RESEARCH ACTIVITIES OF THE PAN AMERICAN FOOT-AND-MOUTH DISEASE CENTER

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At the first meeting in June 1962, of the Advisory Committee on Medical Research of the Pan American Health Organization, a report was presented on the past, present and future program of the Pan American Foot-and-Mouth Disease Center (PANAFTOSA).

For the second meeting of the Advisory Committee a report is now presented of the progress made since June 1962.

**Diagnosis, Virus Isolation and Identification**

Seven distinct immunologic types of foot-and-mouth virus have, so far, been isolated of which three are found in South America: Vallée O, Vallée A and Waldmann C. Within each of the types, subtypes occur, the antigenic behaviour of which is sufficiently different to be of great importance in the selection of virus strains for the preparation of vaccine. The Center provides assistance to the national laboratories of the countries affected with foot-and-mouth disease by studying the characteristics of newly isolated viruses whenever it is suspected that a subtype difference may be involved.

During the period under review three examples of this work can be cited. The first concerned a severe outbreak of foot-and-mouth disease that occurred in the State of Zulia in the west of Venezuela against which the vaccine in current use gave no protection.

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Examinations conducted at the Center revealed the existence of a significant difference between the strain of virus isolated from this outbreak and the strains of the same type (Vallée A) hitherto encountered in Venezuela. This identification of the virus strain from this outbreak coincided with another feature of the research program then taking place in Venezuela to which reference is made later, the combined results of which led to the largest application to date in the Americas of a modified live virus vaccine against foot-and-mouth disease.

The presence in the west of Venezuela of this subtype Zulia of type A caused some concern in Colombia and the Center immediately provided a supply of the strain specific serum for use in the complement-fixation test in order to permit the Colombian institute to verify whether this virus was also present in their territory. Following an outbreak of type A infection in Colombia early in 1963 a detailed examination was made at the Center of a collection of virus samples from the field, without, however, encountering anything unusual.

In connexion with the assistance being provided by the Center to the national foot-and-mouth disease program in Argentina, periodic revisions are made of the results of the identification of virus strains from field outbreaks in that country. This resulted, recently, in the detection of a strain of type Vallée A that would appear to have a broader than usual antigenic coverage of subtype differences. The recommendation has, therefore, been made that the possibility of this strain being very useful for the preparation of vaccine be tested by immunity experiments in cattle.
This work of virus identification involves the use of the complement-fixation test, serum protection or serum neutralization tests and cross-immunity tests in animals. Efforts are continually being made to improve the sensitivity of these tests. Two examples of recent progress at the Center can be cited in this connexion.

Serum protection tests for the detection of antibody can be performed in guinea pigs or, as is more usual, in mice. In the test using mice it has been the practice to employ the unweaned mouse. In a comparison of the use of guinea pigs, unweaned mice and young adult mice it was found that young adult mice and appropriately adapted virus strains provided the most sensitive system for the detection of antibody.

The sensitivity of the complement-fixation test is such that it is very satisfactory for the identification of virus types by the use of strain and type specific hyperimmune sera but unless a very good antigen is available its use is very limited for the detection of antibody in the sera of convalescent or vaccinated animals. Reference is made later to the initiation of the use of a diploid cell line BHK 21 but mention may be made at this point that it has been found that foot-and-mouth disease virus passaged in BHK 21 cells possesses a high complement fixing titre and preliminary results indicate that an antigen prepared in this way may permit the detection by the complement-fixation test of antibody in the sera of convalescent or vaccinated cattle.

The diagnostic service is not only concerned with the identification of the virus types of foot-and-mouth disease. Vesicular
stomatitis is a disease affecting cattle, swine and horses. It is clinically so similar to foot-and-mouth disease that differential diagnosis in species susceptible to both infections requires a laboratory examination. Vesicular stomatitis occurs in the United States of America, Mexico, Central America, Panama, Venezuela, Colombia, Ecuador and Peru. From Panama north there is no foot-and-mouth disease and in these countries, therefore, it is most important that a correct diagnosis be made as rapidly as possible in the case of any outbreak of vesicular disease in cattle or swine. The Center provides this diagnostic service for the countries of Central America and Panama.

Because of its more benign nature, the problem of vesicular stomatitis has never received much attention but the losses and inconvenience that it can cause, especially in dairy cattle, merits consideration. The epizootiology of the disease is not well understood and although two immunologic virus types (Indiana and New Jersey) have been recognized for many years, there are other differences becoming apparent between the two types. For example, it has proved possible to infect *Aedes aegypti* with the Indiana type but not with the New Jersey. The recent isolation of Cocal virus in Trinidad and Brazil with its marked serological relationship to the Indiana type of vesicular stomatitis complicates the picture as vesicular stomatitis has never been recorded in domesticated livestock in Trinidad nor Brazil. Epizootiological studies in Panama, however, have now shown that antibodies to vesicular stomatitis can be detected with a
relatively high frequency in domesticated and wild animals from areas in which no clinical outbreaks have been observed.

In order to draw attention to the interest and importance of intensifying research on vesicular stomatitis, the Center organized a symposium in November 1962, in Mexico City with participants from the veterinary services of all the affected countries and also with the assistance of Professor R. P. Hanson of the University of Wisconsin and Dr. A. H. Jonkers of the Rockefeller Foundation Laboratory in Trinidad. The participation of the Center in a research program on vesicular stomatitis in its laboratories in Rio de Janeiro must be somewhat restricted because of the apparent absence of the clinical disease in Brazil. Work confined to the bench and to small animals is, however, being undertaken. For example, we have recently demonstrated that the wild rodent *Dasyprecta aguti* is susceptible to both vesicular stomatitis virus types and we hope to examine equine and bovine sera for antibodies from areas of Brazil where the environmental conditions are similar to those in countries where vesicular stomatitis is a clinical entity.

**Immunization of Susceptible Livestock**

In countries in which foot-and-mouth disease is enzootic the basis for controlling the disease is the systematic application of vaccine in cattle. The vaccines in use are inactivated virus vaccines and in South America, because of the incidence of the various types, these vaccines must be bivalent or trivalent. The inactivated virus
vaccine in foot-and-mouth disease is a relatively poor product, the
duration of immunity after primary vaccination being no more than
four or five months.

There is obviously a great necessity for research on improved
methods of immunization. The program of the Center has been concen-
trated on improving the existing inactivated virus vaccines and on
the development of a modified live virus vaccine.

**Inactivated virus vaccines**

The principal points which are being investigated with regard
to inactivated virus vaccines are systems of virus multiplication
for production of antigen, methods of inactivation of the virus, the
use of adjuvants, techniques for control of vaccine quality, and the
application of vaccine.

Virus for the preparation of foot-and-mouth disease vaccine
was, at one time, produced exclusively by the inoculation of cattle.
In many cases this was, and is, done in slaughterhouses using cattle
destined for supply of meat. Techniques of culture of virus have
been developed to be used in place of inoculation of cattle and one
important feature of the Center's program is to have these adopted
and to eliminate the inoculation of cattle for virus production.

The most widely used method of culture of foot-and-mouth dis-
ease virus, the Frenkel method, involves the use of normal cattle
tongue epithelium. In many parts of South America the local
availability of tongues is insufficient for the scale of vaccine production required. It is extremely important, therefore, to find alternative methods. The use of monolayers of pig or calf kidney cells has been studied at the Center for some years but not with completely satisfactory results due, in part, to the variability in quality and susceptibility of primary cultures. In the last few months the use of the established cell line BHK 21 is yielding more promising results and reference is made to this later.

In connection with the control of vaccine production, the search has continued for tests of vaccine potency that do not require the use of cattle. A test using young adult mice has been developed at the Center but it is not universally applicable because of the use of saponin as an adjuvant in the preparation of vaccine. This product causes too severe a local reaction at the site of inoculation in mice. A recent publication from the USDA Plum Island Animal Disease Laboratory reported good development of antibody in adult fowls following their inoculation with foot-and-mouth disease antigens with the suggestion that their use might be applicable in tests of vaccine potency. Their use for this purpose is now being studied at the Center especially for saponin vaccines. This work is still in progress but it has been shown that there is no undesirable reaction following the inoculation of fowls with saponin vaccine.
One great advantage possessed by the Center compared with some other foot-and-mouth disease institutes is the ability with which work in the laboratory can be combined with work in the field. This, for example, does not apply to institutes in countries in which the disease is not enzootic.

It is appropriate, therefore, to refer to a program that has been in progress in collaboration with the Brazilian Ministry of Agriculture since the beginning of 1962. The program is the demonstration of the effect of the systematic application of inactivated foot-and-mouth disease vaccine on the control of the disease. Using vaccine produced at the Center, a total of approximately 10,000 cattle have been maintained almost completely free of infection for the sixteen months that the program has been in operation.

**Modified live virus vaccines**

The techniques used, so far, in foot-and-mouth disease research for the development of modified live virus vaccines have been the adaptation of virus strains to various host or tissue culture systems with continued serial passage of the virus until pathogenicity for cattle has been reduced to a very low level. The systems used at the Center are young rabbits, young mice, day-old chicks and chick embryos.

The subtype differences between virus strains referred to earlier is, apparently, of as great importance with modified live virus vaccines as with inactivated virus vaccines. For this reason, the work of modification of strains has not been confined to one example from each of the three immunologic virus types in the Americas. The technique of modification has also not been confined to the use of one
system. A selection of strains is, therefore, being accumulated that have received different manipulations and that have different characteristics.

Until the beginning of 1962 the cattle accommodation available for the use of the Center's research group was very limited and it was, thus, not possible to determine whether all the virus strains being handled has reached a satisfactory degree of modification. The bringing into use of two new cattle stables at the beginning of 1962 has enabled this work to proceed more rapidly.

The most notable advances that have been made within the last year concern virus types Vallée A and Waldmann C.

For the last two or three years the Center has been conducting a program of collaborative research on the development of modified live virus vaccines with the Veterinary Research Institute of the Ministry of Agriculture of Venezuela. This program consists, basically, of the conduct of immunity experiments in cattle in Venezuela with virus strains that have already given some indication, following tests in the Center and in the field in Brazil, that they might be suitable for use as modified live virus vaccines. In August 1962, the Center was engaged in one of these experiments in Venezuela in collaboration with the national Veterinary Research Institute. The virus strain under study was a strain of Brazilian origin: of type A, modified by serial passage in chick embryos (strain A Cruzeiro). When this work was in progress a serious outbreak of foot-and-mouth disease occurred in the State of Zulia due to a type A virus against which the local type A inactivated virus vaccine afforded no protection due to a subtype difference.
Trials were immediately set up to determine whether the strains A Cruzeiro modified live virus vaccine would protect against the A Zulia field strain. Although the strains were not antigenically the same, there was sufficient similarity for A Cruzeiro to give a relatively satisfactory level of protection against A Zulia although the virus dose had to be larger than in the case of a homologous virus challenge.

In view of the emergency presented by this temporarily uncontrollable field outbreak, the same joint Venezuelan Center team proceeded to establish a "crash" program of modified live virus vaccine production. This was then developed by the staff of the Venezuelan Institute and a level of production of 200,000 doses per week was achieved by October. The application of the vaccine in the field in the area of the infection and on the routes of movement of livestock was started at the end of October and beginning of November. By March 1963, a total of 1 1/2 million doses of vaccine had been applied and field observations are in agreement that the course of the outbreak was dramatically halted. It must be appreciated that this was an emergency program and that some standards had to be lowered, as, for example, that with regard to the nonpathogenicity of the modified virus. Nevertheless, a valuable experience has been gained and an analysis of the results will be of importance in planning future development.
Another feature of the program of the Center during the last year has been to obtain a satisfactorily modified strain of type C. Most effort had previously been concentrated on the two most prevalent types, namely, 0 and A.

In the contacts maintained with other institutes engaged on foot-and-mouth disease research, arrangements were made to test at the Center a modified strain of type C developed in the laboratories of the French Ministry of Agriculture. Although this strain proved to be of an acceptably low level of pathogenicity, the immunity produced was disappointing, at least against the Brazilian C type virus used in the challenge of the immunity of the vaccinated cattle.

Work was, in the meantime, being continued on the modification of a Brazilian C type strain (C Rezende) by serial passage in mice and also in rabbits. The results that are now being obtained with this C Rezende strain are proving to be very satisfactory and the phase of passing from the laboratory to the field can now be planned.

Having reached this stage in the development of vaccines of strains representative of each of three virus types present in South America, work can now pass on to the study of the simultaneous application of vaccine of more than one immunologic type.

In addition to this basic work of the development and testing of modified viruses, many other points have had to be studied with regard to the preparation of the vaccine, its storage and
distribution, and its application. It has also been necessary to con-
sider such questions as the stability of the modification, the necessi-
ty of finding markers for use in confirmation of identity of modified
strains and the development of tests for control of vaccine production.
The study of these points is still in progress.

The application of tissue culture techniques

Tissue culture techniques are used at the Center for an increas-
ing variety of purposes such as titration of virus, detection of an-
tibodies, multiplication of virus for preparation of vaccine and in
the search for virus markers.

The most frequently used cell system in foot-and-mouth disease
research has been primary cultures of calf or pig kidney cells. Many
attempts have been made to establish cell lines from these tissues
that retain their susceptibility to the virus. There seems little
doubt that susceptibility to foot-and-mouth disease virus can be cor-
related to the persistence of diploid cells. The ease with which
serial passage of calf or pig kidney cells results in a heteroploid cell
line has constituted the main obstacle to the use of an established cell
line in this work.

Attempts are in progress at the Center to establish diploid cell
lines by, for example, clone selection and the best result to date has
been to carry one line to the 12th passage.

In the meantime, however, a very satisfactory cell line has be-
come available that was developed in the Virology Department of the
University of Glasgow, Scotland. This is the line BHK 21 established
from baby hamster kidney. A sample of these cells were received at the
Center in October 1962, since when extensive studies have been made of their suitability for use in routine and research work in foot-and-mouth disease.

BHK 21 cells are highly susceptible to foot-and-mouth disease and to vesicular stomatitis viruses. It is interesting to note that with foot-and-mouth disease virus strains, at least, the complement fixing titre of virus passaged in BHK 21 cells is notably higher than after passage in calf or pig kidney cells. This, in itself, has suggested useful applications for such antigen preparation in the complement fixation test.

The use of tissue culture systems for the production of virus for preparation of vaccine is a point of great interest, especially in the development of modified live virus vaccines. As has already been mentioned, the modified strains are produced by serial passage in young rabbits, young mice, day-old chicks or chick embryos. Although feasible on a limited scale, the use of these hosts for the production of virus is not very practicable when the preparation of millions of doses of vaccines is considered. Mention has also already been made of some restriction in the applicability in certain countries of the Frenkel system of virus culture for the preparation of inactivated virus vaccines. The production of virus in a tissue culture system has, therefore, received considerable attention in foot-and-mouth disease research in recent years. Most work has, so far, been done on the use of monolayers of primary cultures of calf or pig kidney cells but with the feeling that the use of a culture of suspended cells of an established line would yield better results.
Since receiving the BHK 21 cell line, experiments have been initiated on the use of these cells in suspended culture for the production of virus. The results to date are insufficient to show whether high virus titres can be obtained with regularity but the use of this material for the preparation of vaccine is about to be tested.

A great deal of work is done at the Center on the examination of cattle sera for antibodies in connexion with the selection of cattle for experimental use and also in following the results of vaccination. This has been done by the use of a serum protection test in unweaned mice. The research program has included the study of serum neutralization tests in tissue culture in order to reduce the necessity of using the large number of mice necessary for these antibody determinations. One test that has been studied, for example, has been the metabolic cup test using calf or pig kidney cells. Better results are now being obtained, however, by the use of BHK 21 cells in a serum neutralization test judging the result on the presence or absence of a cytopathogenic effect.

The development and the use of modified live virus vaccines has necessitated a search for markers that can be used, either to select a modified strain or to identify a modified strain in the control of its production. Tissue culture techniques are being used for this purpose but, so far, apart from some differences in certain cell systems of the cytopathogenic effect of modified compared with field strains of virus, no new marker of great utility has yet been discovered.
Epizootiologic survey of Tierra del Fuego

A report of the current research program of the Center would not be complete without a reference to the above survey which is part of a joint program between Argentina, the United States of America and the Center.

The Center accepted the responsibility for the planning and supervision of the execution of this survey which has the object of determining whether or not foot-and-mouth disease is present in the island of Tierra del Fuego. This information is required in order to assist the United States Department of Agriculture in deciding whether importations of frozen lamb could be accepted without risk of introduction of the disease.

The United States Department of Agriculture has agreed that the basis of the survey should be the examination of serum samples from the animals on the island for the presence or absence of antibodies against foot-and-mouth disease.

In addition to the responsibility of the planning and the supervision of the collection of serum samples, the Center has accepted the responsibility for the examination of the samples.

The collection of samples was begun on the Chilean side of the island in the beginning of January 1963, and was terminated on the Argentine side in April. A total of more than 25,000 samples have been collected and during the whole of this period one or other member of the staff of the Center was present in the island.

The intention is to examine these samples by the use of a serum neutralization test employing BHK 21 cells and final arrangements are being completed to commence this task in May. Although a
total of more than 25,000 samples have been collected it is not intended, in the first place, to examine more than about 10,000. The remainder were collected to permit a more extensive revision of any flock to be made that seemed necessary without the requirement of returning to the island.

The other sections of the joint program in which the Center will participate have been delayed because of a number of factors and another investigations, for example, the survival of foot-and-mouth disease virus in salt beef is not expected to start before June 1963.