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LABORATORY AND VACCINATION STUDIES WITH DRIED SMALLPOX VACCINES

A report to the World Health Organization

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with a statistical note on the trial of smallpox vaccine

by

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On behalf of the World Health Organization a laboratory study of the heat stability of four commercially produced dried smallpox vaccines was recently made in laboratories in Copenhagen, Elstree and Paris. The results of this study will be reported by J. O. Irwin elsewhere. It was decided that another batch of the vaccine found to be most heat stable should be investigated further and that at the same time a vaccine which had given promising results in a different unpublished study should be similarly investigated. The aim of the new studies was, (a) to measure the effect of storage at 37°C and 45°C for varying periods by two laboratory methods - rabbit skin scarification tests, and pock counts on the chorio-allantois of chick embryos - and by vaccination success rates in young adults not previously vaccinated, and (b) to compare the results of the laboratory tests with the vaccination success rates.

MATERIALS AND METHODS

Storage and Issue of the Vaccines

The two vaccines under test were designated "P" and "Q" and were held at the Lister Institute, Elstree. A portion of each vaccine was stored at -10°C. The remainder of each vaccine was divided into two lots, one of which was incubated at 37°C and the other at 45°C. After storage for 4, 8, 16, 32 and, (vaccine P only), 64 weeks, samples were removed from the incubators and stored at -10°C until used for vaccinations and the laboratory tests. These were carried out as soon as practicable after removal of the samples from the incubators and in no instance was the interval more than three weeks.

The laboratory tests were made at Elstree and at Liverpool and the vaccinations were carried out by one of us (R. M. Cross) on R.A.F. personnel at a station in the Midlands of England. The samples sent to Liverpool and the R.A.F. station were dispatched in insulated boxes containing ice-cans. In each laboratory and vaccination test, a sample of a standard batch of glycerinated lymph of known potency held throughout at -10°C was used as a control. Before the main trials began, and again at the end of the whole study, the potency of the two dried vaccines and the control batch of glycerinated lymph, all stored at -10°C, was checked by the vaccination of groups of men and by full-scale laboratory tests.

Vaccination Studies

Thanks to the co-operation of the Directorate of Hygiene and Research of the Royal Air Force, the Headquarters Technical Training Command, and in particular of Air Commodore J. Hill, the principal medical officer there, sufficient men were made available for vaccination.

The studies were carried out on groups of young men as they entered the R.A.F. Before the men were vaccinated a history of previous vaccination was taken and the arms were inspected for scars. Those with no previous history and no scars - about two-thirds of the numbers available - were then assembled in line and were directed in strict rotation into one of a number of vaccine groups. The number of vaccine groups so formed varied with the number of vaccines being tested on a particular day. Each vaccine group consisted of about 100 men (table 1). When there was a slight shortage of men the procedure for their allocation to groups was modified so as to reduce the numbers vaccinated with the glycerinated control. When it was known that vaccine Q was losing potency (see below) the numbers vaccinated with it were reduced. The vaccinator was issued with samples of vaccine identified with code numbers, the exact meaning of which was known only to the issuing laboratory. The vaccinator therefore was unable to tell which samples had been stored at 45°C and at 37°C. He could distinguish the dried vaccines from the glycerinated control and, owing to the different shape of the containers, he could distinguish between the dried vaccines.

The dried vaccines were packed in 25-dose ampoules. Each ampoule was used to vaccinate not more than 25 men and any vaccine remaining in the ampoule after 25 vaccinations was discarded. All vaccinations were made by a single linear scratch one-quarter of an inch (6-7 mm) long. Arms were inspected on the seventh day after vaccination and the result was recorded as "vesiculation" or "no vesiculation". No other distinction was made. In the absence of vesiculation men were revaccinated with a routine batch of glycerinated vaccine lymph.

TABLE 1. VACCINATION RESULTS

Period of storage (weeks)*	Vaccine P stored at				Glycerinated lymph stored at		Vaccine Q stored at			
	37°C		45°C		-10°C		37°C		45°C	
	Number vaccinated	Percentage with vesiculation	Number vaccinated	Percentage with vesiculation	Number vaccinated	Percentage with vesiculation	Number vaccinated	Percentage with vesiculation	Number vaccinated	Percentage with vesiculation
4	121	100	121	100	112	100	114	96	117	47
8	86	100	85	100	73	100	83	96	nt	-
16	98	100	92	100	104	100	96	72	nt	-
32	109	100	114	100	57	100	30	10	nt	-
64	100	100	100	100	48	100	nt	-	nt	-

nt = not tested

* As a check on the original potency of the vaccines, at the beginning of the study 204 men were divided into three groups and vaccinated with the two test vaccines stored at -10°C and the glycerinated lymph control. Vesiculation was obtained in all the men in each group. A similar test was made with the three vaccines stored at -10°C at the end of the study. All the vaccinations were again successful.

Vaccine Q gave a successful vaccination rate of less than 50 per cent. after storage for four weeks at 45°C. To avoid having too many unsuccessful vaccinations among the men, the lot stored at 45°C was not used again for vaccination though laboratory tests with it were continued. The lot of vaccine Q held at 37°C showed a considerable drop in potency after storage for 16 weeks and when the time came to test it at 32 weeks only 30 men were vaccinated with it - again to avoid too many unsuccessful vaccinations in the units.

Laboratory Tests

(a) Rabbit skin scarification

The titrations were made in duplicate on clipped backs of two rabbits in each of the two laboratories. The dilutions of vaccine were made in McIlvaine buffer, 0.01 M phosphate at pH 7.4. Volumes of 0.1 ml of the appropriate dilutions were spread over areas of approximately 5 cm^2 and the skin lightly scarified through the fluid. The dilutions normally tested were threefold from 1:1000 to 1:27 000 but when samples showed deterioration they were also tested in dilutions of 1:10 and 1:100. The control glycerinated lymph was titrated in each rabbit in order to control the susceptibility of the test animal to vaccinia virus. The lesions were recorded on the fourth, fifth and, if necessary, the sixth day.

(b) Chorio-allantoic membrane titrations

Dilutions of the vaccines for chorio-allantoic titrations were prepared as follows: the vaccine was reconstituted in the appropriate volume of sterile reconstituting fluid (40 per cent. glycerol in McIllvaine buffer, 0.01 M phosphate at pH 7.4), and dilutions were made in buffer containing 10 per cent. broth and 50 units penicillin per ml. An initial dilution of 1/100 was made by adding 0.1 ml of vaccine to 9.9 ml of diluent. Subsequent serial tenfold dilutions were made by transferring 0.5 ml to 4.5 ml of diluent. A fresh pipette was used for each transfer and was discarded as soon as 0.5 ml had been delivered without "washing out". No special precautions were taken to keep the dilutions colder than the surrounding temperature although the diluent was at +4°C at the start of a dilution series. Nor was any attempt made to keep the dilutions in the dark; direct sunlight, however, was not allowed to fall on the tubes.

TABLE 2. TITRATIONS OF DRIED SMALLPOX VACCINE "P"

Storage conditions	Where titrated	CAM titre (i.u. /ml)	Rabbit scarification titre 1/1000	1/3000	1/9000	1/27 000	Vaccination success rate per cent.
-10°C	A	3.5×10^8	c	c	c	sc+	100
			c	c	c	sc+	
	B	4.9×10^8	c	c	sc	3	
			c	sc	sc	4	
4 weeks at 37°C	A	4.8×10^8	c	c	c	sc+	100
			c	c	sc+	sc+	
	B	3.5×10^8	c	c	c	7	
			c	sc	6	3	
8 weeks at 37°C	A	4.2×10^8	c	sc+	sc	5	100
			c	sc+	3	0	
	B	3.7×10^8	c	c	sc	4	
			c	c	sc	2	
16 weeks at 37°C	A	6.5×10^8	c	c	c	sc	100
			c	c	sc-	2	
	B	3.6×10^8	c	c	sc	1	
			c	c	c	8	
32 weeks at 37°C	A	3.6×10^8	c	sc+	sc+	?	100
			c	c	sc+	1	
	B	1.8×10^8	c	c	sc+	8	
			c	c	sc	7	
64 weeks at 37°C	A	2.5×10^8	sc+	sc-	9	3	100
			c	c	sc-	6	
	B	3.8×10^8	c	c	9	2	
			-	-	-	-	
4 weeks at 45°C	A	4.6×10^8	c	c	c	7	100
			c	c	sc+	6	
	B	4.7×10^8	c	sc	8	-	
			c	c	6	-	
8 weeks at 45°C	A	3.2×10^8	c	sc+	sc-	6	100
			c	3	2	3	
	B	2.9×10^8	c	c	sc	4	
			c	sc	5	5	
16 weeks at 45°C	A	4.6×10^8	c	c	c	sc+	100
			c	c	7	0	
	B	3.0×10^8	c	c	6	1	
			c	c	c	6	

TABLE 2. TITRATIONS OF DRIED SMALLPOX VACCINE "P" (continued)

Storage conditions	Where titrated	CAM titre (i.u. /ml)	Rabbit scarification titre 1/1000	1/3000	1/9000	1/27000	Vaccination success rate per cent.
32 weeks at 45°C	A	1.8×10^8	c	c	sc+	7	100
			c	c	sc+	0	
	B	1.3×10^8	c	c	sc-	3	
			c	c	sc	8	
64 weeks at 45°C	A	6.2×10^7 or 1.6×10^8	sc+	sc-	5	1	100
			c	sc+	sc	3	
	B	1.3×10^8 or 2.3×10^8	c	c	5	2	
			-	-	-	-	

i.u./ml	=	infective units/ml by pock count on chorio-allantoic membrane
c	=	confluent lesion, 100% of area
sc+	=	semiconfluent lesion, 70-80% of area
sc	=	semiconfluent lesion, 50-70% of area
sc-	=	semiconfluent lesion, less than 50% of area
numbers	=	discrete vesicles
A	=	samples titrated in Lister Institute
B	=	samples titrated in Liverpool
-	=	no test

Where necessary, preliminary titrations of deteriorated vaccines were made using three embryos per dilution at 10^{-2}, 10^{-4}, and 10^{-6}. Once the range had been determined, five or six 12-day chick embryos were used for each dilution tested. They were each inoculated on the dropped chorio-allantoic membrane with 0.1 ml of the appropriate dilution. The embryos were then incubated at 35°C for 44 to 48 hours, the dropped areas of the membranes removed and washed, and the lesions counted. Titres were expressed as infective units per millitire (i.u./ml) of undiluted vaccine and were computed from the mean pock count at the lowest dilution giving discrete, countable pocks.

RESULTS

Vaccination Results

The results are given in table 1. All the 394 men given the control batch of glycerinated lymph were successfully vaccinated. Vaccine P, with which a total of 1026 men were vaccinated, was still giving 100 per cent. success rates after storage for 64 weeks at 37°C and 45°C. Vaccine Q was much less satisfactory - only 47 per cent. of the 117 vaccinations with the sample kept at 45°C for four weeks resulted in vesiculation. No further observations were made with vaccine Q at this temperature. At 37°C it gave success rates which fell from 96 per cent. at eight weeks to 72 per cent. at 16 weeks and 10 per cent. at 32 weeks. Vaccine P therefore was eminently satisfactory, but vaccine Q deteriorated very rapidly at 45°C and less rapidly at 37°C.

Laboratory Titrations

The results of laboratory titrations are set out in tables 2 and 3. The agreement between the results by pock counting in the two laboratories is good for vaccine P. With vaccine Q the results obtained in Liverpool (laboratory B) up to eight weeks were usually significantly lower than those obtained at Elstree (laboratory A). Vaccine Q was less homogeneous after reconstitution than vaccine P

TABLE 3. TITRATIONS OF DRIED SMALLPOX VACCINE "Q"

Storage Conditions	Where Titrated	CAM titre (i.u./ml)	Rabbit scarification titre 1/10	1/100	1/1000	1/3000	1/9000	1/27000	Vaccination success rate per cent.
-10°C	A	1.5×10^7	-	-	c	c	sc+	6	100
			-	-	c	c	c	8	
	B	2.9×10^6							
4 weeks at 37°C	A	3.4×10^7	-	c	sc+	sc-	6	1	96
			-	c	c	6	1	3	
	B	1.5×10^6	-	-	6	0	0	0	
			-	-	4	0	0	0	
8 weeks at 37°	A	3.6×10^6	c	c	c	sc	5	0	96
			c	sc+	5	0	0	0	
	B	8.5×10^5	-	c	6	1	0		
			-	sc	6	4	3		
16 weeks at 37°C	A	4.8×10^5	c	sc	6	0	0	-	72
			c	sc+	2	2	0	-	
	B	10.3×10^5	-	c	c	1	0	-	
			-	sc	sc	1	0	-	
32 weeks at 37°C	A	5.1×10^4	3	0	0	0	-	-	10
			?	0	0	0	-	-	
	B	10.2×10^4	4	1	0	-	-	-	
			5	0	0	-	-	-	
4 weeks at 45°C	A	1.6×10^6	-	sc-	3	2	0	1	47
			-	sc	2	1	0	0	
	B	1.0×10^5		2	0	0	0	-	
				4	0	0	0	-	
8 weeks at 45°C	A	3.5×10^4	sc-	0	0	0	0	0	-
			sc-	0	0	0	0	0	
	B	4.7×10^4	sc	0	1	0	-	-	
			5	3	0	0	-	-	
16 weeks at 45°C	A	-	1	0	0	0	-	-	-
			0	0	0	0	-	-	
	B	-	0	0	0	0	-	-	
			0	0	0	0	-	-	
32 weeks at 45°C	A	-	0	-	-	-	-	-	-
			0	-	-	-	-	-	
	B	-							

i.u./ml = infective units/ml by pock count on chorio-allantoic membrane.
c = confluent lesion, 100% of area
sc+ = semiconfluent lesion, 70-80% of area
sc = semiconfluent lesion, 50-70% of area
sc- = semiconfluent lesion, less than 50% of area
numbers = discrete vesicles
A = samples titrated in Lister Institute
B = samples titrated in Liverpool
- = no test

and it appeared that the different results were due to slight differences in technique in the making of dilutions in the two laboratories. The results of tests by scarification on the rabbit skin showed as good agreement as could be expected from this test. The results taken together reflect the stability of vaccine P and the deterioration of vaccine Q.

Relation Between Laboratory Titrations and Vaccination Success Rates

Dr C. C. Spicer of the Standards Laboratory, the Central Public Health Laboratory, Colindale, London, N.W.9, has provided the following report on the relationship between the laboratory and vaccination results:

"The results of the experiment demonstrate a clear relationship between the pock counts of preparations of smallpox vaccine and the percentage of successful vaccinations which they will produce. However, only the figures for vaccine Q preserved at 37°C are available for determining the nature of the relationship, because vaccine Q at 45°C only gave one pock count in the useful range, and vaccine P always gave 100 per cent. "takes" (successful vaccinations). It has been possible to derive a curve relating pock count to percentage "takes" for vaccine Q at 37°C. The rabbit scarification titres are correlated with the pock counts, but are not so accurate and have not been used in the analysis. Their general relationship with the pock counts is similar to that between plate counts and dilution counts on a bacterial suspension. In the latter, which is widely used for estimating faecal coliform organisms in water, the numbers are estimated from the proportions of positive and negative tubes and not be actual counting, and this is essentially a process of very low precision.

"The method of probit analysis was used, as it has been found applicable to other dose response curves of this kind. It is not suggested that more than a reasonable approximation has been obtained, which can be applied in similar studies. The method assumes basically that the probability of a "take" at any given pock count follows a known mathematical formula. Knowing the percentage "takes" corresponding with a number of different counts it is then possible, using standard statistical methods, (see e.g., Finney 1952) to calculate the constants of the distribution and hence the percentage "takes" to be expected at any pock count.

"The analysis shows that vaccine Q preserved at 37°C gives 50 per cent. "takes" at a pock count of about 3.0×10^5. Theoretical pock counts corresponding to some other percentage "takes" are given in table 4 and figure I. Taking into account the approximate nature of the analysis it would be safe to say that pock counts greater than 10^8 are an effective guarantee that a vaccine should give 100 per cent. "takes". The figures given in table 4 refer only to counts of about the same accuracy as those of the present investigation. If counts of lower accuracy are used then the rise from 0-100 per cent. "takes" will be less steep, while more accurate counts will give a steeper slope. An attempt has been made to give an upper limit to the steepness of the slope using a method outlined in the statistical part of this note. This ideal limit is the relationship between pock count and per cent. "takes" that would be found if the counts were perfectly accurate. The only variation remaining is then due to differences in susceptibility to "taking" among the human subjects of the experiment. Such an ideal is not likely to be obtained in practice but it is worth bearing in mind that, with data of this kind, the accuracy of the pock counts systematically affects the relationship between them and the percentage "takes".

TABLE 4. THEORETICAL RELATION BETWEEN POCK COUNT AND PERCENTAGE "TAKES"

% takes	Pock counts
99	1.3×10^7
90	2.4×10^6
50	3.0×10^5
10	3.7×10^4
1	6.8×10^3

"The data on vaccine P relate only to 100 per cent. "takes" but there is a decline in the pock count which is significant statistically and is also more marked at 45°C than at 37°C."

DISCUSSION

The final test of new smallpox vaccines is their capacity to prevent smallpox in those inoculated, but as it is already known that successful vaccination gives a high degree of protection against disease, the ability of a vaccine to elicit a typical primary response in the unvaccinated can be accepted as evidence of potency unless information to the contrary is obtained. Further proof of the efficacy of a vaccine would be resistance to subsequent challenge with a potent vaccine after successful vaccination with the test vaccine. In the light of information already obtained in this trial it is hoped to determine this resistance to challenge a year after successful vaccination with the two dried vaccines and with the glycerinated vaccine, the potencies of which have been lowered by exposure to heat to a point when they give approximately 50 per cent. successful primary vaccination rates. These observations should show whether or not vaccines that are losing their potency produce an immunity as satisfactory as that provided by vaccines of full potency; they will incidentally provide evidence of the titre actually required to produce 50 per cent. vaccination success rates. Since successful vaccination, even if it follows the use of a vaccine that has deteriorated, is likely to produce a firm immunity these observations should provide the required evidence of resistance to subsequent challenge after the use of the test vaccines.

The titration of smallpox vaccine by pock counting on the chorio-allantois of the chick embryo has been shown to be more reliable than titration by scarification on the skin of rabbits. Apart from the relative crudity of the method used in titration by scarification on the rabbit skin, it is obvious from the tests on duplicate rabbits in both laboratories that there is considerable variation in the susceptibility of individual rabbits to the virus; this is also influenced by the state of the animals' skin since it is a matter of experience that rabbits which are moulting and growing a new coat of fur are unreliable. It appears that, within

certain defined limits of error, titration by pock counting measures the number of infective units of virus present in a vaccine; even when comparable dilutions of a control vaccine of known potency are used, titration on the rabbit skin can only be safely used to provide a standard of minimum acceptable potency. The results obtained by pock counting in different laboratories show satisfactory agreement with each other and there is a direct relation between the titres obtained by this method and the vaccination success rates. It has been possible to decide on a minimum titre (10^8 i.u./ml) at which a vaccine may be expected to give the highest possible number of successful vaccinations.

Vaccine P showed much greater resistance to storage at 37°C and 45°C than vaccine Q. The difference was not due to the moisture content, because the amount of residual moisture in the two preparations was about the same, vaccine Q containing slightly less than vaccine P. Vaccine Q appeared to be a relatively crude preparation which was less homogeneous when reconstituted. Some of the ampoules of this material showed slight discolouration after storage at 45°C and when reconstituted tended to sediment more quickly than samples stored at 37°C or -10°C. This may indicate some denaturation at the higher temperature. No similar macroscopic change was noticed with vaccine P.

Both dried vaccines were much more resistant to heat than any ordinary glycerinated lymph. Although it showed some fall in potency, vaccine Q still produced a 96 per cent. vaccination success rate after eight weeks at 37°C and after 16 weeks at this temperature it had only fallen to 72 per cent. This vaccine did not, however, withstand 45°C for four weeks since the success rate fell to 47 per cent. At the termination of the trial after 64 weeks, vaccine P showed a definite deterioration in potency by laboratory tests at both 37°C and 45°C but it was still sufficiently potent to produce 100 per cent. vaccination success rates. It is difficult therefore to set a limit to the life of vaccine P under tropical conditions. Seven other batches of dried vaccine prepared by the same method as vaccine P were stored at 45°C for eight weeks and each was then used to vaccinate about 27 men. All seven gave 100 per cent. success rates, indicating that vaccine P was not exceptionally heat resistant. If stored in an ordinary refrigerator at about +4°C both dried vaccines should retain their potency indefinitely.

SUMMARY

In a vaccination and laboratory study two dried vaccines were tested at intervals of 4, 8, 16 and 32 weeks after storage at both 37°C and 45°C. One of them was tested after 64 weeks at these temperatures; this vaccine (vaccine P) gave 100 per cent. successful vaccination rates after all periods of storage at both temperatures. The other (vaccine Q) deteriorated within four weeks rapidly at 45°C and less rapidly, but very substantially at 37°C. There was no clear evidence of the cause of this deterioration, but there was a suggestion of denaturation of some of the samples stored at the higher temperature.

The laboratory results - rabbit skin scarification tests and chorio-allantoic membrane pock counts - ran parallel as far as could be ascertained with the vaccination success rates. The pock count was found to be the more accurate method of laboratory titration. Statistical analysis of the results was easier with the pock count than with the rabbit scarification test. Seven other batches of vaccine prepared in the same way as vaccine P also gave 100 per cent. vaccination success rates after storage for eight weeks at 45°C so that vaccine P used in the trial was not an exceptional batch.

Vaccines which gave a pock count of 10^8 infective units per ml will give the highest possible rate of successful primary vaccinations.

REFERENCE

Finney, D. J. (1952) Statistical Methods in Biological Assay, London.

STATISTICAL NOTE ON THE TRIAL OF SMALLPOX VACCINE

by

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The probit analysis of these data is only approximate owing to the errors in the pock counts and the problem really involves fitting a regression line in which both variables are subject to error. The usual analysis, which assumes that the dose is accurately known, gives a reasonable graduation of the observed percentage "takes" but differs significantly according to the X^2 test. The equation relating probit of percentage "takes" to the log of the mean pock counts from the two laboratories is:

$$Y = 1.42X - 2.77$$

where

Y = probit of % "takes"
X = log mean pock counts

This curve gives a rough estimate of the percentage "takes" to be expected when the pock counts are determined with about the same accuracy as those of the present trial. It must be emphasized, however, that more accurate counts would probably give a steeper response curve and less accurate counts a flatter one. The mathematical reason for this is that the slope of the probit-log dose curve is equal to the reciprocal of the standard deviation of the distribution of susceptibility in the population under test. The presence of an inaccuracy of counting adds a further component of variability so that if σ_1^2 is the variance of susceptibility and σ_2^2 is the variance of counting, the slope of the curve becomes $1/\sqrt{\sigma_1^2 + \sigma_2^2}$ instead of $1/\sigma_1$ while the 50 per cent. point is unaltered. Formally, if the nominal dose K has a variance σ_2^2, and the distribution of susceptibility has a mean μ and variance σ_1^2, then the average response at a nominal dose K is given by the integral:

$$R = \int_{-\infty}^{+\infty} e^{-\frac{(x-K)^2}{\sigma_2^2}} \frac{dx}{\sigma_2\sqrt{2\pi}} \int_{-\infty}^{\frac{x-\mu}{\sigma_1}} e^{-\frac{1}{2}t^2} \frac{dt}{\sqrt{2\pi}}$$

$$= \int_{-\infty}^{\frac{K-\mu}{\sqrt{\sigma_1^2+\sigma_2^2}}} e^{-\frac{1}{2}t^2} \frac{dt}{\sqrt{2\pi}}$$

In individual experiments there may be deviations from the probit relationship indicated by this integral, but these will not be systematic and on the average a linear relation between probit and log-dose should hold.

The log mean pock counts of both vaccines were analysed to test the significance of the decline in pock count with time and also the relative rates of decline at 37°C and 45°C. The method used was to fit regression lines to the results at 45° and 37° relating log mean pock count to time in such a way that both lines passed through a common count at the beginning of the experiment. The mathematical technique was to fit a multiple regression of the form

$$y = a + b_1 x_1 + b_2 x_2$$

where

y = log pock count

a = the estimated initial count

and x_1 and x_2 are dummy variables such that x_1 refers only to preservation times at one temperature and x_2 to the other. The constants b_1 and b_2 are the decay constants at the two temperatures.

The equations relating log pock count to preservation time for vaccine P were found to be:

$$Y_{45} = 8.63 - 0.0085\ x_1$$

$$Y_{37} = 8.63 - 0.0026\ x_2$$

The regression at 37°C is not statistically significant but at 45° is highly significant, and greater than that at 37°. The analysis of variance was as follows:

	S.S.		Mean sq.
Regression	0.2545	2	0.1272
Residual	0.0753	8	0.00941
Total	0.3298	10	

An exactly similar analysis was made on the results for vaccine Q for which the regression equations were:

$$Y_{45} = 7.13 - 0.311\ x_1$$

$$Y_{37} = 7.13 - 0.072\ x_2$$

Both these regression coefficients are highly significant and differ significantly from one another. The corresponding analysis of variance was:

	S.S.		Mean sq.
Regression	5.5162	2	2.7581
Residual	0.22566	4	0.06415
Total	5.7728	6	

After about eight weeks vaccine Q deteriorated much more rapidly than would be expected from the calculated straight line, and the counts at 16 weeks have not been included in the analysis. Apart from this the regressions give a very good fit to the observed results.

Using the fitted curves it is possible to make an estimate of the ideal probit-log count relationship. If it can be assumed that the deviations of the observed counts from the fitted lines is due almost entirely to errors in the pock counts and hardly at all to random variations in survival, then the "true" log counts can be calculated from the equations of the lines. The probits of the observed percentage "takes" can then be related to these counts by the usual method. The probit line obtained in this way should have a steeper slope than the uncorrected line, while the position of the 50 per cent. should be more or less unaffected. This is in fact found to be the case, its equation being:

$$Y = 1.605\,X - 4.002$$

The fit measured by X^2 is greatly improved ($X^2 = 2.5$ on 3 degrees of freedom) and justifies the use of the rough probit line as a graduation of the curve relating percentage "takes" to the uncorrected counts. The reciprocal of the slope of the ideal probit line provides an estimate of the standard deviation of the distribution of susceptibility to the vaccine, so that $\sigma_1 = 1/1.605 = 0.623$. The uncorrected probit line has a slope of 1.42 and theoretically

$$1.42 = 1 \Big/ \sqrt{(0.623)^2 + \sigma_2^2}$$

whence the error of counting is estimated to be $\sigma_2^2 = 0.118$, which does not differ significantly from the estimate 0.064 which occurs in the analysis of variance table for vaccine Q. It should be noted that the variance of the counts for vaccine P is significantly less than those on vaccine Q. This is probably due to the physical nature of vaccine Q which makes it more difficult to reconstitute than vaccine P.

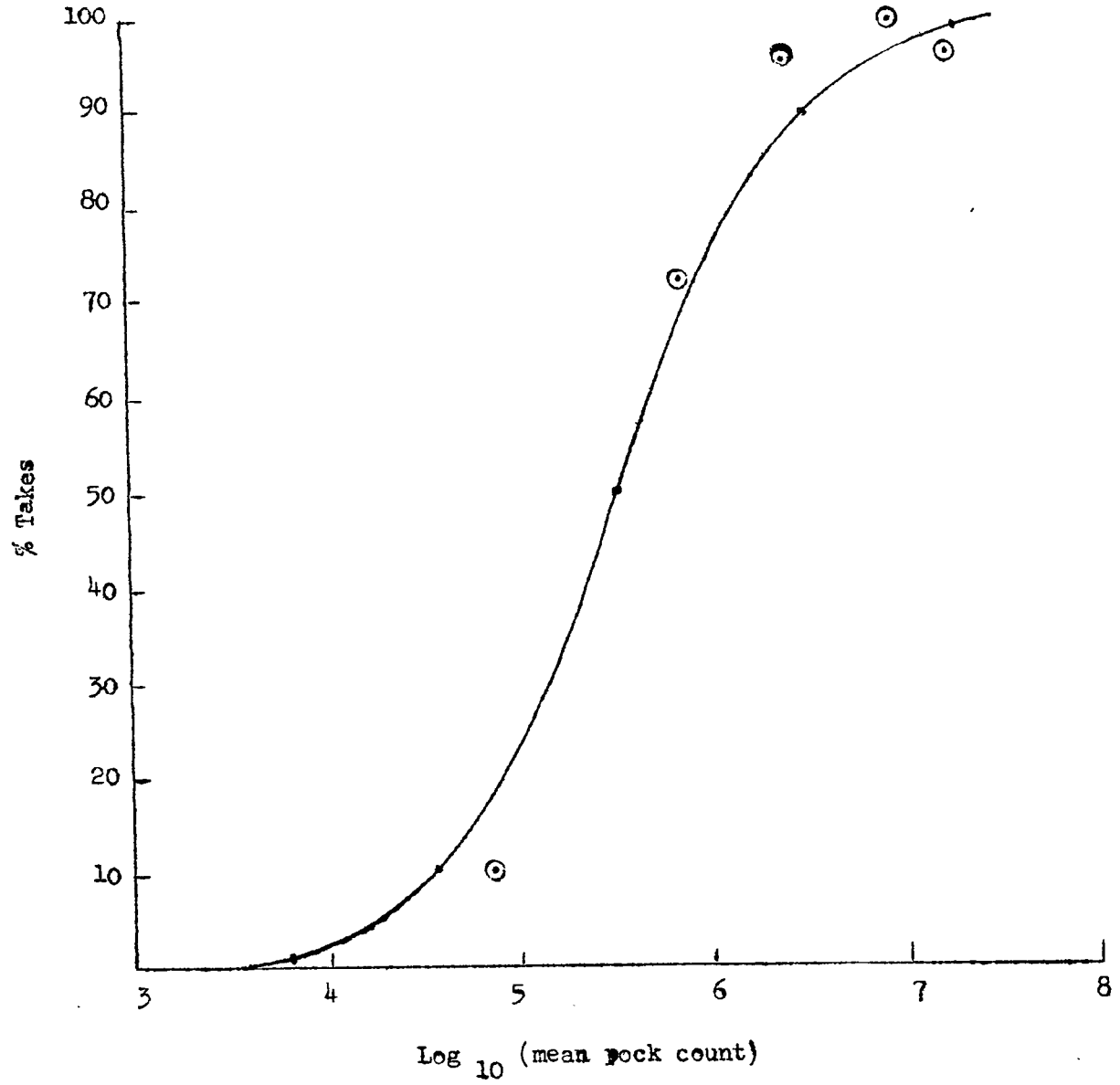
The analysis reported above is necessarily approximate and the suggested probit curve relating pock count to percentage "takes" is only a rough guide which may be useful in any future studies. If more or less accurate counts are available the estimated ideal curve would be more reliable if used in the way indicated.

An alternative method of analysis is in terms of the individual log pock counts. This gives essentially similar results, but a more elaborate analysis of variance is possible which indicates that the decline in pock count of vaccine P at 37° is statistically significant. In addition the line relating the mean log pock counts to probit of percentage "takes" has a steeper slope and one which is much closer to that of the ideal curve. The equation of this line is:

$$Y = 1.67\ X - 4.17$$

The estimate of the error of counting to which this slope leads is $\sigma_2 = 0.03$ which again does not differ significantly from the observed value but in this case is rather lower. It was thought best to give a fuller account of the analysis which demonstrates most clearly the effect of errors of counting, so as not to underestimate the disturbances likely to be met in future trials. Both analyses indicate that vaccines with pock counts of about 10^8 will give 1/1000 or less failures, while counts of about 10^9 are virtually 100 per cent. effective.

Fig. I


100
90
80
70
60
50
40
30
20
10
% Takes
3
4
5
6
7
8
Log 10 (mean pock count)