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THE PROBLEM OF MASS VACCINATION AGAINST YELLOW FEVER

by

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I. Development of the Vaccines in Current Use

For the active immunization of man against yellow fever, methods devised by SAWYER, KITCHEN and LLOYD^{18, 19} (1931, 1932) and by LLOYD¹¹ (1935) consisted in the inoculation of a strain of virus, the pantropic properties of which had been modified by laboratory techniques, together with sufficient immune or hyper-immune serum (as determined by preliminary titration in rhesus) to prevent the virus from entering the circulating blood of the inoculated subject. In these serum-virus methods, the antigenic element in the vaccine used by Sawyer and his collaborators was developed from the French strain of virus modified by mouse brain passage and known as the French neurotropic yellow-fever virus; that used by Lloyd was developed from the Asibi strain grown in tissue culture containing minced mouse embryonic tissue plus 10 per cent normal monkey serum in Tyrode's solution and known as 17E; the immune serum was obtained from persons who had recovered from yellow fever or had developed protective antibody against the virus following vaccination; the hyperimmune serum was produced in horses, rabbits, monkeys or goats.

Other considerations aside, these serum-virus methods, although efficient, were not adapted to mass vaccination, because of the large quantities and the prohibitive cost of the immune or hyperimmune serum which would be required for the completion of such immunization programmes.

Attention was, therefore, directed to discovering methods whereby large-scale immunization could be effected by the administration of active modified virus, without the simultaneous employment of immune serum. Appropriate investigations led to the development of the two different virus vaccines which are in current use: the French neurotropic and the 17D.

1. The French neurotropic virus vaccine

The neurotropic strain used in this vaccine is a derivative of the pan-tropic French strain of yellow-fever virus which, during prolonged serial brain-to-brain passage in mice, had been shown by THEILER²³ (1930) to have lost its ability to produce visceral yellow fever in rhesus monkeys - animals which, when inoculated extraneurally with the modified virus, ordinarily developed only a mild non-fatal infection and consequent solid active immunity. At the same time, however, the modified strain had acquired an enhanced neurotropic virulence for both mice and monkeys, producing in these animals, when injected intracerebrally, a rapidly developing fatal encephalitis. The fact, however, that the prolonged passage of the original virus in mouse brain had effected a marked reduction of its viscerotropic affinity was held to make the use of the strain, thus modified, feasible for human vaccination, with the result that since 1934 immunization by means of this strain, without the simultaneous injection of immune serum, has been extensively practised, particularly by French workers in tropical Africa.

At first the virus vaccine was administered by subcutaneous inoculation. Thus LAIGRET^{9,10} (1934) prepared a vaccine consisting of the mouse brain virus attenuated by exposure, in glycerine, to a temperature of 20°C and dried in the presence of sodium phosphate; three injections of virus exposed to this temperature for four days, two days and one day respectively were given at twenty-day intervals. Later, in order to reduce the number of injections and thereby to make immunization more widely applicable, NICOLLE and LAIGRET¹³ (1935) introduced a single-dose method of vaccination, employing mouse-brain virus which, after one day's exposure in glycerine to 20°C and subsequent desiccation, was, with the object of retarding the diffusion of the virus from the site of inoculation, coated with a layer of egg yolk. The Laigret mouse-adapted virus, prepared from the French strain at its 130th to 185th passage in mice, was the first to be used on a relatively wide scale. Thus in French West Africa between June 1934 and December 1935 there were 9,592 persons immunized by the three-injection method, while by 1937 there had been over

24,000 persons immunized in that territory by one or other of the Laigret methods referred to.

Subcutaneous inoculation with the mouse-adapted virus did not, however, for various reasons, adequately meet the requirements of the French Authorities who, confronted with the problem of yellow-fever control in their vast colonial territories in tropical Africa, recognized as of paramount importance the need for mass immunization of the indigenous populations there.

Search for a vaccine which would be at once safe, effective, easily administered and inexpensive, was, therefore, pursued, particularly at the Pasteur Institute, Dakar, and resulted in the development by PELTIER, DURIEUX, JONCHERE and ARQUIE^{16, 17} (1939, 1940) of the neurotropic yellow-fever virus at its 238th passage through mouse brains, which could be applied to the skin by mild scarification, thereby replacing subcutaneous inoculation and overcoming such major difficulties inherent in mass immunization programmes as the provision, in adequate quantities, of syringes and needles thoroughly sterilized.

Since 1940 this "scratch" method of immunization has been adopted for use, mainly by the French Authorities and particularly in French tropical Africa, wherein to date over 36 million vaccinations have been so performed.

The technique of preparing and administering the vaccine in question was described by PELTIER¹⁴ in 1946, when it was stated that the vaccine was made from the brains of mice inoculated with the French strain of virus at its 256th to its 258th passage in mice. The vaccine is commonly called the Dakar vaccine.

There can be no doubt about the high immunizing property possessed by this mouse-brain virus applied by scarification either alone or, as is commonly practised in French tropical Africa, in combination with smallpox vaccine.

2. 17D virus vaccine

Because of the view held by such workers as FINDLAY⁴ (1934) and THEILER and WHITMAN²⁷ (1935) that the increased neurotropism, for mice and monkeys, of the mouse-adapted French strain rendered that strain, if used alone, potentially dangerous for human vaccination, search was made to discover a method for modifying yellow-fever virus in such a way as to reduce not only its viscerotropism but also its neurotropism. The desired modification was finally achieved by the prolonged cultivation in tissue in vitro, by LLOYD, THEILER and RICCI¹² (1936) and by THEILER and SMITH^{25, 26} (1937 a and b), of the highly virulent pantropic Asibi strain. These workers, using successively minced mouse embryo-Tyrode's solution (18 passages), whole chick embryo-Tyrode's solution (58 generations), and thereafter a medium in which the tissue component was minced chick embryo, from which the brain and spinal cord had been removed before mincing, initiated the branch known as 17D - a variant which showed, not only a loss of neurotropism for mice and monkeys alike, but also a markedly diminished viscerotropism for the latter animals. Thus in mice, although the strain could still produce encephalitis, it could do so only after a somewhat increased incubation period; in monkeys, it had altogether lost its ability, when inoculated intracerebrally, to produce fatal encephalitis. Monkeys inoculated extraneurally with this 17D virus had no fever or other signs of illness; their blood contained but minimal amounts of virus; they were shown to have developed specific antibodies and they were solidly immune to highly virulent pantropic strains.

The loss of both viscerotropic and neurotropic affinities, as demonstrated in monkeys, made this variant, in the opinion of American and English workers, the virus of choice for human vaccination.

Vaccine is prepared from developing chick embryos - fresh, fertile, hen eggs after seven to nine days' incubation being inoculated with 0.05 cc of the 200th to 300th subpassage material. The vaccine is, and since 1942 has been, of the aqueous-base (serum-free) type; the technique of its production is described by HARGETT, BURRUSS and DONOVAN⁷ (1943).

For mass immunization 17D vaccine has since 1937 been administered by subcutaneous inoculation and to this end over 40 million doses have been distributed. In South America alone, between 1937 and 1950, there were eight million persons protected by this vaccine, while in certain British and other territories in Africa it has also been extensively employed.

II. The Dakar and the 17D vaccines compared

In the following paragraphs the two vaccines currently employed in mass vaccination campaigns will be compared from the points of view of their relative efficiency, safety, ease of administration, and cost.

(a) Efficiency

In 1945 a comparison was made between the scarification method using the Dakar vaccine and the subcutaneous inoculation of 17D vaccine; the findings published by UNRRA²⁸ (1946) - the body which had initiated the necessary investigations - showed that 98.94 per cent of positive results were obtained in the sera of a group of 210 French soldiers scarified with the Dakar vaccine, as compared with 64.29 per cent in the sera of a comparable group inoculated subcutaneously with 17D vaccine. The findings represented a combination of the results of neutralization tests made on the sera at three different laboratories, located respectively at Dakar, Montana and Rio de Janeiro. At Dakar and Rio de Janeiro an intracerebral test was employed, while at Montana the tests were made by the intraperitoneal technique. Of the sera tested by the intraperitoneal technique, 100 per cent of those vaccinated with Dakar vaccine and 97.96 per cent of those vaccinated with 17D were positive. The apparent discrepancies in results with sera of those receiving 17D vaccine, when the sera were tested in the three different laboratories, may be explained by the fact that at Montana use was made of a more sensitive mouse-protection test than that employed at the two other laboratories, for it is well recognized that the more delicate intraperitoneal test may indicate the presence of neutralizing antibodies which are not demonstrable by an intracerebral test. Although, then, the results of the above investigation showed that the response induced

by the Dakar vaccine led to the formation of more antibody per person than that evoked by the 17D vaccine, nevertheless experience has abundantly proved that the immunity induced by 17D vaccine is adequate for protection.

(b) Safety

In this respect SMITHBURN²¹ (1951) observes: "The use of the neurotropic virus, which is known to be more pathogenic for man, rhesus monkey, and mouse than is the 17D virus, may be a potential hazard. Even though encephalitis has not been prevalent among persons vaccinated by this (the Dakar) method, the possibility cannot be ignored that it may on occasion occur". [That it can occur has been recently exemplified by two serious outbreaks of encephalitis: one in Costa Rica during 1951, the other in Nigeria during 1952, where respectively 12 cases, with 3 deaths, and 83 cases, with 32 deaths, were reported]. "The use of mouse brain virus seems", according to Smithburn, "to be a more objectionable feature. There is always the possibility that the yellow-fever virus may become contaminated with another virus that the mouse may be harbouring - lymphocytic choriomeningitis, for example - with resultant accidental infection in recipients of the vaccine. Lastly, there is also the potential hazard, whenever mammalian tissue is employed in a vaccine, of allergic demyelinating encephalomyelitis."

As regards 17D: theoretically the use of a strain, which has been rendered essentially avirulent neurotropically as well as viscerotropically, while still retaining in large measure its antigenic potency, should preclude the occurrence of cases of severe reactions involving the central nervous system. To judge from a perusal of the relevant literature, this assumption has in the main been confirmed. Exceptions, however, have been: (i) a case recorded by SOPER and SMITH²² (1938) as having occurred one month after vaccination, with definite meningeal signs, the relation of which to vaccination was considered very doubtful; and (ii) a serious outbreak of encephalitis during 1941 in Brazil, where, as reported by FOX, LENNETTE, MANSON and SOUZA AGUIAR⁵ (1942), 254 cases, with one fatality, occurred among 69,843 persons vaccinated with certain lots of vaccine prepared from several substrains

of the original 17D virus. Investigations carried out by these authors proved that a sudden alteration in character of the 17D virus had taken place during a very small number of subcultures away from the parent stem. The demonstrated variation in pathogenicity of different substrains of the 17D virus and the consequent variation of the response in man inoculated with these substrains led to the standardization of the manufacture of 17D vaccine - a procedure which ensured that all the vaccines used were initiated from primary and secondary seed batches of known character only. Since that time no case of encephalitis has been recorded in literature as following the use of 17D virus vaccine; indeed, according to THEILER²⁴ (1948), any reactions which have occurred have been, as a rule, extremely mild.

In regard to (a) and (b) SMITH²⁰ (1951) states: "It appears ... that the Dakar vaccine produces a greater degree of immunity as measured by serum antibody response. This is accompanied by a greater danger of serious neurologic reactions from the neurotropic vaccine, as well as the risk of extraneous infections from latent viruses of mice that may be pathogenic for man. It appears reasonable, therefore, in view of the satisfactory experience with 17D vaccine in large-scale immunization campaigns over a period of 13 years, that its greater safety would recommend it above the Dakar vaccine for general use". That the number of reactions after vaccination with Dakar vaccine is greater than that of those which occur after the use of 17D vaccine is stated by THEILER²⁴ (1948) in a recent article.

(c) Ease of administration

One point greatly in favour of Dakar vaccine for mass vaccination is that immunization is effected by simple application of virus to cutaneous scarifications. This obviously is to be preferred to any method involving the administration of vaccine by means of syringes and needles which require rigorous sterilization prior to use.

(d) Cost

Dakar vaccine is also less expensive to produce than 17D. In the Dakar vaccine, which incidentally has the advantage of a greater simplicity of preparation than 17D, the whole virus-infected mouse brain is used, and, according to PELTIER¹⁴ (1946), one-tenth of a brain yields 100 doses of vaccine. In the manufacture of 17D vaccine only the supernatant fluids of the embryo suspensions are employed and the virus-rich sediments, which are about one-third by volume, are discarded, with a considerable loss of potential vaccine virus (DICK¹ 1953). It is true that in the Americas the cost of producing 17D vaccine has been estimated at about 0.025 US dollar per dose in New York and about 0.025 US dollar per dose in Rio de Janeiro (SOPER and SMITH²² 1938); this figure is low, but when the cost of application is added, mass immunization by means of 17D becomes, according to SMITH²⁰ (1951), "a burdensome expense".

From the foregoing it emerges that for mass vaccination:

(a) Dakar vaccine has much to recommend it, not only by virtue of its demonstrated effectiveness as a method of immunization, but also because of its cheapness and simplicity of preparation, as well as its ease of administration. On the other hand, two main objections to this vaccine have been voiced, because of the possibility that: (i) the mouse brains employed in its preparation may be contaminated with a virus pathogenic for man although latent in mice (e.g. lymphocytic choriomeningitis), or may be the cause of demyelinating encephalomyelitis; (ii) the use, as antigen, of a virus with enhanced neurotropic properties may be followed by serious reactions involving the central nervous system;

(b) 17D vaccine, although eliciting an antibody response quantitatively less than that evoked by Dakar vaccine, nevertheless confers an immunity which is adequate for protection. Moreover, since the standardization, in 1942, of the seed virus used in its preparation, any reactions following its administration have been, as a rule, extremely mild. On the other hand, 17D vaccine has, particularly for mass vaccinations, certain disadvantages, of which the chief

are: (i) its cost of preparation; and (ii) the necessity of using, for its administration, large numbers of sterilized syringes and needles.

III. Suggestions for improving the vaccines in current use

From a consideration of the relative merits of the Dakar and 17D vaccines, the ideal for mass immunization would appear to be a method combining the advantages, and eliminating the disadvantages, of both. To this end - the development of a vaccine, which would be not only safe and efficient but also comparable with the Dakar vaccine in its suitability for mass vaccination in the field and in its low cost of production and application - investigations into the possibility of employing 17D virus vaccine by scratch were begun in 1947 at the Yellow Fever Research Institute in Lagos, Nigeria. HAHN⁶ (1951) describes the mode of preparation of the vaccine and the results obtained from its use. The vaccine, produced by grinding the whole virus-infected chick embryos with gum arabic solution and desiccating the homogenized mixture to powder form, proved, when reconstituted in sterile distilled water at the time of use, easy of administration by the scratch technique.

From results obtained both in the laboratory and in a field trial at Kumba Fiango, British Cameroons, where 3,808 of the inhabitants were immunized by this method, Hahn concluded that the 17D strain of yellow-fever virus could be applied by scratch "with the production of a level of immunity of the same order as that resulting from subcutaneous inoculation of the virus". Further evidence in this sense is adduced by HORGAN²⁹ (1950, 1951), by DICK¹ (1952), and by DICK and HORGAN² (1952) in respect of 153 African volunteers immunized by this method in Uganda. No untoward reactions were reported either by HAHN⁶ (1951) or by DICK and HORGAN² (1952) respectively.

Alternative to the use of a 17D vaccine produced as described above by Hahn, it has been suggested by DICK¹ (1952) that "studies should be made on the antigenicity of 17D mouse-brain virus as a scarification vaccine". [The objection to the use of a mouse-brain preparation, because of the possibility that the mouse brains employed in making the vaccine might become contaminated with a virus pathogenic for man although latent in mice, is considered by Dick to be perhaps somewhat academic,

since PELTIER¹⁵ (1948) does not record the occurrence of any such accident in a total of 20,053,338 vaccinations for which Dakar mouse-brain vaccine was used.⁷

In his summary of yellow-fever vaccination by scarification, DICK¹ (1952) suggests that by using either (a) a crude (see above) extract of chick embryos infected with 17D virus or (b) 17D mouse-brain virus, "a preparation of 17D vaccine could be administered by scarification which might prove to be a satisfactory method of mass vaccination of persons living in endemic yellow fever areas. The use of such a preparation would reduce the cost of the vaccine, and the dispensing with syringes would facilitate mass vaccinations and reduce the chance of the syringe transmission of disease." [The danger of transmission of disease by the syringe is dealt with by HUGHES⁸ (1946) and by EVANS and SPOONER³ (1950)]⁷.

IV. Summary

1. The development of the two vaccines in current use for mass immunization against yellow fever has been described.
2. The relative merits of the Dakar and the 17D vaccines have been discussed.
3. Recent work on, and suggestions for, the development of a vaccine to fulfil the prerequisites for mass vaccination - safety, efficiency, ease of administration and lowness of cost of production and application - have been indicated.

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