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Cutaneous responses to smallpox revaccination with calf lymph and the effect of fluorocarbon purification of the vaccine

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For several years the routine smallpox revaccination procedures at a central inoculation unit were arranged to assess the relation between vaccine titre (pock count) and success. Calf lymph batches were applied, diluted and undiluted, over a log titre range of 7.0–9.8. The dose–effect relationship did not appear to fit a linear equation on a log-probit scale, except in the lower part of the titre range. Plotted on this scale, the take rates of nearly all vaccine specimens of the required strength ($> 10^8$ PFU/ml) were lower than anticipated by linear extrapolation from low titres. Differences between batches were noted. These findings relate to pulp processing without purification. Fluorocarbon extraction of the calf skin harvest resulted in a 4-fold increase of vaccine volume with commensurate virus dilution; it also gave clearly higher take rates than parallel nonpurified vaccine specimens, whether at original strength or at 4-fold dilution.

In 1962 a study was published on the relationship between vaccine titre (log pock-forming units per ml—PFU/ml) and take rate in primary vaccination against smallpox (Polak et al., 1962). This report presents the results of a similar study, undertaken as a continuation of the earlier work, to assess the same relationship in revaccination.

As the study population was highly heterogeneous with regard to history of previous vaccination against smallpox, some subdivision was considered advisable. This, it was felt, should make it possible to distinguish between different levels of immunity, indicated by ED_{50} values that increase, in theory, from the basic level as estimated from primary vaccination trials to a 1–2 logs higher virus concentration for recently revaccinated persons. The slopes of these log-probit lines would express the heterogeneity within subgroups, the most homogeneous subgroup having the steepest slope. In theory no higher value for the slope can be expected than is found in primary vaccination.

The results of a study of Swedish recruits were compatible with that approach. Espmark (1965a, 1965b) found the ED_{50} in primary vaccination to be 0.965 log lower than in revaccination, while the slopes were estimated at 2.09 and 1.60 respectively.

In a technically somewhat different study of primary vaccination of Dutch recruits (Polak et al., 1962), a linear relation was also found, with log ED_{50} estimated at 6.12 PFU/ml and the slope at 1.58 (combined data for a calf lymph and a sheep lymph batch). The present data on revaccination were collected as an extension of that study; however, longer inoculation scratches were made and the vaccinator was different, so that comparisons must be conditional.

MATERIALS AND METHODS

Vaccines

All vaccines (Table 1) were prepared in the Smallpox Vaccine Laboratory of the Rijks Instituut voor de Volksgezondheid, Netherlands, from the third calf skin passage of the Elstree strain from the Lister Institute of Preventive Medicine, England. Particulars of production and of potency testing

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Table 1. Summary of smallpox vaccines used in four studies

Study	Period (month/year)	Calf pulp	Nature of vaccine	Initial titre (log PFU/ml)	Vaccine specimens applied in the study	
					Batch code	Titres [a]
1	4/65–8/66	6414	glycerolated	9.8	B	9.8 9.1 8.4 7.7 7.0
2	8/67–4/69	6701	glycerolated	9.7	D_1	9.7 8.8 7.9 7.0
		6669 I	glycerolated	8.8	D_2	8.8 7.9 7.0
3	4/69–2/70	6713-18	freeze-dried	8.4	E_1	8.4
		6804-06	freeze-dried	9.2	E_2	8.3
4	2/70–3/71	6669 II	glycerolated	8.8	F	8.8 8.3
		6669 II	fluorocarbon purified, glycerolated	8.3	FA	8.3

[a] Reductions from initial titres were obtained by suitable (serial) dilution.

are given below in addition to the data provided elsewhere by Hekker et al. (1972).

In the production of glycerolated vaccines B, D_1, D_2, and F, a quantity (say x grams) of pulp was treated with $2x$ ml of 1% phenol solution. After being passed through a coarse sieve, it was then mixed with $3x$ ml of 80% glycerol and 10% peptone so that final concentrations of 40% glycerol and 5% peptone were obtained. In the production of freeze-dried vaccines E_1 and E_2 glycerol was replaced by 10% sorbitol, the final concentration of both sorbitol and peptone being 5%. However, for vaccine E_2 a further volume of 5% peptone and 5% sorbitol in McIlvaine's buffer was added in order to obtain, for reasons not relevant here, a relatively low vaccine potency.

In the production of purified vaccine FA, x grams of pulp were mixed with x ml of Arcton 113 (trifluorotrichloroethane) and $10x$ ml of McIlvaine's buffer. After centrifugation, the aqueous supernatant was treated with a small volume of 10% phenol solution, so that the working concentration of phenol was 0.3%. After incubation at 22°C for 24 hours, a 1 : 2 dilution with 80% glycerol and 10% peptone was prepared. This process of fluorocarbon extraction results in a dilution about four times higher than the procedures described in the previous paragraph and hence a log titre reduction of about 0.6.

The vaccines were used in revaccination both undiluted and in selected dilutions (batches B, D_1, D_2, and F), only undiluted (batches E_1 and FA), or only in dilution (batch E_2). The dilutions were prepared in the Smallpox Vaccine Laboratory with 40% glycerol in McIlvaine's buffer with 5% peptone. In this way a number of vaccinia virus suspensions were obtained, each defined by the batch code listed in the sixth column of Table 1 and by its ultimate titre in log PFU/ml. These suspensions, for use in the field, will be referred to as " vaccine specimens ".

Vaccine specimens were stored at −70°C in bottles containing about 0.25 ml. Every 5–6 weeks a number of bottles were taken from Bilthoven to Rotterdam, a 1½-hour trip, and stored in a refrigerator at −20°C. Each Monday, the 5 bottles to be used for revaccinations from Monday to Friday were raised in temperature to +4°C; they were returned to −20°C at the end of the week. The bottles were identifiable only by a code number indicating the week and day of use. Thus all revaccinations on any one day were performed with one predetermined vaccine specimen and the coding system was designed to ensure that the vaccine specimens were applied at random within the population of each particular study. However, this randomization was aimed at the day of revaccination and not at the revaccinated persons individually, in order to reduce the risk of error. The vaccine specimens were as a rule distributed evenly over the days of revaccination; departures from this rule are of minor importance, except in study 4.

The potency of vaccine specimens was regularly tested on the chorioallantoic membranes (CAM) of chick embryos before and during field use. The adopted titres, given in tenths of log PFU/ml, can for all practical purposes be accepted as accurate. From a series of CAM tests during the course of

study 4, the standard deviation of the log titre averages per vaccine specimen is estimated at 0.032. The vaccine specimens appeared to be highly stable during field use. The procedure for potency testing may also be considered accurate, as substantiated by the results of potency tests of the WHO International Reference Preparation for Smallpox Vaccine (log titre about 8.4 after reconstitution with 0.25 ml of fluid) and of local reference preparations. The average log titre loss, estimated from parallel tests on vaccine samples returned from the field after 5–6 weeks and on laboratory counter samples, was 0.055 during study 4. It seems highly probable that on the actual day of field use the loss of titre was much lower.

Study population

All persons applying for revaccination to the Rotterdam Municipal Health Service and found eligible under the usual rules were entered in the trials by the vaccinator (J. H.) provided that they appeared able and willing to report again after about 1 week for reading of the cutaneous reaction.

The reasons for attending for revaccination were diverse: they included the requirement to produce a valid certificate for international travel on business, for pleasure, or on emigration, and the desire to stimulate protective immunity to variola in so-called high risk groups. The study population was, therefore, highly heterogeneous. In order to reduce the impact of heterogeneity, individuals were grouped according to time interval since their previous vaccination. This admittedly rather crude criterion was simple to use and appeared to be efficient. The time interval was calculated in years by subtracting the year of previous vaccination from the year of revaccination in the study.

Intervals were grouped as follows: <3, 3, 4–5, 6–9, 10–17, 18–33, 34–50, and ≥51 years. The first group was, of course, small. The next five groups, encompassing the intervals from 3 to 33 years, were most useful for our purposes. Some additional information was obtained from the last two groups, but except when low potency vaccine specimens were used they always showed take rates of or closely approaching 100%.

Standard population

The take rates per vaccine specimen of the five main interval groups (3, 4–5, 6–9, 10–17, and 18–33 years) were analysed separately. Differences between these take rates, as to both interval group and vaccine specimen, were consistent. In order to characterize the effectiveness of each vaccine specimen by a single figure, the observed take rates (percentages) of the five main interval groups were averaged per specimen. Such an average represents the estimated take rate (percentage) in a standard population as formed by equal contributions from each of the five main interval groups. It might therefore be called the " equivalent average take rate " by analogy with the equivalent average death rate sometimes calculated in vital statistics.

Vaccinations and readings

Vaccinations were performed by making two linear scratches about 6–7 mm long in the skin of the deltoid area. The results were read after 6–8 days. A " major reaction " as defined[1] by the WHO Scientific Group on Smallpox Eradication (1968) in one or two sites was considered to be evidence of successful revaccination. All vaccinations and readings were done by J. H.

In studies 1 and 2, if no major reaction was observed, a repeat vaccination with calf lymph from the routine production was performed as some trial vaccine specimens were below the required titres of 5×10^7 PFU/ml and 10^8 PFU/ml (WHO Study Group on Requirements for Smallpox Vaccine, 1959; WHO Expert Group on Requirements for Biological Substances, 1966).

Statistical analysis

Independence between interval since previous vaccination and vaccine specimens or batches was tested in $m \times n$ contingency tables by calculating χ^2 values.

Vaccine specimens were compared as to take rate per interval group in 2×2 tables. The test results of these tables for the five main interval groups were combined, using normal approximation.

For probit analysis and analysis of variance, the usual procedures were followed.

RESULTS

For each of the seven calf skin batches applied in four studies (see Table 1), Table 2 shows the number of valid observations according to interval in years since previous vaccination. In addition to the grand total of 4 746, nearly 200 other individuals were

[1] A vesicular or pustular lesion, or an area of definite induration or congestion surrounding a central lesion that may be a scab or ulcer.

Table 2. Number of revaccinations with seven vaccines

Study	Vaccine batch	Interval (years) since previous vaccination									Rejected data
		0–2	3	4–5	6–9	10–17	18–33	34–50	51+	total	
1	B	27	104	162	115	109	108	204	88	917	49
2	D_1	13	95	147	143	120	139	175	87	919	42
	D_2	19	95	101	121	82	116	102	68	704	17
3	E_1	10	65	63	65	51	60	55	24	393	33
	E_2	10	45	50	40	42	58	43	31	319	21
4	F	10	67	109	85	77	96	74	58	576	9
	FA	22	91	172	120	110	189	134	80	918	23
	Total	111	562	804	689	591	766	787	436	4 746	194

Table 3. Success rate according to vaccine specimen and interval since previous vaccination *

Vaccine	log PFU/ml	Interval (years) since previous vaccination								Success % in standard population (intervals of 3–33 years)
		0–2	3	4–5	6–9	10–17	18–33	34–50	⩾51	
B	9.8	5/6	12/20	9/17	13/25	14/21	14/16	39/39	10/10	63.8
	9.1	3/4	10/22	23/38	13/20	17/20	15/18	41/41	20/20	67.9
	8.4	2/5	15/21	24/38	15/19	21/22	25/29	42/42	26/26	79.0
	7.7	3/4	10/21	17/27	20/28	18/22	18/23	35/42	12/12	68.4
	7.0	4/8	8/20	18/42	14/23	13/24	15/22	24/40	13/20	53.2
aver. success %			52.9	56.5	65.6	76.6	80.7	88.7	93.0	
D_1	9.7	0/4	17/26	28/39	27/34	29/34	39/41	43/46	19/19	79.4
	8.8	2/4	13/21	19/23	28/33	26/31	20/23	32/32	21/21	80.0
	7.9	3/3	10/17	30/44	32/41	28/33	27/30	44/44	22/22	76.0
	7.0	1/2	9/31	11/41	18/35	16/22	34/45	41/53	21/25	51.1
aver. success %			53.8	62.3	73.4	81.7	86.9	92.7	96.0	
D_2	8.8	1/7	14/32	15/27	32/50	19/26	24/31	32/34	25/25	62.8
	7.9	2/7	21/33	20/34	26/35	24/36	38/46	31/32	19/19	69.2
	7.0	2/5	8/30	14/40	19/36	11/20	26/39	26/36	20/24	47.2
aver. success %			44.7	49.8	63.7	64.9	75.6	87.7	94.4	
E_1	8.4	4/10	28/65	36/63	44/65	38/51	57/60	54/55	23/24	67.5
E_2	8.3	4/10	22/45	25/50	33/40	35/42	54/58	41/43	31/31	71.6
aver. success %			46.0	53.6	75.1	78.9	94.1	96.8	97.9	
F	8.8	2/5	16/31	22/42	24/39	24/34	39/45	35/36	21/22	64.6
	8.3	4/5	15/36	33/67	26/46	32/43	44/51	37/38	36/36	61.6
FA	8.3	10/22	56/91	119/172	90/120	96/110	173/189	132/134	78/80	76.9
aver. success %			51.6	56.9	64.4	77.4	88.2	97.7	97.7	

* Take rate denominator: revaccinations read after 6–8 days; take rate numerator: revaccinations read as successful.

entered in the studies. However, as the results of inoculation could not be read after 6–8 days by J. H., the data were rejected.

The take rates produced by each of the 17 vaccine specimens are shown by interval group in Table 3.

Time interval since previous vaccination

The figures given in Table 2 were used to test for independence between the main interval groups (3–33 years) in the successive studies 1–4. The result, shown in the first line of Table 4, makes it clear that the study population shifted significantly during the course of several years with regard to its previous experience with smallpox vaccine. Except for the 10–17 year interval group, all groups showed marked variation in their contributions to the different studies.

Within each study, however, as Table 4 indicates, the denominators of Table 3 do not demonstrate dependency between the main interval groups and vaccine specimens. We may therefore assume that random distribution of vaccine specimens over interval groups within each study was largely achieved, although the specimens were allocated by vaccination day and not to individuals.

Take rate and time interval since previous vaccination

In each study the success percentage, averaged for each vaccine specimen, increased regularly with the interval since previous vaccination (Table 3). There were differences even between the 34–50 and ⩾51 year groups, which may be considered as evidence of some lasting immunity after about 40 years.

Table 4. Tests on distribution of five main interval groups (3, 4–5, 6–9, 10–17, 18–33 years since previous vaccination) in $m \times n$ contingency tables

	χ^2	Degrees of freedom	P
Between studies 1–4	33.8	12	0.001
Within studies:			
1. Vaccine B (5 specimens)	15.8	16	0.5
2. Vaccine D_1 (4 specimens)	17.1	12	0.1
Vaccine D_2 (3 specimens)	12.6	8	0.1
Between vaccines D_1 and D_2	5.3	4	0.3
3. Vaccine E_1 (1 specimen), vaccine E_2 (1 specimen)	3.3	4	0.5
4. Vaccine F (2 specimens), vaccine FA (1 specimen)	7.0	8	0.5
Total	61.1	52	

Take rates in the standard population

The success percentages observed for each vaccine specimen in the five main interval groups were averaged in order to obtain take rates for the standard population (see Materials and Methods). The figures are given in the last column of Table 3 and displayed graphically in Fig. 1.

Fig. 1 also shows the smooth curve of the relationship between vaccine titre and success percentage, assuming linearity in log-probit scale, a 50% effective log titre of 7.0 PFU/ml and a positive slope of 0.67.

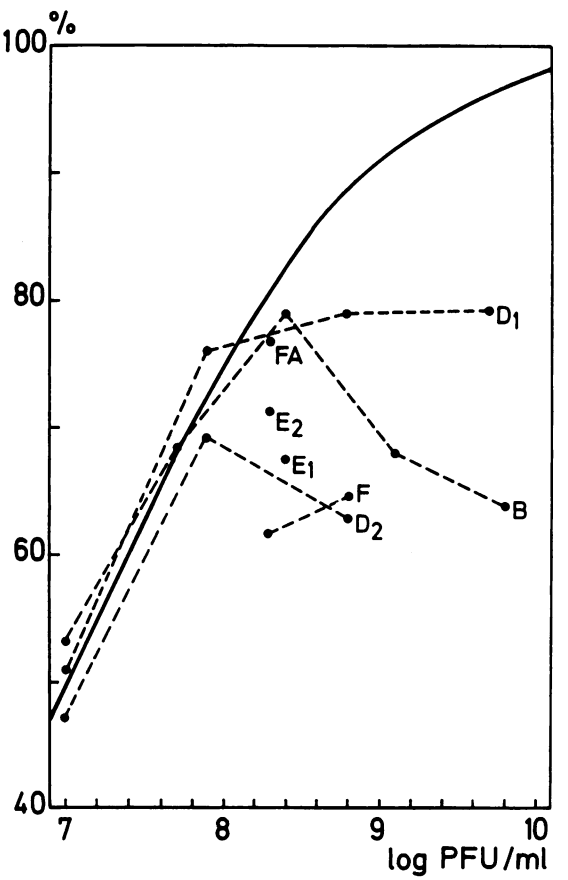

Fig. 1. Success percentages in the standard population according to titre for 17 vaccine specimens from 7 vaccine batches. The smooth curve represents the log-probit line with ED_{50} = 7.0 and slope = 0.67.

Take rates with vaccines B, D_1, and D_2

There is good agreement between vaccines B, D_1, and D_2 as to the take rates obtained with low titre specimens. The smooth curve in Fig. 1 seems to give an adequate picture of the titre/success relationship in the standard population for vaccine specimens in the 7.0–8.4 log titre range. However, it is clearly unsatisfactory for the take rates obtained at log titres 8.8 (vaccines D_1 and D_2), 9.1 (vaccine B), 9.7 (vaccine D_1), and 9.8 (vaccine B). The effects of undiluted or slightly diluted specimens from batches B, D_1, and D_2 seem to have been deficient in view of the results obtained with more highly diluted specimens, since one would expect linearity in the log-probit scale for the complete range of titres. This conclusion regarding the five main interval groups combined in the standard population also holds for each of these interval groups, although at varying levels of success.

Moreover, at log titre 8.8 vaccine D_1, diluted from log titre 9.7, appears to be more effective than vaccine D_2, as Fig. 1 indicates. Table 5 gives five pairs of success percentages. In each interval group vaccine specimen D_1–8.8 shows higher figures and according to the combined test of five 2×2 tables the difference has a low probability ($P = 0.001$). The differences between vaccines D_1 and D_2 at titre levels 7.9 and 7.0 are much less obvious (Fig. 1) and cannot be considered significant (Table 5).

Table 5. Success percentage by interval groups as compared in various pairs of vaccine specimens

Vaccine specimen and log PFU/ml	Interval (years) since previous vaccination					Combined double-tailed P value in five 2×2 tables [a]
	3	4–5	6–9	10–17	18–33	
D_1–8.8	62	84	85	84	87	0.001
D_2–8.8	44	56	64	73	77	
D_1–7.9	59	68	78	85	90	0.1
D_2–7.9	64	59	74	67	83	
D_1–7.0	29	27	51	73	76	0.7
D_2–7.0	27	35	53	55	67	
B –9.8	60	53	52	67	88	0.005
D_1–9.7	65	72	79	85	95	
D_2–8.8	44	56	64	73	77	0.8
F –8.8	52	52	62	71	87	
F –8.3	42	49	57	74	86	<0.001
FA–8.3	62	69	75	87	92	

[a] Derived from the success rates shown in Table 3.

While vaccines D_1 and D_2 were both applied by random allocation in study 2, the divergent success percentages for vaccine specimens B–9.8 and D_1–9.7 (Fig. 1 and Table 5) were obtained in study 1 and study 2 respectively. The latter specimen, although its titre was slightly lower, provided the highest results in all five main interval groups ($P = 0.005$).

Take rates with vaccines E_1 and E_2

Both E_1 and E_2, the vaccines applied in study 3, were freeze-dried products reconstituted in bulk by the Smallpox Vaccine Laboratory. Vaccine E_2 was diluted 6-fold in order to obtain equal titres for both specimens. However, titrations concurrent with field use showed a slight difference, the actual log titres being 8.4 and 8.3 for E_1 and E_2 respectively.

Results of revaccination are given in Table 3. Significant differences between the two specimens are not discernible. In general, these reconstituted freeze-dried vaccines were not more effective than specimens of vaccines B, D_1, and D_2 with a log titre 0.5 lower (Fig. 1).

Take rates with vaccines F and FA

Vaccines F and FA in study 4 were related to vaccine D_2 in study 2, as the same harvest from the calf skin served as the source of vaccinia virus, although after further storage for 30 months at −25°C.

Vaccines D_2 and F were manufactured as duplicates according to the same procedures. For both, the adopted log titre of the undiluted preparation was 8.8. Vaccine FA was purified by fluorocarbon treatment, which implied that the ultimate product was diluted about 4-fold as compared with vaccine F. The log titre adopted for vaccine FA was 8.3.

In the field, two specimens of vaccine F (log titres 8.8 and 8.3) [1] and one specimen of vaccine FA (log titre 8.3) were used for revaccination. Within a period of 5 weeks (25 days of revaccination) F–8.8, F–8.3, and FA–8.3 were allocated to 3, 4, and 12 days respectively, since much weight was given to observations with the fluorocarbon-treated specimen. In fact, FA–8.3 was applied in 2 subspecimens according to whether or not the underlying portion of calf skin pulp had been treated with phenol, but

[1] Specimen F–8.3 was obtained by 1 : 4 dilution from specimen F–8.8. This dilution would imply a 0.6 difference. In parallel CAM tests, the mean difference (0.57, standard error 0.031) in log titre between these specimens was compatible with the value. However, on the basis of the complete range of CAM test results, it seemed better to accept the 8.8 and 8.3 titres, and therefore the 0.5 difference.

as this influenced neither the titres nor the take rates, only the combined results are presented. The remaining 6 days in the 25-day period were used for revaccinations not relevant to this paper.

The take rates obtained with vaccine specimens D_2–8.8 and F–8.8 show only minor differences (Table 5, Fig. 1) that fall well within the variation attributable to chance for each of the five main interval groups as well as for the combined groups. Specimen F–8.3 gave take rates of about the same levels (Table 5). Vaccine specimen FA–8.3, in contrast, yielded clearly higher levels; the effects of this purified specimen were significantly better than those of the nonpurified specimen F–8.3. For the standard population, fluorocarbon treatment of the calf skin harvest resulted in an increase in the success rate from 61.6% to 76.9%, as illustrated by Fig. 1.

DISCUSSION

The desire to estimate two parameters, ED_{50} and slope, that would characterize a linear relation between log titres of vaccine and probits of success percentages prompted the trials reported here.

The study population was heterogeneous, not only as to vaccination history but also as to age and other attributes. This is quite usual outside the realms of infant and recruit vaccination.

It appears that the criterion used to distinguish groups of less heterogeneity, i.e., the time interval since previous vaccination, was efficient. Take rates were positively correlated to time interval (Table 3). However, the titre–take rate relation showed major deviations from a linear equation in log-probit scale.

In summary:

(*a*) linearity seemed reasonably satisfactory only for diluted vaccine specimens with log titres from 7.0 to 8.0–8.5;

(*b*) take rates for vaccine specimens either undiluted or at low dilutions in the log titre range from about 8.3 to almost 10.0 did not fit on a linear extrapolation from the 7–8 log titre range, but were too low;

(*c*) in the upper titre range the take rate levels seemed to depend more on some undefined quality of the calf skin harvest than on the titre (see vaccines D_2 and F from one calf skin pulp as compared with vaccine D_1 from another pulp at log titre 8.8 in Fig. 1).

In view of this nonlinearity in the complete range of titres explored, the selection of low titre specimens in order to estimate the parameters of a linear log-probit equation from their success percentages is arbitrary. If a supposed quality of the pulp is indeed responsible for reduced take rates from vaccine specimens B–9.8, B–9.1, and D_2–8.8, a similar mechanism may or may not have been in operation for specimens B–8.4 and D_2–7.9 (see Fig. 1). Even vaccine specimens with a log titre of 7.0 cannot be free of suspicion.

However, we present in Table 6 the results of probit analyses by interval group of all observations at log titres 7.0, 7.5, 7.7, 7.9, and 8.4. The underlying take rates are shown in Table 3 except in the case of log titre 7.5, the results for which were provided by an additional study discussed in the final section of this paper.

Table 6. Results of probit analysis of all observations at log titres 7.0, 7.5, 7.7, 7.9, and 8.4

Interval group (years)	$ED_{50} \pm$ s. d.[a]	Slope $\pm$ s. d.[a]	χ^2	Degrees of freedom	P
3	7.65 ± 0.110	0.81 ± 0.194	0.69	3	0.9
4–5	7.47 ± 0.109	0.64 ± 0.150	3.76	3	0.3
6–9	6.85 ± 0.237	0.60 ± 0.185	1.31	3	0.7
10–17	6.65 ± 0.298	0.69 ± 0.210	2.56	3	0.5
18–33	5.69 ± 0.726	0.48 ± 0.189	4.88	3	0.2

[a] S. d. = standard deviation.

The ED_{50} decreases as the interval since previous vaccination increases, all five slope values differ significantly from zero and all χ^2 values are compatible with linearity. All this does not disprove interference by the same mechanism thought to be responsible for disturbing the linearity in the full titre range from 7.0 to 9.8. For instance, it might be that the rather low slope values, particularly in the higher interval groups, should be understood as signs of such interference.

While the above discussion referred to observations on glycerolated vaccines, the results with freeze-dried vaccines E_1 and E_2 also seem to indicate interference with the vaccination take. On the whole, the efficacy of freeze-dried specimens at log titres of 8.3 and 8.4 was no higher than that of glycerolated vaccines B, D_1, and D_2 at 7.7–7.9.

Fluorocarbon extraction of calf skin harvest

Pulp extraction with fluorocarbon (Arcton 113) appears to confer higher effectiveness on the vaccinia virus suspension. Vaccine specimen FA–8.3 clearly induced higher take rates than parallel specimens D_2–8.8, F–8.8, and F–8.3. In fact, the results from FA–8.3 fit well with the log-probit lines as defined in Table 6. But again, it cannot be asserted that the data for vaccine specimen FA were free from interference.

Investigations to test whether linearity can be obtained for a purified calf lymph of this type over a broad log titre range from 7.0 to 9.0 and above seem to be indicated. If so, there is less reason for continued suspicion that an interfering mechanism reduces vaccine effectiveness. A study for this purpose has been initiated.

The advantages of a fluorocarbon extraction phase in the production of calf skin smallpox vaccine go beyond the partial removal of debris. In comparison with undiluted vaccine F, the yield in volume per gram of pulp was 4-fold for fluorocarbon-treated vaccine FA, without apparent loss of infective particles. With a log titre about 0.6 lower, vaccine FA was successful in 77% of the standard population, against 65% for undiluted vaccine F. As take rate differences per interval group between vaccine specimens D_2–8.8, F–8.8, and F–8.3 could be attributed to chance variation, the results for these specimens were amalgamated and compared with those obtained for FA. The respective success percentages, shown in Table 7, are a clear argument for including the extraction procedure as part of the routine production of calf skin smallpox vaccine.

Table 7. Success rates and percentages for vaccine specimens from a single calf skin harvest, according to whether or not extracted with fluorocarbon

Interval (years) since previous vaccination	Specimens D_2–8.8, F–8.8, F–8.3 (not extracted)		Specimen FA–8.3 (extracted)	
	Success rate	%	Success rate	%
0–2	7/17	41	10/22	45
3	45/99	45	56/91	62
4–5	70/136	51	119/172	69
6–9	82/135	61	90/120	75
10–17	75/103	73	96/110	87
18–33	107/127	84	173/189	92
34–50	104/108	96	132/134	99
⩾51	82/83	99	78/80	98

Table 8. Success rates induced by vaccine C, according to log titre and interval since previous vaccination

log PFU/ml	Interval (years) since previous vaccination								Success % in standard population (interval of 3–33 years)
	0–2	3	4–5	6–9	10–17	18–33	34–50	⩾51	
8.5	3/7	12/37	38/62	31/48	38/53	40/46	80/82	37/37	63.4
8.0	1/3	11/25	30/66	27/42	32/42	34/38	82/83	34/34	63.9
7.5	0/10	23/54	39/70	24/41	33/46	37/40	53/60	33/37	64.2
aver. success %		39.7	54.2	62.5	73.2	89.6	94.9	96.4	

Additional observations

The data in Table 3 support the main points in the above paragraphs. Results of an early trial in 1962–64 with glycerolated calf lymph (vaccine A) are not shown as the registration was incomplete. The outcome was in line with the later findings given in Table 3.

In 1966–67 an interim study was carried out with glycerolated calf lymph (vaccine C) produced in sub-batches with and without 5% peptone, each at log titres 8.5, 8.0, and 7.5. In combined 2×2 tables for the five main interval groups, the take rate appeared to be independent of peptone, both for each titre separately and for the amalgamate ($P > 0.05$). The observations are therefore presented in Table 8 irrespective of the presence of peptone. Data of specimen C–7.5 were included in the probit analysis reported in Table 6, although the success percentages of specimens C–8.0 and C–8.5 were disregarded because of their poor fit (an arbitrary decision in favour of linearity). In the standard population success percentages obtained with vaccine C did not increase with the titre; it may be assumed that an interfering mechanism was again active. However, in the highest interval groups ($\geqslant$ 34 years) some effect of titre increase was noted.

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RÉSUMÉ

RÉACTIONS CUTANÉES À LA REVACCINATION ANTIVARIOLIQUE À L'AIDE D'UN VACCIN OBTENU CHEZ LE VEAU ET EFFETS DE LA PURIFICATION DU VACCIN PAR LE FLUOROCARBONE

Durant plusieurs années, les techniques de revaccination antivariolique appliquées dans un centre de vaccination ont été adaptées en vue d'évaluer le rapport entre l'activité des vaccins (mesurée par numération des unités infectantes sur membrane chorio-allantoïde d'embryon de poulet) et le taux de prise (réactions majeures). Les résultats ne fournissant pas la relation linéaire escomptée sur l'échelle logarithme-probit, on a utilisé une série de vaccins antivarioliques, dilués ou non dilués, dont la gamme des titres, en valeurs logarithmiques, allait de 7,0 à 9,8. Pour tenir compte de l'hétérogénéité de la population participant à l'étude, on a analysé les taux de prise en fonction du temps écoulé depuis la vaccination précédente (0–2, 3, 4–5, 6–9, 10–17, 18–33, 34–50 et 51 ans et plus).

De 1965 à 1971, on a utilisé quatre lots de vaccin glycérolé non purifié (B, D_1, D_2 et F), deux lots de vaccin lyophilisé (E_1 et E_2) et un lot de vaccin glycérolé traité par le fluorocarbone (FA). Certains vaccins ont été employés à des dilutions différentes de sorte qu'au total 17 préparations vaccinales, caractérisées par leur lot et leur titre, ont été examinées sous le rapport de leur efficacité pour la revaccination.

On relève une corrélation positive entre les taux de prise et le temps écoulé depuis la vaccination précédente. Les pourcentages moyens de réactions positives dans les cinq groupes principaux de population (sujets vaccinés depuis 3 à 33 ans) représentent le nombre estimatif de prises par 100 individus appartenant à une population standard hypothétique et donnent les caractéristiques d'efficacité pour la revaccination de chaque préparation vaccinale.

En cas d'emploi de vaccin glycérolé non purifié, on n'observe pas de relation linéaire entre le logarithme des titres et le pourcentage de prise exprimé en probits. Un rapport satisfaisant n'apparaît que pour les vaccins dilués dont le logarithme des titres est de 7,0 à 8,0–8,5. Les taux de prise des vaccins non dilués ou peu dilués, dans la gamme des titres de 8,3 à 10,0, ne correspondent pas aux taux obtenus par extrapolation des taux relatifs aux titres 7–8, mais leur sont inférieurs. Dans la gamme des titres élevés, les taux de prise semblent plutôt fonction d'une propriété non identifiée du vaccin que du titre.

L'étude des réactions provoquées par les vaccins lyophilisés E_1 et E_2 fait également apparaître un taux de prise insuffisant.

Les taux de prise obtenus après revaccination par le vaccin glycérolé traité au fluorocarbone (vaccin FA) de titre 8,3 sont considérablement plus élevés que ceux observés après emploi de vaccins non purifiés, de titre égal ou supérieur, de même origine (vaccin D_2 de titre 8,8; vaccin F de titres 8,8 et 8,3). On en conclut que le traitement de la suspension de virus vaccinal par le fluorocarbone est un procédé recommandable qui permet d'obtenir des taux de prise plus satisfaisants.

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