be increased epidemiological analysis and interpretation of collected data, with every effort made to avoid duplicate records. Analysis must emphasize the trend of the disease over time for places of incidence, most severely affected age groups and sex, social factors involved, etc. Maps should also be prepared showing the geographic distribution of the endemic foci.

Antibody Levels. Some positive cases can be monitored clinically and serologically to determine the levels and duration of specific post-treatment antibodies in the serum.

Special Epidemiological Studies. Serologic studies using the FAT or ELISA should be done to evaluate the prevalence and distribution of infection. These data can be entered on maps of irrigation zones, land use, and phytogeography.

Search for Reservoirs: It is recommended that the following studies be attempted:

- a study of the flora and fauna in the endemic area,
- special serologic and parasitologic studies in stray dogs to find signs of infection,
- examination of foxes and rodents in endemic and adjacent areas, and
- isolation and characterization of the leishmania in these animals.

Entomologic Studies. A well-conducted entomologic study should be done in an attempt to distinguish the vectoral densities of the different species of phlebotomi, their geographic and seasonal distributions, the preferred host of each species, and the vectoral capacity.

Vaccines: The Way Ahead[1]

Communicable diseases caused by parasites, bacteria, and viruses continue in the 1980s to impose a major burden of morbidity, mortality, and disability on the world's populations. The greatest hope of reducing this toll lies in immunization.

Acute diarrheal diseases are widespread and are the cause of more than 4.5 million deaths a year in children less than six years old; acute respiratory infections cause over 2 million deaths a year; while malaria, schistosomiasis, and other tropical diseases in warmer climates and tuberculosis—mainly in poorer areas—are major scourges.

Future improvements in environmental and nutritional standards may reduce the incidence and severity of some of these diseases. New drugs and antibiotics for prophylaxis and therapy will, of course, continue to be developed, but many will lose their efficacy as the infecting organisms become resistant.

Passive immunity, conferred by the injection of preformed specific antibody, can last only while the antibody remains in the recipient. Such therapy or prophylaxis is expensive and brings its own range of undesirable side effects and is therefore not envisaged for general use.

In contrast, vaccination against viral and bacterial agents appears to be of wide applicability and is generally a safe procedure. Once economical methods are developed for their preparation, vaccines may be made in large quantities and usually cheaply. For example, trivalent live poliovaccine may cost a fraction of a dollar per dose. Immunity induced by one to three doses of vaccine is generally long lasting. Vaccination against parasitic diseases has still to be fully developed, but recent vaccines against malaria show considerable promise.

Mankind is now on the threshold of a new era in the technology of vaccine development and production, which stems from important advances in biotechnology, in particular recombinant DNA and cell fusion techniques. It offers hope of producing vaccines for many of the diseases that are yet uncontrolled and also of developing vaccines that are more effective, safer, and more cost-effective than those in current use.

[1]Reprinted from *World Health Forum* 4(4):408-413, 1983.

Conventional Vaccines

Classical immunoprophylaxis of bacterial and viral diseases may make use of inactivated vaccine or live attenuated vaccine. Sometimes both may be used simultaneously.

Inactivated vaccines consist of organisms treated physically or chemically to abolish infectivity and to remove many contaminating materials. Such vaccines have been sucessful against tetanus, diphtheria, pertussis, rabies, influenza, tick-borne encephalitis, Japanese encephalitis, and—more recently—meningococcal infections. Leprosy may soon be included in this list.

The efficient production of an inactivated vaccine generally requires, firstly, that the organism should be capable of cultivation *in vitro* so as to produce immunogenic quantities of the antigen; secondly, that an available inactivation procedure will destroy infectivity and/or toxicity, but retain antigenicity; thirdly, that the antigen can be suitably purified; and, finally, that the end product should elicit an immune state in man.

A unique case is hepatitis B vaccines. So far the only hepatitis B vaccines available are based on hepatitis B surface antigen (HBsAg), the viral surface antigen from human plasma, treated to remove unwanted plasma components and infectivity. Ultimate progress in the control of hepatitis B and its associated illnesses may depend on the new biotechnology.

The use of live attenuated organisms as vaccines has the potential advantage that the immune response generated is likely to resemble more closely that induced by natural infection, in contrast to the responses to inactivated vaccines. Live vaccines against tuberculosis, poliomyelitis, measles, rubella, mumps, and yellow fever are widely used. Live attenuated influenza vaccines are potentially valuable, but further research is required to establish their safety and efficacy.

A live attenuated vaccine must not induce significant disease, but it must replicate and induce an immune state. Further, attenuated vaccines must be genetically stable, since the appearance of genetic revertants of the original vaccine strain during production or in the vaccinee is clearly undesirable. Such an event has never yet been documented, but it should be emphasized that no live vaccine can be regarded as being absolutely safe. The degree of risk involved in the use of such a vaccine, however, is very much less than that arising from the disease it prevents. For live polio vaccines, a single case of vaccine-associated paralytic illness appears on average once every three million doses of vaccine administered.

Inactivated and live vaccines are prepared in batches using established seed strains of the organisms, and appropriate in-process control tests on the final product must be rigorously applied to ensure the safety and efficacy of the product. The World Health Organization formulates specific requirements for virtually all vaccines in regular use.

Although great progress has been made in the prevention and control of viral and bacterial diseases by the approaches described above, certain limitations are apparent as described in the following paragraphs.

Some organisms either do not grow *in vitro* or produce only small amounts of antigen. For example, the only source of hepatitis B antigen is human plasma from chronically infected persons. The production of the vaccine is technically complex and the yield is small. For products derived from human plasma there is a risk of contamination with pathogens present in donors. Indeed, the recent emergence of acquired immunodeficiency syndrome has focused much attention on the safety of blood-derived medicinal products.

The production of inactivated vaccines against highly pathogenic agents, such as those of African hemorrhagic fevers, may be hazardous to those engaged in this work.

There may be technical difficulties in detoxifying or inactivating vaccines. For example, in the early attempts to develop inactivated measles vaccine the viruses were treated with formalin. The resulting vaccine gave only partial or short-term protection but induced an abnormal immune state in recipients such that they developed severe atypical measles when subsequently infected with wild measles virus. Retrospective analysis suggested that formalin destroyed the fusion protein, one of the essential immunizing components of the virus.

As knowledge of the genetic basis of attenuation is meagre, vaccine strains have to be selected on arbitrary criteria. Live vaccine strains may have the potential to revert to virulence or to lose immunogenic activity. With greater knowledge of the genetic basis of virulence and the use of the new biotechnology, the selection of vaccine strains should improve.

Some viruses are associated with cellular transformation and potentially with the induction of malignancy. This is true of certain herpes viruses. Attenuated vaccines against these agents therefore call for rigorous safety tests.

Owing mainly to the complexity of the etiological

agents, little progress has been achieved in the control of parasitic diseases using conventionally produced vaccines. However, the new biotechnology may facilitate the development of vaccines against certain major parasitic diseases, in particular malaria.

Vaccine Development and the New Biotechnology

The new biotechnology has yielded two techniques of particular significance—the manipulation of defined coding sequences of DNA and their controlled expression in appropriate host cells and the use of cell fusion technology to produce immortal hybridoma cell lines that secrete monoclonal antibodies.

Recombinant DNA Technology

Genes that code for a specific product can be isolated and propagated by the insertion of naturally occurring or synthetic genetic material into a suitable vector organism, followed by the selection of individual clones of the vector that carry the required gene—the process of gene cloning. Most gene cloning work has been carried out in plasmids of *Escherichia coli*. Key steps in the process involve the insertion of the gene into the vector with the aid of highly specific restriction endonuclease enzymes, which cleave the vector DNA at predetermined sites, and ligases, which recombine the gene insert into the vector.

Techniques for the controlled expression of relevant microbial or cellular genes, after insertion into suitable vector systems, are now available. Using these methods, suitable cell systems can be made to produce defined microbial proteins or oligopeptides representing, for example, the epitopes of microorganisms relevant to immunization. These methods have the potential to be harnessed for the large-scale production of materials for use as vaccines and to provide the most powerful tools for vaccine development and production.

Some major applications of recombinant DNA technology are therefore:

- production of nucleic acids of defined microbial specificity for use as diagnostic reagents and tools for epidemiological research;
- modification of microbial genomes for the production of stable, safe, attenuated mutants as live vaccines;
- detailed identification of the chemical structure of antigens so that selected parts of the molecules can be synthesized by chemical methods and used as vaccines (synthetic peptide vaccines);
- production, by controlled gene expression in suitable vector organisms, of defined microbial proteins or oligopeptides for use as vaccines or as tools in diagnosis and epidemiological research; and
- production of "synthetic" antimicrobial antibodies, an approach that is considered feasible but has not yet been explored.

Cell Fusion Technology

In 1975, Köhler and Milstein first reported the production of monoclonal antibodies from hybrid cells obtained by fusing mouse myeloma cells with lymphocytes from immunized animals. Such techniques establish immortal cell clones, which continually secrete large amounts of antibodies against specific antigens of bacterial, viral, or parasitic origin. Monoclonal antibodies serve as highly specific tools for the analysis of the gene sites of microbial antigens in order to identify those of greatest potential value for inclusion in vaccines. They are also of value in the purification of antigens by affinity chromatography for use in vaccines.

Among useful applications of hybridoma technology are:

- preparation of monoclonal antibodies for the analysis of microbial antigens, so that antigenic structures relevant to immunogenesis may be identified and used to produce vaccines;
- production on a large scale of defined antimicrobial monoclonal immunoglobulins for use in passive immunoprophylaxis or therapy or as diagnostic reagents;
- production of immunoglobulin linked to antimicrobial or anticellular toxins (i.e., targeted drugs) for use in therapy; and
- preparation of clones of immunocompetent cells (B cells, T cells) that have a role in immunological research and potentially in disease control.

Parallel Research

Since some of the products of the above genetic engineering procedures may be small molecules that are themselves only poorly antigenic, it may be necessary to develop acceptable adjuvants, immunopotentiators, and antigen "carriers". The best use of the new vaccines will also depend on developments in immunology. Recent knowledge of the immune response to microbial antigens in man was reviewed by a WHO scientific group in July 1982 *(1)*.

Both humoral (immunoglobulin) responses and cell

mediated responses play a role in immunity and recovery from microbial infection. Indeed, the immune response depends on a complex network of interdependent components, and its characteristics differ widely for different types of microorganism. There is now, however, much new information on the cells involved in the immune response.

Some Priority Applications

In virology, an important application of the new biotechnology is the development of vaccines against agents that have not so far been cultivated or that grow poorly. Examples are the hepatitis B virus and rotavirus. The latter agent is a major cause of severe diarrheal disease in infancy but it grows too poorly to permit the preparation of conventional vaccines. By use of the new methods, vaccines against dangerous pathogens, such as African hemorrhagic fever viruses could probably be formulated without the risks of using infectious viruses.

The influenza virus presents special problems for immunoprophylaxis because of its high degree of antigenic variability. The modern techniques can be used analytically to study the problem of variation and to seek alternative approaches to vaccine design or chemotherapy. Further information is needed on host factors in infection, not only for influenza but also for other human respiratory viruses such as respiratory syncytial virus before rational immunoprophylactic approaches can be developed.

With certain medically highly important viruses such as the herpes viruses, latency and reactivation occur and are complicating factors in attempts at immunoprophylaxis. Some herpes viruses may also be associated with oncogenicity. So far, attempts to develop vaccines against these agents have made little progress. Methods of genetic engineering and modern immunology should be used vigorously to study the host/virus interaction and to develop a rational approach to control. This may call for entirely new concepts.

The development of small, synthetic peptides mimicking the antigenic structures of certain viruses has already provided encouraging results. Viruses that may be mimicked in this way include hepatitis B virus, foot-and-mouth disease virus (an agent with many of the features of poliovirus), and rabies virus. This field of work is worthy of careful exploration and development to determine the value of the peptides as vaccines.

Although many bacterial diseases are well controlled by the use of toxoid vaccines, the structural and antigenic complexity of bacteria and parasites is much greater than that of viruses. For some important bacterial agents vaccines are of low efficacy and acceptability, while little progress using conventional approaches has been made in developing vaccines against parasitic diseases. Genetic engineering methods are therefore of particular value for the control of disease caused by these agents and should be applied as a matter of urgency. For bacterial diseases, research is needed into vaccines against tuberculosis, pertussis, gonococcal infections, and leprosy. In the field of tropical medicine, the main priorities for vaccine development are the major parasitic diseases—malaria, filariasis, schistosomiasis, leishmaniasis, and trypanosomiasis.

A new vaccine development program started by the World Health Organization will commission research in selected areas where progress in disease control is dependent on the production of new vaccines, the improvement of existing vaccines, or the elaboration of specific drugs and other substances, such as interferons and modulators of the immune system. The Organization now has an unparalleled opportunity to guide the efforts of the biotechnology and pharmaceutical industries towards the diseases for which effective vaccines are most needed and of bringing the fruits of research within reach of everyone.

Reference

(1) Viral vaccines and antiviral drugs: report of a WHO Scientific Group. Geneva: World Health Organization, 1983. (Technical Report Series No. 693).

(Source: Dr. G. C. Schild, Head of the WHO Collaborating Center for the Standardization of Viral Products, and Head of the Division of Viral Products at the National Institute for Biological Standards and Control, London, England and Dr. F. Assaad, Director of the Division of Communicable Diseases at WHO, Geneva, Switzerland.)

Editorial Comment

This article draws attention to the potential benefits that the new biotechnology can bring to public health by generating new tools for the more efficient prevention and control of communicable diseases. A key application is the development of new vaccines using recombinant DNA technology. These vaccines can fill the great need for currently unavailable immunogens against several important diseases, or can replace existing vaccines which are not satisfactory. Favorable re-

sults recently documented in a clinical evaluation of a hepatitis B vaccine made by recombinant DNA procedures[2] clearly illustrate that practical applications of this new generation of vaccines are not far from reach. Another important application of the new biotechnology involves the production of nucleic acids of defined specificity for use as diagnostic reagents and tools for epidemiological research. The utilization of cell fusion technology has resulted in the production of monoclonal antibodies, some of which are already used for diagnostic purposes in several countries of the Region.

Modern biotechnology has created new opportunities that call for PAHO's involvement in the field. PAHO can be instrumental in selecting priorities for vaccine development, improvement, and evaluation. Moreover, the Organization can play a crucial role in transferring vaccine and reagent production technology to developing countries.

Although biotechnology offers considerable promise in the production of immunobiologics, conventional techniques still prove valuable. In this context, PAHO has played an active role in coordinating and supporting the development of new vaccines and the improvement of existing ones using conventional approaches. Substantial progress has been achieved in developing a live attenuated vaccine against Argentine hemorrhagic fever, and the first trials in volunteers are to be conducted in the near future. Another example of PAHO's involvement is the progress made in modernizing the current 17-D yellow fever vaccine produced in embryonated eggs by Colombia and Brazil. In addition, plans are under way to develop a thermostabilized yellow fever cell culture vaccine, which will represent an important technological achievement.

[2]Scolnick, E. M. et al. Clinical evaluation in healthy adults of hepatitis B vaccine made by recombinant DNA. *JAMA* 251(21):2812-2815, 1984.

Reports on Meetings and Seminars

Society for Epidemiologic Research Annual Meeting

The Seventeenth Annual Meeting of the Society for Epidemiologic Research (SER), 13-15 June 1984, was hosted by the University of Texas School of Public Health in Houston, Texas.

The meeting was structured around six symposia with the following topics: epidemiology of aging; infectious agents and chronic disease; controversies in reproductive outcomes; human biology and epidemiology; methods in occupational epidemiology; and epidemiology of injuries. Almost 200 papers were presented dealing with these and other issues such as cancer, maternal and child health, cardiovascular disease, blood pressure, diabetes, infectious diseases, and methods.

The 1985 and 1986 SER meetings will be held in Chapel Hill, North Carolina and in Pittsburgh, Pennsylvania, respectively.

International Symposium on *Salmonella*

An international symposium on *Salmonella* was held in connection with the annual meeting of the American Veterinary Medical Association on 19-20 July 1984 in New Orleans, Louisiana, USA. The symposium was broad in scope and included a review of the worldwide *Salmonella* situation with regard to livestock production, meat and poultry processing, and public health.

One of the principal aims of the symposium was to identify practical ways of preventing or reducing *Salmonella* infections in major food animal populations and of curbing contamination of products derived from such animals. The gathering also considered the impact of *Salmonella* on world trade, the utility of various national and regulatory programs for controlling the species, and the problems posed by important host-adapted organisms such as *S. dublin* and *S. gallinarum*.

Overall, the symposium was a vehicle for developing a more coherent understanding of approaches that are useful in seeking to contain the worldwide *Salmonella* problem. A separate one-day meeting on food hygiene, which included papers on *Salmonella*, was held the day before the symposium began.

Additional information may be obtained by contacting Dr. G. H. Snoeyenbos, General Chairman, International Symposium on *Salmonella*, Paige Laboratory, University of Massachusetts, Amherst, Massachusetts 01003, USA.