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STUDIES WITH DRIED AND GLYCERINATED SMALLPOX VACCINES OF FULL AND DIMINISHED POTENCIES

by

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SYNOPSIS

In a further vaccination and laboratory study with two dried smallpox vaccine (designated P & Q) and one glycerinated vaccine, the potency of samples of each vaccine were deliberately lowered by exposure to heat. Similar groups of volunteers were vaccinated with the fully potent vaccines and with those of reduced potency. All those who developed a vesicular response to this vaccination were challenged a year later by revaccination with a potent vaccine and the results obtained in the different groups were compared. No significant difference could be detected in the response to challenge between the groups. These results indicate that if vesiculation is obtained with a vaccine, even though potency has been reduced to a point when it produces less than 50% successful primary vaccinations, satisfactory protection will result one year later. It also appears that successful vaccination with a dried vaccine confers as good immunity as that obtained from glycerinated lymph. The results obtained indicate that a vaccine with a pock count of 10^6 infective units/ml will produce about 40% successes in primary vaccination.

In the report on Laboratory and Vaccination Studies with Dried Smallpox Vaccine (Cockburn et al. 1957) made on behalf of the World Health Organization, it was said that in addition to the ability of a vaccine to elicit a primary vesicular response in the unvaccinated a further proof of its efficacy would be the resistance of the

successfully vaccinated to subsequent revaccination with a potent lymph. It was decided to use this method of investigation to determine whether those who had developed a vesicular response to vaccination a year earlier with vaccine of low potency had the same degree of immunity as those successfully vaccinated at the same time with vaccine of high potency. These observations would, incidentally, provide confirmatory evidence of the titre actually required to produce 50% vaccination success rates.

MATERIALS AND METHODS

From information gained in the previous studies (Cockburn et al. 1957) it was possible to produce both dried and glycerinated vaccines giving a successful primary vaccination rate of about 50%. Accordingly two batches of dried vaccine (vaccine "P" and vaccine "Q" of the previous studies) and a batch of normal Lister Institute glycerinated lymph were set aside for the study. Part of each batch was kept for the vaccination of control groups and was stored at -10°C at the Lister Institute. The rest of each batch was subjected to heat before use, as described below:

1. Dried vaccine "P". Because of the great resistance to heat shown by this type of dried vaccine (Cockburn et al. 1957; Cross, Kaplan & McClean, 1957) it was impossible readily to diminish the titre of the WHO trial batch to the desired point. A special batch was therefore prepared. An elementary body suspension derived from sheep pulp was diluted in 5% peptone solution to a titre of 3.7×10^7 infective units (i.u.)/ml and dried in 0.25 ml volumes. The titre after drying was 2×10^7 i.u./ml. The ampoules were then immersed in boiling water for 60 minutes - a procedure which reduces the titre about tenfold (Cross, Kaplan & McClean, 1957). The titre of the vaccine after boiling was 4×10^6 i.u./ml.

2. Dried vaccine "Q". Storage at 45°C for 4 weeks reduced the titre of the vaccine to the required level of about 10^6 i.u./ml (see Bull. Wld Hlth Org. 16, 63). This procedure was repeated.

3. Lister Institute glycerinated vaccine. Ten ampoules of the control glycerinated vaccine used in the first part of this trial were held at 37°C for 7 days. The contents were pooled and titrated by pock count. The titre was 1.7×10^6 i.u./ml. The vaccine was then dispensed in 0.25 ml amounts in sealed ampoules.

Laboratory Tests

The potency of all the vaccines used in this study were titrated on the chorio-allantoic membrane of the developing chick by the method described in the previous study (Cockburn et al. 1957).

Vaccinations

Tests were made on groups of R.A.F. apprentices kept under observation for at least one year, using vaccines sent to the R.A.F. Station in insulated boxes containing ice cans. Precautions against the inclusion in the study of those previously vaccinated and against any bias in the selection of individuals were the same as those used in the previous study. The method of vaccination was also the same. The arms were inspected on the seventh day after the primary vaccination and the results were recorded as "vesiculation" or "no vesiculation"; no other distinction was made. Those with vesiculation were revaccinated one year later with potent vaccine; their arms were inspected on the fourth as well as the seventh days to detect any accelerated reactions. In all those groups vaccinated with the vaccines of lowered potency equal numbers were done with each deteriorated vaccine on any one day. Smaller control groups were vaccinated at the same time with the unheated lots of the dried and glycerinated vaccines. These control groups were also challenged a year later.

RESULTS

The results with both the unheated and heated vaccines are shown in the table. The percentage success rate after primary vaccination and after subsequent challenge are shown for the separate groups vaccinated with the different vaccines and the results for all the unheated and all the heated vaccines are also given. The control groups vaccinated with the unheated vaccines are smaller than those receiving the deteriorated samples, but the uniformity of the results with each type of unheated vaccine permit the numbers in the groups to be added together and thus give a sufficiently large single control group.

The results obtained in the earlier trial indicated that any vaccine giving a pock count greater than 10^8 infective units/ml could be relied on to produce 100% takes in primary vaccinations. Statistical analysis of the results after storage suggested

that 99% takes would be obtained with a vaccine with a pock count of 1.3×10^7 and 50% takes with a pock count of 3.0×10^5. In fact, vaccine "Q" after 4 weeks at 45°C when the pock count had fallen to between 1.0×10^5 and 1.6×10^6 produced 47% takes. In this study the potency of the heated "P" vaccine was reduced to 4×10^6, the heated "Q" vaccine to 1×10^6 and the heated glycerinated vaccine to 1.7×10^6. The percentage success rate obtained with all three heated vaccines when summed was 42%. This suggests that the titre required to give 50% takes is somewhat higher than the 3.0×10^5 i.u./ml deduced from statistical examination of the results in the earlier trial.

When the combined results of challenge with potent vaccine a year after successful vaccination with all three potent vaccines are compared with the combined results of challenge after successful use of the corresponding deteriorated vaccine there is no significant difference between the two groups; nor are there any significant differences between the responses to challenge in any of the six individual groups. In each group there was a small number whose response was completely negative and a similar small number that produced a vesicle which did not crust over until the twelfth day, thus resembling a primary response, but the bulk of responses to challenge (79-85%) were of the accelerated type, indicating substantial protection. In the small number (5-7%) in which the reaction proceeded in a similar manner to a primary reaction the vesicle could quite easily be distinguished from that of a primary reaction by its very characteristic appearance. The different types of reaction to vaccination met with in those of different susceptibility is to be the subject of a further paper.

The results indicate that if successful vaccination follows the use of a vaccine of low potency the immune state, as estimated by revaccination a year later, cannot be distinguished from that following vaccination with a vaccine of normal potency; there is also no detectable difference after a year between those vaccinated with glycerinated lymph and those who received dried vaccine whether fully potent or deteriorated. It can therefore be assumed that those successfully vaccinated with the dried vaccines in this and the earlier trials were satisfactorily protected.

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VACCINATION RESULTS WITH VACCINES OF FULL AND DIMINISHED POTENCY
AND SUBSEQUENT CHALLENGE

Vaccine	Primary vaccinations			Challenge of successful primary vaccinations after one year with potent vaccine		
	Number vaccinated	Results	Percentage with vesiculation	Number revaccinated	Results	Percentage
"P" Dried Unheated	16	16 V	100	16	2 Negative 12 AR 2 V	12.5 75 12.5
"P" Dried Heated	97	52 Negative 45 V	46	45	2 Negative 39 AR 4 V	4 87 9
"Q" Dried Unheated	20	20 V	100	20	3 Negative 15 AR 2 V	15 75 10
"Q" Dried Heated	99	60 Negative 39 V	39	39	4 Negative 34 AR 1 V	10 87 3
Glycerolated Unheated	11	11 V	100	11	0 Negative 10 AR 1 V	0 91 9
Glycerolated Heated	39	24 Negative 15 V	37	15	1 Negative 12 AR 2 V	7 80 13
All Unheated	47	47 V	100	47	5 Negative 37 AR 5 V	10 79 10
All Heated	235	136 Negative 99 V	42	99	7 Negative 85 AR 7 V	7 85 7

Negative = no local reaction

AR = accelerated reaction. Vesicle appears on 4th day - does not extend beyond the original 1/4" scratch - considerable itching - crusts over by the 8th day.

V = vesicle appears on 4th to 5th day - extends to double the 1/4" scratch - does not crust over until about the 12th day.