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THE COMBINED USE OF THE VIRUSES OF YELLOW FEVER AND VACCINIA BY THE SCRATCH METHOD FOR IMMUNIZATION AGAINST YELLOW FEVER AND SMALLPOX

by

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The combined use of these two viruses by the Scratch method for mass immunization was first proposed by PELTIER, DURIEUX, JONCHERE and ARQUIE [7,8] (1939, 1940), after they had found that the application of the French neurotropic strain of yellow-fever virus to cutaneous scarifications resulted in the development of immunity to yellow fever, and after they had established proof that by this method vaccination against both yellow fever and smallpox could be performed simultaneously without accident. Acceptance of the proposal by the French Authorities led successively to the administration, by cutaneous application, of this combined yellow-fever-smallpox vaccine to 100,000 inhabitants of Senegal in 1939, to certain of the military and civilian inhabitants of the territory of French West Africa in 1941, to 14,330,735 of the total population in that territory (approximately 16 million) between 1942 and 1946, and to 17,000,000 inhabitants of that territory by 1948 (PELTIER, [5,6] 1947, 1948).

The yellow-fever virus component of the combined vaccine is made from the brains of mice infected with neurotropic yellow-fever virus at its 256th - 258th passage through mice (PELTIER, [4] 1946). After desiccation, the mouse brains are ground up and mixed with sterile inert powder (brain powder - 1 volume, inert powder - 2 volumes). After dehydration, the powder is distributed in ampoules, each containing 1/10 of a brain = 100 doses of vaccine. The antivariolous component is dried smallpox vaccine. For use, the two components are placed in a mortar and mixed together before adding 2 cc of neutral sterile gum arabic solution for further mixing. Of the resultant suspension 2 drops are placed on the skin in the deltoid region and through each drop two parallel scarifications are made, each 0.5 cm in length. The gum dries and provides a protective covering.

This combined vaccine, because of its demonstrated effectiveness, its ease of administration, and its low cost of production and application, has proved highly successful in meeting the requirements of such a campaign as that waged in French West Africa, which has entailed vaccination of the entire population of that territory with the two viruses administered simultaneously, once every four years, "in order to insure immunity in all the newborn and to re-immunize any persons who may have lost their immunity to either virus". (SMITHBURN,[10] 1951). This campaign has been described by SMITH[9] (1951) as being "highly successful" and "one of the most ambitious public health measures ever put into effect".

Because of the fact, however, that the yellow-fever virus component of this combined vaccine contains, as antigen, a strain of yellow-fever virus with highly developed neurotropism, certain workers have considered its use to constitute the potential hazards for human immunization, particularly for children (e.g. serious post-vaccination reactions involving the central nervous system). In consequence, with a view to replacing, if possible, this neurotropic virus by one which had been rendered essentially avirulent, neurotropically as well as viscerotropically, while still retaining in large measure its antigenic potency, investigations into the possibility of employing 17D virus by scratch were commenced in 1947 at the Yellow Fever Research Institute in Lagos, Nigeria. Once the immunizing power of 17D vaccine administered by scarification had been established by several experiments (HAHN,[3] 1951; DICK,[1] 1952), HAHN[3] (1951) developed a combined yellow-fever - smallpox vaccine for cutaneous application. The 17D component of the vaccine is prepared in the usual way to the stage of homogenizing the infected chick embryos; a 50 per cent solution of gum arabic is then added to give a final concentration of gum of 15 per cent, followed by 1/5th volume of phenolized vaccine lymph; the combined product is now mixed and dispensed into ampoules in amounts of 0.5 or 1 cc. The ampoules are then rapidly shell frozen and attached to a desiccator; after 24 hours' desiccation, they are filled with dry nitrogen, sealed off and stored at 0°C. After reconstitution in 0.5 or 1.0 cc of distilled water, as the case may be, the reconstituted vaccine is mixed thoroughly and allowed to stand for 15-30 minutes. Pasteur pipettes are used for mixing and placing a drop on the arm skin; 2 scratches 6-8 mm long are then made through the drop.

The protection afforded by scarification with 17D virus is evidenced by the results and reported by HAHN[3] (1951). During a field trial at Kumbo-Fiango, British Cameroons,

4,431 of a total population of 5,368 were vaccinated - 3,808 by scratch, 623 by subcutaneous inoculation. Blood specimens were obtained from 116 persons immediately before vaccination; of that number 77 were vaccinated by scratch, 39 by subcutaneous inoculation. Of the total 116 pre-vaccination sera, 102 gave a negative protection test.

Three months after the vaccinations, an attempt was made to secure sera from the 102 in question, but only 67 could be located, of whom 41 had been vaccinated by scratch and 26 by injection.

Of the 41 vaccinated by scarification, 38 gave a positive protection test: i.e. 92.7%

Of the 26 vaccinated by subcutaneous inoculation, 23 had a positive protection test: i.e. 88.5%.

Later, a further 68 blood specimens were secured from persons vaccinated by the scratch method, and of that number, 65 or 95.6% gave a positive protection test; also, of 23 further blood specimens taken from persons vaccinated by subcutaneous inoculation, 20 or 87% gave a positive protection test.

Further evidence in this respect is adduced by DICK[1] (1952), who, in Uganda, compared the immunity response following the administration of (a) the Dakar vaccine by scratch, (b) the 17D vaccine by subcutaneous inoculation and (c) the 17D vaccine by scratch. Among groups of volunteers, who were found to have no demonstrable yellow-fever antibody prior to vaccination and whose post-vaccination sera were available for examination 28 to 32 days after vaccination:

(a) 55 out of 56 vaccinated with Dakar vaccine developed protective antibody, i.e. 98.2%

(b) 64 out of 68 vaccinated with 17D vaccine by subcutaneous inoculation developed protective antibody, i.e. 94.1%; and

(c) 85 out of 91 vaccinated with 17D vaccine by scratch developed protective antibody, i.e. 93.4%.

In so far as the immunity response to the administration by scarification of the yellow-fever virus component in Hahn's combined vaccine is concerned, HAHN[3] (1951) reported that in one experiment, in which his mixed 17D yellow-fever-vaccinia vaccine was employed, 12 out of 12, i.e. 100% of those vaccinated, who had had negative pre-

vaccination sera, developed yellow-fever antibody by the sixth week after vaccination.

On the other hand, when a combined vaccine similar to that of Hahn was administered by scarification to a selected group in Uganda, DICK and HORGAN[2] (1952) failed to find a like percentage of antibody response to the yellow-fever virus component.

In this study the vaccines employed were: (a) the yellow-fever vaccine was one prepared in the laboratories of the International Health Division of the Rockefeller Foundation, New York and was that used by Dick in his experiments recorded above; (b) the vaccinia virus was the standard calf lymph prepared at the Medical Research Laboratory, Nairobi, Kenya; it was suspended in a 50% glycerol with phenol in a final concentration of 0.5%. A gum arabic solution was used to suspend the 17D virus and the smallpox vaccine for combined application by the scarification technique. It was also used to suspend the calf lymph in another part of the experiments (see below).

Fifty African women were made available for the test and placed in two groups of 25 each. Tests were carried out as follows:

Group I. The contents of 1 ampoule of yellow-fever vaccine were rehydrated in a mixture of 1.0 ml of the gum arabic solution and 1.0 ml of calf lymph, and thoroughly mixed in a mortar, with the aid of a pestle. 0.02 ml of this mixture was delivered from a tuberculin syringe as 2 drops on to the deltoid region of the arm of each of the 25 women included in this group. Two scarifications, each approximately 1 cm long, were made through each drop.

Group II. Each of the 25 women in this group were inoculated subcutaneously with 0.5 ml of the contents of 1 ampoule of the yellow-fever vaccine suspended in 50 ml of distilled water, and each was then immediately vaccinated at the same site by scarification, following the method described above, with 0.02 ml of a mixture of calf lymph and 1.0 ml of the gum arabic solution.

Results: Among those who had been found to have no demonstrable yellow-fever antibody prior to vaccination and whose post-vaccination sera were available 28 days after vaccination:

In Group I, 14 out of 21 scarified with the mixed vaccine had by that time developed yellow-fever antibody, i.e. 66.7%;

In Group II, 22 out of 22 inoculated subcutaneously with 17D vaccine and then vaccinated by scarification with a mixture of calf lymph and gum arabic solution had by that time developed yellow-fever antibody, i.e. 100%.

In regard to those in Group I whose sera were negative 28 days after vaccination, all 7 were again bled two months later, but none of them had developed positive sera by that time.

Thus, whereas Hahn found that, following the administration by scratch of his mixed yellow-fever vaccinia vaccine, 100% of those so vaccinated developed yellow-fever antibody by the sixth week after vaccination, Dick and Horgan found that only 66% of those who were scarified with their mixed vaccine had by that time developed yellow-fever antibody.

Although, in connexion with these divergent results, there were differences in the preparations employed, it is noteworthy that in Dick's previous experiments in Uganda - to which reference has been already made in this paper - scarification with 17D vaccine alone, using the same batch as in the combined vaccine of Dick and Horgan mentioned above, had immunized 93.4% of those vaccinated. Moreover, from experiments described in their paper by DICK and HORGAN[2] (1952), it was concluded that any significant reduction in the titre of the 17D virus component in their combined vaccine due to contact with phenol, glycerol or gum arabic in the concentrations present and under the conditions of their study was very unlikely. These authors suggest that the difference in the immunity response of the two groups in their study may be due to some local factor which prevented invasion of susceptible cells of the skin by the 17D vaccine virus in some cases. The results of their study led these authors to the following conclusion: "while there is good evidence for the efficiency of 17D vaccine as an immunizing agent when administered by scarification (HAHN[3] 1951; DICK[1] 1952), the present study indicates that the percentage of those who became immune after vaccination with the mixed vaccine used in this trial is not sufficiently high to suggest that this type of mixed vaccine should be used routinely". On the other hand, "if Hahn's results are confirmed, then it would seem that a highly efficient mixed vaccine is available for use".

Finally it may be mentioned that in the groups vaccinated by Hahn and by Dick and Horgan with a mixed vaccine, there was no evidence that the yellow-fever virus component had interfered with the vaccinia virus component. In neither series was there any post-vaccination reaction involving the central nervous system.

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