VACCINATION BY SCARIFICATION WITH A COMBINED 17D YELLOW FEVER AND VACCINIA VACCINE

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The immunizing power of 17D yellow fever vaccine administered by scarification has been established by several experiments (Hahn, 1951; Dick, 1952). Hahn has also claimed satisfactory results with a combined 17D yellow fever-vaccinia vaccine administered by scarification. It is the purpose of this paper to report the results of a small study which was made in Uganda with a combined vaccine of a type similar to that employed by Hahn.

PREPARATIONS EMPLOYED

Vaccines. The yellow fever vaccine used was of batch 1760 prepared in the laboratories of the International Health Division of the Rockefeller Foundation, New York. This was the same batch of vaccine as was used in the scarification experiments with 17D vaccine already described (Dick, 1952). Each ampoule of vaccine contained at least $3{\cdot}3 \times 10^6$ mouse intracerebral LD_{50} of virus at the time of this study.

The vaccinia vaccine used was the standard calf lymph prepared by Dr G. L. Timms, Medical Research Laboratory, Nairobi, Kenya. It was suspended in 50 % glycerol with phenol in a final concentration of 0·5 %. Titrations of the lymph on the skin of rabbits gave semi-confluent to heavy confluent takes at dilutions of 1:10,000, no higher dilutions being tested. The technique of titration was that used at the former Government Lymph Institute, Colindale, London.

Gum arabic. The preparation of the gum arabic solution employed followed the method used at the Pasteur Institute, Dakar (Durieux, personal communication), except that powdered gum acacia B.P. *Elect.* was used instead of gum arabic of Senegal. Gum arabic solutions were used to suspend 17D yellow fever virus in vaccination experiments using a scarification technique in which 17D vaccine alone was used (Hahn, 1951; Dick, 1952), and have been used extensively to suspend both neurotropic yellow fever vaccine virus alone and combined with smallpox vaccine (Peltier, 1948). There is no evidence that gum arabic has any deleterious effect on yellow fever virus in the concentration and under the conditions of the human vaccination experiment to be described.

EXPERIMENTAL

As will be described, a mixture of yellow fever vaccine, vaccine lymph and gum arabic was used to vaccinate one group of people. In order to test whether the glycerol or phenol of the vaccine lymph might significantly reduce the titre of the

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https://doi.org/10.1017/S0022172400019653 Published online by Cambridge University Press

17D vaccine in this mixture, the following experiment was done: aliquots of serial dilutions of some rehydrated 17D vaccine were added to (*a*) equal quantities of vaccine lymph, (*b*) vaccine lymph which had been inactivated at 56° C. for 2 hr., and (*c*) a buffered solution of 0·2% bovine albumin (Dick & Taylor, 1949). The mixtures were allowed to stand at room temperature for 10 min. and then mice in groups of six were inoculated intracerebrally with one or other of the mixtures. A contact time of 10 min. at room temperature was chosen in order to reduplicate the *in vitro* contact time of the mixtures in the human experiment. The results of this experiment are shown in Table 1.

Table 1. *Mortality in mice inoculated intracerebrally with* 17D *vaccine*

17D vaccine dilution	Mortality ratios of mice inoculated with 17D vaccine plus		
	lymph	inactivated lymph	bovine albumin
10^{0}	6/6	N.t.	N.t.
10^{-1}	12/12	12/12	12/12
10^{-2}	12/12	12/12	11/11
10^{-3}	11/12	7/10	12/12
10^{-4}	5/12	1/12	5/12
10^{-5}	0/11	1/12	0/11
10^{-6}	0/12	0/11	0/11
LD_{50}	3·8	3·4	3·8

Numerator = number of mice which died; denominator = number of mice inoculated; N.t. = not tested.

From these results there was no evidence that the phenol or glycerol would significantly reduce the titre of yellow fever virus (calculated by the method of Reed & Muench, 1938) under the conditions of the human experiment to be described.

Considerable experience has shown that all ampoules of batch 1760 of yellow fever vaccine stored under the same conditions at this Institute have, at any one time, the same titre. Since, for scarification in the vaccination experiment to be described, the contents of one ampoule of 17D vaccine were suspended in 2·0 ml., and the dose for each scarification was 0·02 ml., and since for subcutaneous inoculation 0·5 ml. of the contents of one ampoule suspended in 50 ml. of distilled water were used, it is considered that the dose of virus inoculated (approximately 33,000 LD_{50}) was the same as that placed on the arm through which the scarifications were made. That this is greatly in excess of the quantity required to immunize was shown by one of us (G.W.A.D.) in an earlier experiment in which serial tenfold dilutions of one ampoule of 17D vaccine were made in distilled water, and alternate groups, each of three volunteers who had been bled prior to vaccination, were inoculated (*a*) subcutaneously with 0·05 ml. of each dilution, or (*b*) by four sacrifications, each approximately 1 cm. long through two drops of each dilution of a total volume of 0·05 ml. Each dilution of the vaccine was inoculated intracerebrally into mice after the vaccinations had been made and the LD_{50} was found to be 3·5 per 0·05 ml. (A number of mice died immediately after inoculation in this

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titration, presumably due in part to the quantity of the inoculum or to the lack of isotonicity of the inoculum.) The vaccinated volunteers were again bled 28 days after vaccination and their sera tested for antibody with the results shown in Table 2.

Table 2. *Comparison of immunizing power of serial dilutions of 17D vaccine administered by inoculation or by scarification*

Dilution of vaccine	Number of those with negative pre-vaccination sera who developed antibody after vaccination by	
	Inoculation	Scarification
10^{0}	2/2	3/3
10^{-1}	3/3	2/2
10^{-2}	1/2	2/2
10^{-3}	1/3	1/3
10^{-4}	0/2	0/2
10^{-5}	0/3	0/3

Numerator = number with antibody 28 days after vaccination, denominator = number with negative pre-vaccination sera.

Although the numbers involved are small, this experiment suggests that even with minute doses of virus (32 mouse LD_{50}) the scarification route of inoculation is highly efficient.

Vaccinations. On 19 June 1951, blood samples were taken from 50 African adult female patients in the Mulago Mental Hospital, Kampala, Uganda. Their names were recorded and their previous smallpox vaccination state was recorded as the number of vaccination scars (1, 2, etc.) and the extent of the scars (+ or + +).

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 first 25 women (group 1). Two scarifications each approximately 1 cm. long were made through each drop. The patients were kept in the shade until the vaccine preparations had dried and the vaccinated area was then covered with a piece of gauze. The gauze dressing was used in order to discourage any immediate rubbing or washing off of the vaccine.

Each of the second group of 25 patients (group 2) was inoculated subcutaneously with 0·5 ml. of the contents of 1 ampoule of batch 1760 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 1·0 ml. of calf lymph and 1·0 ml. of the gum arabic solution.

The patients were under the daily medical supervision of Dr G. Campbell Young and were examined by the writers 2, 8 and 28 days after vaccination. On the first two examinations, the patients were called at random and the results of the vaccinia vaccination were read and recorded as primary, vaccinoid or immune reactions. On the twenty-eighth day after vaccination, all patients, with the exception of four who had been discharged, were again bled. No general reaction to the vaccinations was observed in any of the patients.

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The sera from the pre-vaccination blood samples and from the samples taken 28 days after vaccination were tested for yellow fever neutralizing antibodies by a sensitive method (Smithburn, 1945) in which the test serum was mixed with a 1% suspension of mouse-brain-passage neurotropic yellow fever virus and inoculated intraperitoneally into either eight 10–14- or six 35–42-day-old Swiss white mice of a stock originated from the Carworth Farms, New York. When the older mice were used, each was given an intracerebral inoculation of sterile 2% starch prior to the intraperitoneal inoculation of the virus-serum mixture. The only modification from the method described by Smithburn was that 0·2% bovine albumin was used instead of 10% serum-saline as diluent. The results were interpreted by the methods we have previously used (Smithburn, 1945). No serum was considered to be positive (i.e. to contain neutralizing antibody) if more than one animal died in a test group of 6–8 mice inoculated with the serum-virus mixture. Specimens which gave inconclusive results were retested whenever sufficient serum was available.

RESULTS

The results of the vaccination experiment are presented in Table 3.

In order to compare quantitatively groups 1 and 2 with regard to previous smallpox vaccination, a numerical index was given to each patient. This index was obtained by multiplying the number of old vaccination cicatrices by the number of +'s recorded for the extent of these cicatrices. Thus a patient with three cicatrices each of + + is recorded as having a pre-vaccination index of 6, while a patient with two scars, one of + and one of + +, is recorded as 3.

It is clear (Table 4) that according to our index the two groups are not very different in their pre-vaccination state, as far as smallpox is concerned, for if the frequency distribution of patients with indices of 0 to 6 in the two groups is compared, it is found that $t = 1{\cdot}171$ with $P = < 0{\cdot}3 > 0{\cdot}2$. Groups 1 and 2 were comparable with regard to their pre-vaccination state of immunity to yellow fever as shown by the results of neutralization tests on pre-vaccination blood samples (Table 3).

There is no significant difference in the response of the patients of groups 1 and 2 to the vaccination with the vaccinia vaccine ($\chi^2 = 2{\cdot}53$, $n = 2$, $P = < 0{\cdot}30 > 0{\cdot}20$). The results of the yellow fever neutralization tests show, however, that of those with negative pre-vaccination sera whose sera were tested 28 days after vaccination, 14 of 21 (66·7%) of group 1, and 22 of 22 (100%) of group 2 developed positive sera. The difference in the response of these two groups is significant ($\chi^2 = 6{\cdot}5$, $P = < 0{\cdot}02 > 0{\cdot}01$).

All of those whose sera were negative 28 days after vaccination were again bled about 2 months later but none of them had developed positive sera by that time.

DISCUSSION

Hahn (1951) has reported in one experiment using a mixed 17D yellow fever-vaccinia vaccine, that 100% (12 of 12) of those vaccinated, who had negative pre-vaccination sera, developed yellow fever antibody by the sixth week after

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vaccination. In our series only 14 of 21 (66·7 %) of those with negative pre-vaccination sera who were scarified with the mixed vaccine had by that time developed yellow fever antibody. The results of this study are, however, not strictly comparable

Table 3. *The results of vaccinations*

Group 1. Vaccinated with combined vaccinia and yellow fever vaccine by scarification					Group 2. Vaccinated by subcutaneous injection with yellow fever and by scarification with vaccinia				
	Vaccinia		Yellow fever			Vaccinia		Yellow fever	
Patient no.	Pre-vac. state	Post-vac. result	Pre-vac. state	Post-vac. result	Patient no.	Pre-vac. state	Post-vac. result	Pre-vac. state	Post-vac. result
1	2	P	−	+	1A	1	P	−	+
2	4	P	−	+	2A	0	V	+	+
3	1	V	−	−	3A	2	P	−	+
4	1	V	−	−	4A	6	I	−	+
5	2	V	−	+	5A	6	I	−	+
6	1	P	−	+	6A	0	P	−	+
7	6	V	+	+	7A	2	V	−	+
8	4	I	−	+	8A	2	I	−	+
9	2	V	−	+	9A	4	V	−	+
10	1	V	−	+	10A	1	I	−	+
11	3	P	−	+	11A	4	I	−	+
12	0	P	−	No test	12A	1	P	−	+
13	3	V	−	−	13A	1	P	Inc.	+
14	2	I	−	−	14A	0	I	−	+
15	4	I	−	−	15A	0	P	−	+
16	3	I	−	+	16A	2	I	−	+
17	2	P	−	No test	17A	1	V	−	+
18	2	I	−	−	18A	2	V	−	+
19	2	V	+	No test	19A	0	P	−	+
20	3	V	−	+	20A	6	I	−	+
21	1	I	−	+	21A	3	V	−	+
22	1	V	−	+	22A	1	P	−	+
23	6	I	−	+	23A	4	I	−	No test
24	6	V	−	−	24A	1	P	−	+
25	3	I	−	+	25A	0	P	−	+

P = primary; V = vaccinoid; I = immune reaction; Inc. = inconclusive result of protection test; − = no neutralizing antibody found; + = neutralizing antibody present.

Table 4. *The distribution of patients developing primary, vaccinoid or immune reactions in groups* 1 *and* 2

Reaction	Group 1	Group 2	Total
Primary	6	10	16
Vaccinoid	11	6	17
Immune	8	9	17

with those of Hahn, since there were differences in the preparations used and in the population groups vaccinated. However, in previous experiments in Uganda (Dick, 1952), scarification with 17D vaccine alone, using the same batch as in the combined vaccine described above, had immunized 93·4 % of those vaccinated.

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From the experiments described above it does not seem likely that some possible reduction in titre of the 17D vaccine due to contact with the phenol or glycerol (which could not be demonstrated for contact periods of 10 min. in experiments in mice) or with the gum arabic, can explain the low immunity rates found after scarification with the mixed vaccine, and it is considered, from the evidence in which dilutions of 17D vaccine were used, that the estimated LD_{50} of 17D virus (33,000) used for each scarification was greatly in excess of the minimum immunizing dose.

It is unlikely that the difference in the yellow fever immunity rates in groups 1 and 2 is due to the fact that a larger number of patients in group 1 may have rubbed off the vaccine. Thus, as has been shown, there was no significant difference in the response to vaccinia vaccine of groups 1 and 2, and furthermore, in group 1 there is no significant difference in those who developed (*a*) primary and vaccinoid, or (*b*) immune responses among those who had positive or negative sera ($\chi^2 = 0{\cdot}95$, $n = 1$, $P = < 0{\cdot}50 > 0{\cdot}30$). If rubbing off of the vaccine was the explanation of the lower yellow fever immunity rates in group 1, then one would have expected that those with negative sera would have shown a significantly different number of immune vaccinia responses as compared with those with positive sera.

There is no suggestion from our experiments that after scarification with 17D vaccine there is a delayed development of yellow fever antibody. Thus in none of the seven persons in group 1 who had not developed antibody by the 28th day, was yellow fever antibody demonstrable in sera taken 2 months later.

There was no significant difference in the reactions of groups 1 and 2 to the vaccinia vaccine and, from the types of response, no suggestion that yellow fever vaccine had interfered with the vaccinia vaccine, although to be certain of this a control group who had been vaccinated with vaccinia only would have been required. Furthermore, there was no evidence of the vaccinia virus interfering with the yellow fever virus in the group 2 patients who were inoculated subcutaneously with yellow fever vaccine. Thus when one vaccine was given immediately after the other (group 2) a good response was obtained to each of them. A possible explanation of the lower yellow fever immunity rates in group 1 may be due to local interference. Further studies are required to elucidate this point, but it may be that when the mixed vaccine is administered the presence of vaccinia in some cases prevents invasion by yellow fever virus. One might speculate that any such inhibition might be a factor of the relative titres of the vaccinia and yellow fever viruses in the mixtures. Some such explanation might explain the much higher percentage of positive yellow fever sera in Hahn's series as compared with those reported above.

If Hahn's results are confirmed, then it would seem that a highly efficient mixed vaccine is available for use. Our mixed vaccine did not produce a sufficiently high immunity rate against yellow fever to justify its recommendation.

There is one additional important point with regard to mixed yellow fever-vaccinia vaccines which requires further study—namely the incidence of reactions. No general reactions to the combined vaccine or double vaccination were noted in any of our patients, of whom 14 developed both primary vaccinia reactions and

https://doi.org/10.1017/S0022172400019653 Published online by Cambridge University Press

positive yellow fever sera. Hahn (1951) records no severe reactions in those he inoculated with his mixed 17 D yellow fever-vaccinia vaccine, of whom 10 out of 12 (with negative pre-vaccination and positive post-vaccination sera) developed 'takes' to the vaccinia component. Both these studies, however, were made in Africans, and it may be that their reaction to such a combined vaccine or to double vaccination is less severe than that of white races. Two mishaps have been recorded in Britain in association with the use of a combined vaccination. The details are: (1) a man was inoculated with yellow fever vaccine 13 days after primary vaccination and developed encephalitis the following day and died 5 days later; (2) a child, aged 6 years, was given a yellow fever inoculation 3 days after primary vaccination. Definite encephalitis developed on the eighth day after vaccination but the child fortunately recovered. It cannot be stated whether the above reactions might not have occurred if no yellow fever vaccine had been given. Seven cases of post-vaccinial encephalitis were reported during the same year, but it is not at present possible to calculate attack rates.

Peltier (1948) states that using the Dakar method, which employs a mixed French neurotropic yellow fever-vaccinia vaccine administered by scarification, the reactions are of the same order as those observed after the use of the French neurotropic vaccine by itself, but that they are observed less frequently in the black than in white races. Unfortunately, no figures are available except in the U.N.R.R.A. evaluation of the Dakar method of vaccination (*Epid. Inf. Bull.* 1946). In that study, however, the number of reactions in those vaccinated with a mixed vaccine are not truly comparable with the number of reactions in the group vaccinated with yellow fever only, since the latter group were subjected to considerable physical strain 4 days after inoculation. A careful follow-up of white people vaccinated with the combined Dakar vaccine might produce some valuable information in this respect. Until more information is available with regard to double or mixed vaccination with yellow fever and vaccinia vaccine it would seem wise to follow the recommendations offered by the Ministry of Health, London (*Monthly Bulletin*, 1949), which outlined the period which should elapse between smallpox and yellow fever vaccination to ensure maximum immunization and freedom from sequelae.

SUMMARY

Comparable groups of adult African women were vaccinated (*a*) with a mixed 17D yellow fever-vaccinia vaccine administered by scarification, or (*b*) by inoculation subcutaneously with 17D vaccine followed by scarification with vaccinia vaccine. There was no significant difference in the response of the two groups to the vaccinia vaccine. In the group inoculated with the mixed vaccine, of those whose sera contained no demonstrable yellow fever antibody prior to vaccination, 66·6% had developed antibody when their sera were tested 28 days later. In the other group 100% had developed antibody by the twenty-eighth day after vaccination.

It is suggested that the difference in the response of the groups in this study might be due to some local interference which prevented invasion by the 17D virus in some cases.

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While there is good evidence for the efficiency of 17D vaccine as an immunizing agent when administered by scarification (Hahn, 1951; Dick, 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.

In none of our patients was any reaction noted to the mixed or double vaccinations. Further information is required on the reaction of both negro and white races to combined vaccinations with yellow fever and vaccinia viruses. It is suggested that a careful follow-up of persons vaccinated by the combined Dakar vaccine might produce some valuable information in this respect.

Our thanks are due to Dr George Campbell Young for his very great co-operation, and to Messrs W. A. Whittaker, L. E. Hewitt and D. Santos for technical assistance.

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(*MS. received for publication* 7. II. 52)

https://doi.org/10.1017/S0022172400019653 Published online by Cambridge University Press