IMMUNOLOGY OF YELLOW FEVER

IMMUNOLOGY OF YELLOW FEVER

KENNETH C. SMITHBURN, B.Sc., M.D.

The Rockefeller Foundation, New York, N.Y., USA

Yellow fever was the first disease in man shown to be caused by an ultra-microscopic filtrable virus.[62, 94] The workers who made this discovery were further successful in establishing yellow fever as the first virus diesease in man known to be transmitted by mosquitos. These epoch-making discoveries had far-reaching effects in the control of the disease, but further advancement of knowledge concerning it was delayed another quarter-century until a susceptible animal host was found.[84] The great strides made in research on this disease since 1927 have gone far in bringing it under control. They have also served as models for work on other insect-borne diseases.

A comprehensive review of the more significant research on yellow fever was published under the editorship of Strode in 1951.[85] The chapter on immunology in that volume covered the subject more extensively than is the purpose of this publication, and included much material not previously published. In this communication the subject will be treated especially in its relationship to vaccination, and the more significant original papers appearing since 1951 will be considered.

Immunity to yellow fever may be either active or passive. Active immunity to the virus may result from natural infection, from experimental infection, or from vaccination. Passive immunity may be the result of transfer of immunity from an immune mother to her offspring, or it may result from the inoculation of an otherwise susceptible individual with protective antibody derived from an immune individual.

Active Immunity

Active immunity resulting from natural infection

Even before a susceptible host for yellow fever virus was known, it was recognized that a human being who contracted yellow fever and recovered became immune as a result of this experience and did not contract the disease a second time in his life.[54, 62, 94] Soon after the rhesus

monkey was found to be susceptible to yellow fever virus,[84] it was observed that the serum of persons recovered from the disease was effective in preventing infection in monkeys [84] when given along with or at about the time of the inoculation with the virus. Advantage was taken of this fact, particularly in the laboratories of the Yellow Fever Research Institute at Yaba, Nigeria, to study the distribution and frequency of yellow fever in various West African countries.[5, 35] It was also observed that protection tests with sera from persons in the acute phase of yellow fever and with convalescent sera from suspect cases could be used for diagnostic purposes. The protection test revealed also that the yellow fever of the African continent and that of South America are the same disease.[34, 36] These uses of immunological methods, developed in 1927 and 1928, have now been superseded by a method which is more convenient, largely by the fact that it is less cumbersome and less expensive—namely, the performance of the same type of test in Swiss mice. Nevertheless, the early experiments with monkeys revealed the scope of immunological methods in diagnostic and epidemiological studies.

Very little is known about the mechanism responsible for immunity to yellow fever, either in man or in the animals which are susceptible to the disease. It is known that the development of immunity is associated with the appearance in the serum of antibody which is capable of neutralizing the virus, but it is not known whether other defensive mechanisms than the humoral are involved. The inoculation of virus into an immune host does not elicit a visible reaction, even though the inoculation be made intradermally in a site where even a mild inflammatory reaction would be quite easily detected. Nevertheless, the virus is quite effectively dealt with in the immune host and can rarely be recovered once it has been injected.

The antibody response evoked in man by natural infection with yellow fever virus occurs fairly rapidly [8] and, even in fatal cases in which death occurs as early as the fourth or fifth day, the antibody can be detected, as a rule, before death. Once this response has taken place in a human being, the individual apparently never loses his immunity. Neutralizing antibody has been demonstrated as long as 75 years after an attack of the disease.[64] Further evidence of the enduring nature of the immune response is the fact that authenticated instances of plural infection in the same individual with yellow fever virus are unknown.

Certain wild animals which are natural hosts of yellow fever in either Africa or South America, in particular the wild primates, exhibit an antibody response to the infection which is in all respects similar to that characteristic of the human being. The duration of the response in monkeys is prolonged, as it is in man. This enduring character of the response, both in the human being and in the wild animal (monkey) host, is taken

advantage of in epidemiological studies to determine the presence and density of infection occurring under natural conditions.[22, 24, 29, 40, 41]

Active immunity following experimental infection

All the characteristics of the response which experience has shown to be typical in human beings infected with yellow fever can be detected in experimentally inoculated primates. If a susceptible monkey is inoculated with a small dose of yellow fever virus or is subjected to the bite of one or more infected mosquitos of a vector species, a typical infection results.[84] If the animal is of a highly susceptible species, such as the Indian rhesus monkey (*Macaca mulatta*), and especially if the infecting virus is an African strain, death is almost certain to result; but if the animal is bled when it is *in extremis*, and the serum is inactivated to destroy any virus which may be present, neutralizing antibody is almost certain to be detectable. In the event of the survival of the animal, antibody is usually detectable three or four days after virus was first demonstrable in the serum. In experimentally inoculated primates, as in human beings and in monkeys naturally infected, the antibody, once it has appeared in the serum, is likely to be present for the remainder of the life of the animal. A monkey which has exhibited such an antibody response is completely refractory to re-inoculation with yellow fever virus, regardless of how susceptible it may initially have been.

Both the human being naturally infected [8] and the monkey experimentally inoculated with yellow fever virus thus exhibit the stage of the disease in which both the virus and the specific antibody are demonstrable in the blood. At this stage of the disease the virus is, of course, diminishing in quantity and the antibody is on the increase. This phase of the infection in monkeys occurs over a 24-48-hour period, between the third and fifth or sixth days after inoculation with any but the very smallest doses of the virus. With minimal doses of virus the entire clinical reaction may be delayed, as was shown by Bauer in experiments in which yellow fever virus was titrated in rhesus monkeys.[2] In those experiments animals receiving minimal infective doses exhibited no response over a long period of time, but when the infection began to take place it was typical in all respects, including the antibody response, of infection with a shorter incubation period.

Active immunity induced by vaccination

The practical aspects of this question are dealt with by Courtois in his contribution to this monograph (see page 105), and we shall concern ourselves here with historical and theoretical aspects of the matter.

The first vaccine against yellow fever was developed by Sawyer, Kitchen & Lloyd.[66] The method they proposed consisted in the inoculation of a measured dose of neuro-adapted yellow fever virus, together with sufficient specific immune serum to nullify any harmful effects which the virus might otherwise have had. This method was tried originally in monkeys and was found to be quite effective. When the method was applied to human beings the earliest recipients of the vaccine were hospitalized and quarantined and kept under very close observation. Although some of them developed mild clinical reactions, none of these was serious. The antibody response was good and the sera of vaccinated individuals showed high protective powers.[67] Field application of the method was greatly restricted, however, owing to the prohibitive amount of specific immune serum required. Convalescent human beings or vaccinated persons were the original sources of supply for immune serum, but when this source became inadequate the hyperimmunization of monkeys [89] or of non-susceptible hosts, such as horses and goats, was undertaken in order to meet the demand. Even these sources would not have been adequate on the large scale to which the vaccine came to be used.

Workers in various countries applied themselves to the problem of developing a vaccine for yellow fever, but the first vaccine to be used widely in the field was the attenuated strain developed by Lloyd, Theiler & Ricci.[48] A strain 17E [47] was first used and then shortly replaced by the 17D strain,[90, 91] which was apparently more nearly fixed in its properties. This strain was extensively studied in the laboratory before its field application was undertaken.[91] The first large experiment in the field with 17D vaccine was that of Smith, Penna & Paoliello in Brazil.[73, 82] These workers concerned themselves especially with the technical aspects of the problem, for instance, the transportation of a thermolabile product and administration of the material to significant population groups within the time which the lability of the material allowed. Originally, bio-assays were also carried out on each lot of vaccine, and sampling of vaccinated persons was undertaken before and after the inoculation to study the antibody response. It was found that demonstrable antibody was evoked in a large percentage of cases by inocula which contained 100 or more LD_{50} of the 17D vaccine. With smaller doses, the response of some individuals was equally good, but the percentage exhibiting a response was lower. Reactions to the vaccine were uncommon, and mild when they did occur.[73, 83] Accordingly, the 17D vaccine was adopted for routine use in Brazil and other South American and Central American countries, as well as in certain parts of Africa.

With the outbreak of the Second World War, the Rockefeller Foundation undertook to supply yellow fever vaccine 17D to the armed forces of the United States of America and allied countries.[85] During the course

of the war, more than 25 million doses of this vaccine were produced in the Foundation laboratories and dispensed to American and allied governmental agencies. In the early years the vaccine was made with a component of non-immune human serum, originally believed to be necessary to protect the virus against deterioration. Vaccine prepared in such a manner was responsible for causing a large number of cases of hepatitis,[23] especially among American military personnel,[69, 70, 93] owing to contamination with the relatively thermostable hepatitis virus contained in the human serum component. Before this, Theiler had already carried out experiments which showed that effective vaccine could be prepared without the use of serum. When this series of hepatitis cases occurred the large-scale production of vaccine prepared without serum was undertaken in the United States Public Health Service laboratories [31] and the laboratories of the Rockefeller Foundation in New York.[a] Since that time no cases of hepatitis have been reported following vaccination with the 17D strain.

The antibody response to 17D vaccine in rhesus monkeys has been studied by Theiler & Smith,[91] and by Smithburn & Mahaffy.[78] Inoculated monkeys seldom exhibit the vaccine virus in their circulation, but they respond by the formation of specific antibody, which is demonstrable in the blood within about seven days following inoculation. Experimental challenge, however, reveals that monkeys vaccinated with 17D virus are refractory to the pantropic virus within about three or four days from the time they receive the 17D vaccine. Thus the animals are actually refractory to the inoculation three to four days prior to the time when antibody may be demonstrated in the blood. Whether this is due to the presence of antibody in quantity too small to be detected by the available methods, or whether it is due to the fact that the developing immunity becomes effective during the incubation period which the pantropic virus requires, is not known.

Administration of the 17D vaccine to man is followed within seven to ten days by the appearance in the serum of demonstrable specific antibody capable of neutralizing yellow fever virus.[78, 91] In field application the percentage of persons responding to the vaccine is very high. Occasional failures occur under field conditions, but, in the experience of the writer, where yellow fever vaccine was administered to newly engaged employees in a field laboratory, and where the responses were studied by protection tests, no failures were observed over a period of several years. Such failures as do occur in field experience are probably due to vaccine of inadequate potency or to faulty technique in its administration.

---

[a] According to Dr F. L. Soper, production of 17D virus vaccine without a human serum component was begun and made standard practice for all yellow fever vaccine produced in Brazil after August 1940 by Dr J. A. Kerr in the laboratory of the Service for Study and Research on Yellow Fever maintained by the Ministry of Health of Brazil and the Rockefeller Foundation. This fact is not generally known.

French scientists, accepting the responsibility of protecting indigenous and other residents in vast overseas territories, much of them situated in the tropical zone of yellow fever endemicity, have worked for many years on the problem of yellow fever vaccines. Their vaccines, exemplified by the products proposed by Sellards & Laigret,[72], Laigret[42, 43] and by Peltier,[57, 58] have invariably involved the use of the French neurotropic strain of yellow fever virus in some form or other. The vaccines successively used by Laigret were preparations of this virus subjected to varying degrees of thermal exposure[43] (in the belief, probably unfounded,[21, 92] that this caused attenuation), or suspended in one or other different menstruum.[55, 56] More recently, the workers at the Institut Pasteur in Dakar have employed the neurotropic virus desiccated in the presence of gum acacia and applied topically together with smallpox vaccine.[58, 59] Many millions of persons have been inoculated in French territories with these vaccines and by this method.[58] The results, interpreted either in terms of antibody response[20] or in terms of the incidence of yellow fever in the territories where the vaccine was thus applied, have been good. Nevertheless, certain potential hazards exist with this method—namely, the possibility that the neurotropic form of virus might cause encephalitis in some of the recipients, or that certain of the viruses which are occasionally endemic in the Swiss mice used for preparation of the vaccine might gain access to the vaccine and cause infection among persons receiving it. No infections of the latter type due, say, to lymphocytic choriomeningitis virus have been reported. However, outside French territories there have been reports[19, 50] of encephalitis following the Dakar vaccine and some of these incidents have unfortunately assumed serious proportions.[50] In order to make use of the attenuated 17D strain, thus avoiding the potential hazard of the more potent neurotropic strain, and at the same time to make use of certain desirable features of the French vaccine, Hahn[30] has prepared 17D chick-embryo vaccine lyophilized with gum acacia and administered topically together with smallpox vaccine and has tested its effects. Results obtained in a field trial were highly satisfactory. It may be that this vaccine will prove to be the one of choice for mass application. These matters will be dealt with elsewhere in this monograph (see page 97).

Passive Immunity

Immunity inherited from a parent

Workers in Brazil and Nigeria observed that infants born of mothers immune to yellow fever had protective antibody against this virus in their blood at birth.[32, 81] That this immunity was not derived by nursing was shown by positive tests obtained with blood specimens collected from the

umbilical cord. Various workers have also found that the offspring of monkeys immune to yellow fever are likewise possessed of protective antibodies when born. It has been shown that this inherited immunity disappears within the first few months of life and that the newborn individual then becomes fully susceptible. This phenomenon is of interest from a number of different standpoints. It is of importance in medicine in that it gives rise to the question whether such individuals should be actively immunized against yellow fever while they are in possession of their inherited immunity. No published experiments along this line have come to our attention. Nevertheless, in other fields in which inherited immunity occurs, it is known that active immunity may be acquired during the period when the inherited immunity is still in existence. Until studies to determine this point in reference to yellow fever can be made, it would seem advisable not to withhold vaccination from infants having antibody derived from an immune mother.

Transfer of immunity by inoculation

During the time when rhesus monkeys were being employed as the animal hosts for yellow fever protection tests, it was found that the serum of an immune individual exerts its protective action in the inoculated animal even if it is given some hours after the introduction of the virus.[3, 15] The preventive action is likewise effective if the serum is given as much as a day or two in advance of the inoculation of virus. Thus the immune bodies taken from one host and transferred to a different host of the same or a different species confer a state of immunity upon the recipient. This type of immunity is quite transient and if the inoculation of immune serum be given too long before or after the introduction of the virulent virus no protection results.[3, 15] It has been also observed that the passive immunity so transferred is of shorter duration if the antibody is derived from a species heterologous to that to which it is introduced.[3]

Attempts have been made to employ such passive immunity in the treatment of yellow fever and other virus diseases by the use of immune or hyperimmune serum. These efforts have not met with notable success. Nevertheless, there is a possibility that practical use of this phenomenon could be made. In the days prior to the development of vaccine against yellow fever, experimental work with this disease was an extremely hazardous occupation. Passive transfer of immunity was in those times often resorted to when some individual had become accidentally infected or exposed to infection with yellow fever virus. Stocks of suitable immune serum were kept in the laboratories for this purpose, and, when occasion required, inoculations were made into individuals who had sustained some laboratory accident exposing them to infection.[12] It is not possible to

state with certainty that such efforts were successful in preventing infections, but there is reason to suspect that they may have been.

Demonstration of Immunity by Means of Protection Tests

The first method devised for the performance of yellow fever protection tests involved the use of rhesus monkeys.[84] In the early surveys in which these animals were used to study the distribution of yellow fever in the human population of West Africa,[5, 35] two rhesus monkeys were used for the testing of each individual serum. Thus the method was extremely costly and its application accordingly limited. However, with the discovery in 1930 by Theiler [86] that yellow fever virus is pathogenic for Swiss mice when inoculated into the brain of these animals the way was laid open to more practical methods for the performance of protection tests. Theiler showed that yellow fever immune serum mixed with the virus, and inoculated intracerebrally, had a neutralizing action and prevented infection in mice.[87, 88] The view was held at this time, however, that immunity could be more effectively demonstrated if relatively large quantities of serum were administered. Sawyer & Lloyd [68] developed the first extraneural protection test technique in order to effect the administration of as much serum as possible. According to their method, 1.5 ml quantitites of concentrated mouse-brain virus suspension were added to 3.0 ml quantities of undiluted serum, and the mixture was inoculated intraperitoneally in amounts of 0.6 ml per individual into mice previously prepared by the intracerebral injection of a small quantity of sterile soluble starch solution. The latter agent caused slight irritation in the brain and brought about the localization of the yellow fever virus which, however, had already been exposed to the in vitro and in vivo action of the serum with which it was mixed. If the serum contained the specific antibody, the mice were protected thereby ; if it did not, fatal encephalitis resulted. This method was used in the International Health Division laboratories of the Rockefeller Foundation in New York,[65, 71] in the laboratories of the Yellow Fever Service in South America,[79, 80] at the Yellow Fever Research Institutes at Lagos [6, 7] and Entebbe,[52, 77] and elsewhere [45, 46] in the intensive worldwide surveys of immunity to this disease. Later, when the protection test came to be used more extensively for other purposes than immunity surveys, more sensitive methods were required. In various studies of the immune response evoked by vaccines against yellow fever, it was found that the antibody response in man is less intense than that which follows natural infection, and in order to detect the response to the vaccine with certainty, more sensitive methods were required ; also, in epidemiological

studies of the role of wild animals in natural cycles of infection, it was found that some animal hosts respond to yellow fever virus less intensively than does man and likewise required the use of more sensitive methods. Consequently, various modifications of the yellow fever protection test were proposed, in particular by Whitman,[95] Bugher,[9, 10] and Smithburn,[74] in order to meet one or more special needs for the test. In principle, all these modifications have much in common, and they differ from the original methods proposed by Sawyer & Lloyd [68] principally in employing a more sensitive balance between the amount of serum and the dose of virus,[74] or better standardized virus preparations,[9] or more sensitive experimental hosts.[10, 74, 95] It was found that a method which was satisfactory in one laboratory [95] might not necessarily be equally satisfactory in another,[74] owing to differences in the susceptibility of the mice used in the different laboratories. Likewise, some of the methods, especially those involving the use of lyophilized virus preparations,[9] were not applicable everywhere owing to lack of facilities for the preparation of the standard virus. As a result of these facts, laboratories in various parts of the world came to use, not so much a standard technique for the yellow fever protection test, as a technique which was found to be suitable for a given laboratory and for the particular investigations in progress there.

There are a great many variables in yellow fever protection tests by whatever methods they are carried out. Mice of different breeds are extremely variable in their response to this virus, as was found years ago by Theiler.[88] Theiler likewise observed that infant mice are much more susceptible to yellow fever virus than adult mice ; [88] these variations in susceptibility were more critically explored in later years by Whitman [95] and Smithburn.[74] Another important variable in the technique of the test is the route by which the animals are inoculated. In adult mice the virus must be introduced intracerebrally ; or, if it is inoculated extraneurally, an intracerebral inoculation of a localizing agent must be given. Even with the use of intracerebral injections of starch solution, however, peripheral inoculation of virus is much less effective and multiple intracerebral infective doses are required to cause illness.[74]

The dose of virus used in the yellow fever protection test is of the utmost importance. By whatever route the virus is introduced, it is possible to overcome the effectiveness of antibody by excessive virus dosage.[74] The *effective* virus dosage is not invariably the same as the number of infective units administered ; multiple intracerebral infective doses are required to produce illness if the virus is introduced peripherally—even with simultaneous brain injections of starch solution. Also, by one route of inoculation the antibody may be more effective against the virus than by another route ; higher antibody titres are obtained with the same serum in intraperitoneal tests in immature mice than in intracerebral tests.[74]

When protection test are being done for the purpose of identifying newly isolated strains of (pantropic) yellow fever virus, the intracerebral route of inoculation is the one of choice, since freshly isolated virus may be of low virulence on peripheral inoculation—even with preparatory intracerebral injection of starch solution (Strode,[85] pp. 226-227).

The strain of virus being used in the tests may also determine certain technical features. For example, J. H. S. Gear, B. de Meillon, and D. H. S. Davis [b] used the 17D virus in protection test surveys in order to avoid importation of the more potent neurotropic virus into South Africa. The tests done with 17D virus yield results comparable to those done with standard neurotropic virus, but require that the mice be observed for a longer period owing to the slower progress on infection with this variant.

False positive results are occasionally obtained in yellow fever protection tests, especially with certain animal sera.[39, 49] Recognition of this fact and the adoption of more severe criteria for testing such sera [9] are essential if confusion is to be avoided in epidemiological studies.

The quality of the serum which is being tested also has an influence on the outcome of the protection tests. The writer has repeatedly titrated yellow fever virus simultaneously in the sera of different species and found the titre to be significantly higher in one than in another. Bugher [9] has also pointed to the necessity of obtaining sound results with non-immune individuals of any species of which the sera are being extensively studied by means of protection tests. He emphasizes that the sera of one species may require to be tested by a somewhat different method (virus dosage) from that used for routine purposes. Furthermore, the sera of certain animal species have been found to have toxic properties for Swiss mice and to require special methods. In our own experience, the serum of the ratel, or honey-badger (*Mellivora capensis* Schreber), behaved in this manner and could not be tested in the undiluted state. Dilution to a concentration of 1 : 10 eliminated the toxic action sufficiently to permit performance of tests with sera of this species.

The distribution of virus in the suspension used is very important and the results will be poor whatever method is used if uniform dispersion is not obtained. When fresh mouse-brain virus is being used, as in the case of intraperitoneal mouse-protection tests, considerable care and patience must be exercised in the preparation of the suspension. In instances in which preserved virus (frozen or lyophilized material) is used, the suspension prepared for stock must likewise be made carefully and certain precautions exercised when this material is re-constituted for the performance of the tests. For instance, lyophilized virus which is rehydrated and

---

b Paper presented at the African seminar on yellow fever at Kampala, Uganda, in September 1953.

titrated immediately is likely to give a lower titre than if the rehydrated material is allowed to stand for 15-30 minutes before the dilutions are prepared for titration. This is probably due to the fact that complete dispersion of virus particles in the liquid does not take place immediately.

It is essential to employ a protein-containing diluent for the virus owing to its sensitivity to electrolytes.[4] The materials most commonly used for this purpose are non-immune serum in 5 % or 10 % concentration,[4] or bovalbumin.[18] The concentration of bovine albumin recommended by Dick & Taylor [18] is, however, inadequate and should be increased to 0.75 %.

The technique of inoculation may likewise be a source of variability in the performance of protection tests, especially if these be done by the intracerebral route. Individuals who are not practised in the technique of inoculation may vary their procedure sufficiently to influence significantly the outcome of the tests. Furthermore, even if the technician is fully experienced and practised, the method which one uses may be sufficiently different from that employed by another to give significant differences in titrations (Strode,[85] p. 191). It is therefore essential that all inoculations in a given test be done by one person and that the method in successive tests be suited to the technique which the inoculator uses.

The ideal for the performance of yellow fever protection tests, or any other immunological tests, would be to adopt a standard technique of procedure which would be rigidly followed in all laboratories where such tests are done. In the case of the yellow fever protection test, however, in the opinion of the writer, this is neither possible nor desirable. Mice used in different laboratories vary sufficiently in their susceptibility, either innately or at different ages, so that a technique such as Whitman's [95] 21-day mouse intraperitoneal test might be quite suitable in one laboratory and impossible in another.[74] The purposes for which the tests are being done may also necessitate variations in technique. When the tests are for survey purposes and it is desired to learn whether yellow fever occurs in a given geographical area, it is important that the test be made sufficiently severe to eliminate any possibility of non-specific or false positive reactions; it is of considerably less importance in such instances to determine precisely the percentage of persons who are immune. In other instances, for example, in studying the results of vaccination to determine how many individuals of a group have exhibited an antibody response or in studies to determine the duration of immunity induced by vaccination, it does seem desirable to employ highly sensitive methods for the protection test, since the detection of minimal amounts of antibody is important. Therefore it may be less important to adopt a standard technique for the protection test than it is to be certain of the competence of the individuals performing the tests and then permit variations of method according to the objectives.

Other Immunological Reactions in Yellow Fever

The other immunological reactions which have been found to be useful in yellow fever investigations are the complement-fixation test and the haemagglutination-inhibition reaction. A number of workers have studied the complement-fixation reaction in yellow fever,[14, 26-28, 33, 44, 60, 61] and a variety of different antigens, some of them highly infectious, have been used in the complement-fixation tests. It is clearly established that an attack of yellow fever gives rise to the formation of complement-fixing antibody, and it is likewise clear that this antibody disappears from the serum of the infected individual, usually within about 6 months.[80] It has also been clearly established that inoculation with 17D vaccine does not evoke a response of complement-fixing antibody.[44] Therefore an individual whose serum contains complement-fixing antibody against yellow fever virus has not only experienced infection with that virus in its virulent form, but has been infected within very recent months. The failure of the serum to give a complement-fixing reaction may occur in an individual who is fully immune either as a result of having had the disease more than six months previously or by virtue of having been successfully vaccinated. Thus in medical or public health work the complement-fixation test could be employed for diagnostic purposes [44, 61] but would be of no value in survey work or in studying the effects of vaccination.

Recently Casals & Brown have employed complement-fixation (personal communication) and haemagglutination-inhibition techniques [13] in studying the relationships of viruses which are known or presumed to be transmitted by insects. Yellow fever virus was included among these agents. The complement-fixation reactions obtained by them with antigens derived from yellow fever virus showed relatively little cross-reaction. Good haemagglutination was obtained at pH 7.0 after incubation at 4°C or 22°C, employing antigens derived from both pantropic and neurotropic yellow fever virus. In the haemagglutination-inhibition tests, yellow fever virus fell into the group B in that its antigen cross-reacted with New Guinea dengue, Ilheus, Japanese B, Ntaya, Russian Far Eastern, St. Louis, and West Nile antisera.[13] In these tests homologous reaction was stronger than any of the heterologous reactions, and the yellow fever immune serum did not react to the same extent as other antisera. It is not yet clear, however, whether the haemagglutination-inhibition reaction would be useful in field epidemiological studies.

Hughes [37] conducted a series of experiments with a view to perfecting a precipitin test for yellow fever investigations. He demonstrated the occurrence of a precipitinogen and the formation of a precipitating anti-

body, but found that both these reagents were of evanescent character, and the test for their presence was therefore of limited usefulness.

Application of Immunological Methods

The most extensive use of immunological methods in yellow fever has been in surveys to define the distribution and the prevalence of this disease in various countries in the world. These surveys covered not only the known zones of endemicity in Africa [6, 7, 52, 71, 77] and South America,[79, 80] but also the remainder of both those continents, as well as North America, Central America, Europe, and parts of Asia.[65] The extent to which the yellow fever protection test was used for survey purposes outstrips that of immunological tests for most other diseases. Extensive use has been made of protection tests in studying the responses to yellow fever vaccine, in particular to determining the duration of that response.[1, 11, 16, 17, 25, c] As a result of these applications of immunological methods, the distribution of the disease throughout the world is quite well defined, as are areas in which the disease has not occurred in the past. And in regions in which the susceptible human and animal hosts exist and in which vector species of mosquitos also occur, but in which the disease has not occurred, epidemiologists of the present day are keeping an ever-watchful eye for possible introduction of this disease.

The studies on the reaction of human beings to yellow fever vaccine have shown that the response is, as a rule, quite effective within one week of the time of inoculation of the vaccine,[11, 82] and that the immunity induced by vaccination is well sustained.[1] Studies made six,[17] nine,[16] and twelve [c] years after inoculation of 17D vaccine have shown a high percentage of the recipients to have demonstrable immunity. Since the 17D vaccine is a living, although attenuated, virus it is possible that the immunity which it induces will be found to be lifelong, as is that induced by an attack of the disease.

Immunological methods, neutralization and complement-fixation tests in particular, have further important use in the identification of viruses,[51, 53] and in studies of their interrelationships.[38, 63, 75, 76] When new strains of virus are isolated, it is necessary to employ a variety of methods in order to determine their identity. Those which are most widely accepted for the identification of newly isolated viruses are the immunological methods. Notable examples of this are the studies of Smithburn [75, 76] and Kerr [38] on the relationships of viruses isolated in recent years in Africa and South America, and the work of Macnamara [50] in identifying as neurotropic

---

[c] See also the contribution by Dr G. Courtois, page 105.

yellow fever virus the agents recovered from the nervous systems of persons exhibiting acute encephalitis following vaccination with the Dakar yellow fever vaccine.

Immunological methods are also important in the diagnosis of yellow fever. When a virus is isolated from blood or, in fatal cases, from the tissues of the deceased person, the method of choice for identification of the agent is the protection test, employing the newly isolated agent against known immune and non-immune sera.

Immunological tests may also be of diagnostic value when the virus has not been isolated. The testing of paired serum samples, one taken early in the illness and the second drawn when convalescence is well under way, may be done either by neutralization or by complement-fixation tests.[44] If the test shows that the illness has evoked the formation of neutralizing or complement-fixing antibodies against yellow fever virus or its specific antigen, the diagnosis is firmly established.

REFERENCES

1. Anderson, C. R. & Gast-Galvis, A. (1947) *Amer. J. Hyg.* **45**, 302
2. Bauer, J. H. (1931) *Amer. J. trop. Med.* **11**, 337
3. Bauer, J. H. (1931) *Amer. J. trop. Med.* **11**, 451
4. Bauer, J. H. & Mahaffy, A. F. (1930) *Amer. J. Hyg.* **12**, 175
5. Beeuwkes, H., Bauer, J. H. & Mahaffy, A. F. (1930) *Amer. J. trop. Med.* **10**, 305
6. Beeuwkes, H. & Mahaffy, A. F. (1934) *Trans. roy. Soc. trop. Med. Hyg.* **28**, 39
7. Beeuwkes, H., Mahaffy, A. F., Burke, A. W. & Paul, J. H. (1934) *Trans. roy. Soc. trop. Med. Hyg.* **28**, 233
8. Berry, G. P. & Kitchen, S. F. (1931) *Amer. J. trop. Med.* **11**, 365
9. Bugher, J. C. (1940) *Amer. J. trop. Med.* **20**, 809
10. Bugher, J. C. (1941) *Amer. J. trop. Med.* **21**, 299
11. Bugher, J. C. & Gast-Galvis, A. (1944) *Amer. J. Hyg.* **39**, 58
12. Burke, A. W. & Davis, N. C. (1930) *Amer. J. trop. Med.* **10**, 419
13. Casals, J. & Brown, L. V. (1954) *J. exp. Med.* **99**, 429
14. Davis, G. E. (1931) *Amer. J. Hyg.* **13**, 79
15. Davis, N. C. (1934) *J. Immunol.* **26**, 361
16. Dick, G. W. A. & Gee, F. L. (1952) *Trans. roy. Soc. trop. Med. Hyg.* **46**, 449
17. Dick, G. W. A. & Smithburn, K. C. (1949) *Amer. J. trop. Med.* **29**, 57
18. Dick, G. W. A. & Taylor, R. M. (1949) *J. Immunol.* **62**, 311
19. Eklund, C. M. (1953) *Bol. Ofic. sanit. panamer.* **35**, 505
20. *Epidem. Inform. Bull.*, 1946, **2**, 618

21. Findlay, G. M. (1934) *Lancet*, **2**, 983

22. Findlay, G. M. & MacCallum, F. O. (1937) *Trans. roy. Soc. trop. Med. Hyg.* **31**, 103

23. Findlay, G. M. & MacCallum, F. O. (1937) *Trans. roy. Soc. trop. Med. Hyg.* **31**, 297

24. Findlay, G. M., Stefanopoulo, G. J., Davey, T. H. & Mahaffy, A. F. (1936) *Trans. roy. Soc. trop. Med. Hyg.* **29**, 419

25. Fox, J. P. & Cabral, A. S. (1943) *Amer. J. Hyg.* **37**, 93

26. Frobisher, M., jr. (1929) *Proc. Soc. exp. Biol. (N.Y.)*, **26**, 846

27. Frobisher, M., jr. (1931) *Amer. J. Hyg.* **14**, 147

28. Frobisher, M., jr. (1931) *J. prev. Med. (Baltimore)*, **5**, 65

29. Haddow, A. J., Smithburn, K. C., Mahaffy, A. F. & Bugher, J. C. (1947) *Trans. roy. Soc. trop. Med. Hyg.* **40**, 677

30. Hahn, R. G. (1951) *Amer. J. Hyg.* **54**, 50

31. Hargett, M. V., Burruss, H. W. & Donovan, A. (1943) *Publ. Hlth Rep. (Wash.)*, **58**, 505

32. Hoskins, M. (1934) *J. Immunol.* **26**, 391

33. Hudson, N. P. (1932) *Amer. J. Hyg.* **15**, 557

34. Hudson, N. P., Bauer, J. H. & Philip, C. B. (1929) *Amer. J. trop. Med.* **9**, 1

35. Hudson, N. P. & Kitchen, S. F. (1930) *J. prev. Med. (Baltimore)*, **4**, 459

36. Hudson, N. P., Philip, C. B. & Davis, G. E. (1929) *Amer. J. trop. Med.* **9**, 223

37. Hughes, T. P. (1933) *J. Immunol.* **25**, 275

38. Kerr, J. A. (1952) *J. Immunol.* **68**, 461

39. Koprowski, H. (1946) *J. Immunol.* **54**, 387

40. Kumm, H. W. & Laemmert, H. W., jr. (1950) *Amer. J. trop. Med.* **30**, 733

41. Laemmert, H. W., jr., Hughes, T. P. & Causey, O. R. (1949) *Amer. J. trop. Med.* **29**, 555

42. Laigret, J. (1934) *Bull. Off. int. Hyg. publ.* **26**, 1078

43. Laigret, J. (1937) *Ann. Méd.* **42**, 463

44. Lennette, E. H. & Perlowagora, A. (1943) *Amer. J. trop. Med.* **23**, 481

45. Liégeois, P. (1944) *Rec. Sci. méd. Congo belge*, **3**, 97

46. Liégeois, P., Rousseau, E. & Courtois, G. (1948) *Ann. Soc. belge Méd. trop.* **28**, 247

47. Lloyd, W. (1935) *Bull. Off. int. Hyg. publ.* **27**, 2365

48. Lloyd, W., Theiler, M. & Ricci, N. I. (1936) *Trans. roy. Soc. trop. Med. Hyg.* **29**, 481

49. MacCallum, F. O. & Findlay, G. M. (1937) *Trans. roy. Soc. trop. Med. Hyg.* **31**, 199

50. Macnamara, F. N. (1953) *Trans. roy. Soc. trop. Med. Hyg.* **47**, 199

51. Mahaffy, A. F., Hughes, T. P., Smithburn, K. C. & Kirk, R. (1941) *Ann. trop. Med. Parasit.* **35**, 141

52. Mahaffy, A. F., Smithburn, K. C. & Hughes, T. P. (1946) *Trans. roy. Soc. trop. Med. Hyg.* **40**, 57

53. Mahaffy, A. F., Smithburn, K. C., Jacobs, H. R. & Gillett, J. D. (1942) *Trans. roy. Soc. trop. Med. Hyg.* **36**, 9

54. Marchoux, E., Salimbeni, A. & Simond, P.-L. (1903) *Ann. Inst. Pasteur*, **17**, 665

55. Mathis, C., Durieux, C. & Mathis, M. (1936) *Bull. Acad. Méd. (Paris)*, **116**, 226

56. Nicolle, C. & Laigret, J. (1935) *C. R. Acad. Sci. (Paris)*, **201**, 312

57. Peltier, M. (1947) *Amer. J. publ. Hlth*, **37**, 1026

58. Peltier, M. (1948) *Vaccin antiamaril et vaccinations antiamariles et antivariolo-amariles par la méthode dakaroise en Afrique-Occidentale Française.* In: *Proceedings of the Fourth International Congresses on Tropical Medicine and Malaria, Washington, D.C., 1948*, **1**, 489

59. Peltier, M., Durieux, C., Jonchère, H. & Arquié, E. (1940) *Bull. Acad. nat. Méd. (Paris)*, **123**, 137

60. Perlowagora, A. & Hughes, T. P. (1947) *J. Immunol.* **55**, 103

61. Perlowagora, A. & Lennette, E. (1944) *Amer. J. trop. Med.* **24**, 235

62. Reed, W., Carroll, J., Agramonte, A. & Lazear, J. W. (1900) *Philad. med. J.* **6**, 790

63. Sabin, A. B. (1949) *Fed. Proc.* **8**, 410

64. Sawyer, W. A. (1931) *J. prev. Med. (Baltimore)*, **5**, 413

65. Sawyer, W. A., Bauer, J. H. & Whitman, L. (1937) *Amer. J. trop. Med.* **17**, 137

66. Sawyer, W. A., Kitchen, S. F. & Lloyd, W. (1931) *Proc. Soc. exp. Biol. (N.Y.)*, **29**, 62

67. Sawyer, W. A., Kitchen, S. F. & Lloyd, W. (1932) *J. exp. Med.* **55**, 945

68. Sawyer, W. A. & Lloyd, W. (1931) *J. exp. Med.* **54**, 533

69. Sawyer, W. A., Meyer, K. F., Eaton, M. D., Bauer, J. H., Putnam, P. & Schwentker, F. F. (1944) *Amer. J. Hyg.* **39**, 337

70. Sawyer, W. A., Meyer, K. F., Eaton, M. D., Bauer, J. H., Putnam, P. & Schwentker, F. F. (1944) *Amer. J. Hyg.* **40**, 35

71. Sawyer, W. A. & Whitman, L. (1936) *Trans. roy. Soc. trop. Med. Hyg.* **29**, 397

72. Sellards, A. W. & Laigret, J. (1932) *C. R. Acad. Sci. (Paris)*, **194**, 1609

73. Smith, H. H., Penna, H. A. & Paoliello, A. (1938) *Amer. J. trop. Med.* **18**, 437

74. Smithburn, K. C. (1945) *J. Immunol.* **51**, 173

75. Smithburn, K. C. (1952) *J. Immunol.* **68**, 441

76. Smithburn, K. C. (1954) *J. Immunol.* **72**, 376

77. Smithburn, K. C., Goodner, K., Dick, G. W. A., Kitchen, S. F. & Ross, R. W. (1949) *Ann. trop. Med. Parasit.* **43**, 182

78. Smithburn, K. C. & Mahaffy, A. F. (1945) *Amer. J. trop. Med.* **25**, 217

79. Soper, F. L. (1937) *Amer. J. trop. Med.* **17**, 457

80. Soper, F. L. & Andrade, A. de (1933) *Amer. J. Hyg.* **18**, 588

81. Soper, F. L., Beeuwkes, H., Davis, N. C. & Kerr, J. A. (1938) *Amer. J. Hyg.* **27**, 351

82. Soper, F. L. & Smith, H. H. (1938) *Vaccination with virus 17D in control of jungle yellow fever in Brazil.* In: *Acta Conventus Tertii de Tropicis atque Malariae Morbis, Amsterdam*, **1**, 295

83. Stefanopoulo, G. J. & Duvolon, S. (1947) *Bull. Soc. méd. Hôp. Paris,* **63**, 990

84. Stokes, A., Bauer, J. H. & Hudson, N. P. (1928) *Amer. J. trop. Med.* **8**, 103

85. Strode, G. K. (ed.) (1951) *Yellow fever,* New York

86. Theiler, M. (1930) *Science,* **71**, 367

87. Theiler, M. (1931) *Ann. trop. Med. Parasit.* **25**, 69

88. Theiler, M. (1933) *Ann. trop. Med. Parasit.* **27**, 57

89. Theiler, M. & Smith, H. H. (1936) *Bull. Off. int. Hyg. publ.* **28**, 2354

90. Theiler, M. & Smith, H. H. (1937) *J. exp. Med.* **65**, 767

91. Theiler, M. & Smith, H. H. (1937) *J. Exp. Med.* **65**, 787

92. Theiler, M. & Whitman, L. (1935) *Bull. Off. int. Hyg. publ.* **27**, 1342

93. Turner, R. H., Snavely, J. R., Grossman, E. B., Buchanan, R. N. & Foster, S. O. (1944) *Ann. intern. Med.* **20**, 193

94. United States of America. 61st Congress: Third Session (1911) *Yellow fever,* Washington, D.C., p. 56 (Senate document 822)

95. Whitman, L. (1943) *Amer. J. trop. Med.* **23**, 17