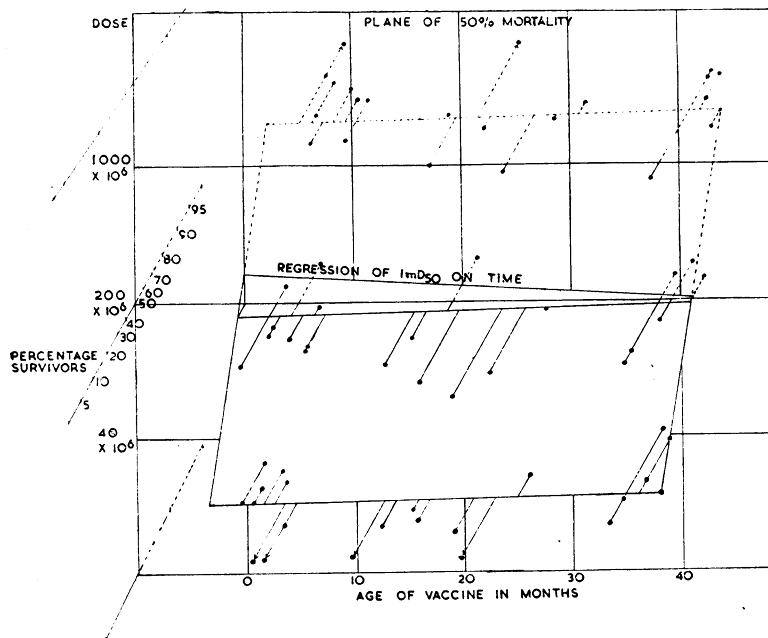

Graph showing dose and age of vaccine, and proportion of mice surviving.

We have observed no deterioration of certain vaccines, and our finding agrees with that reported by Kendrick, Eldering, Hornbeck, and Baker (1955). In particular we have records relating to one vaccine, BIV, stored at 4° C. over a period of three years. In this paper we assess the highest rate of deterioration for this vaccine to be legitimately inferred from mouse-protection tests. The supply of BIV being now exhausted, no further tests are possible with it.

Statistical Note

In Table I are shown all the available results from 22 sets of three doses as the number of survivors out of the total in each group of mice obtained over a period of 41 months; Table II gives the ImD_{50} values. A plane was fitted to log dose, time, and probit of percentage survivors with a single cycle of weighting (Finney, 1947). The regression equation for log dose and time on probits was calculated. The ratio of the two regression coefficients was used as a measure of the rate of deterioration. Heterogeneity was found, as was expected (Ungar and Basil, 1957), and provided for. The lower fiducial limits of this ratio (Fieller, 1944) were calculated for P=0.05 and P=0.01 (single tail) and expressed as log units per annum and half-life in years. The procedure is illustrated in the graph. This is a projected graph in which the dose of vaccine, the age of the vaccine, and the proportion of mice surviving are plotted at right angles. The 66 observed values are marked as solid circles, and for each the deviation from the fitted plane is marked by a line. The plane between dose of vaccine and age of vaccine is also shown at the 50% level. The two planes, the fitted one and the one between dose and age of vaccine, intersect in a line that is the regression of the ImD_{50} dose of vaccine on the age of the vaccine. The slope of this line measures the rate of deterioration.

Results

The deterioration as determined above is not different from zero. Indeed, the sample value indicates a slight increase in activity. Its lower fiducial limit (P=0.05) is 0.05 log unit, or a half-life of 5.76 years. At P=0.01, the lower limit is 0.09 log unit, or a half-life of 3.23 years. Although, as can be seen, an appreciable deterioration may have occurred, the limiting value found is much smaller than the one given in the Whooping-cough Committee's report.

Discussion

The reasons for the deterioration of the vaccine, as given in that report, are a matter of speculation. One could lie in the method of preparing the vaccine—namely, incomplete killing of the organisms and destruction of enzymes. It is known that certain chemicals will act as bacteriostatic or slow bactericidal agents—for example, organic mercurial compounds—without at the same time inactivating proteases. The enzymes can in the course of months affect the antigenicity of the prophylactic, particularly if it happens to have been kept at room temperature.

Another explanation of the alleged deterioration may be found in the assay method. The mice used might vary with time in their response to the antigen, unless precautions are taken to use a specially uniform strain of mice and a stable standard is available for comparison. Further, the challenge strain of *Haemophilus pertussis* used in the method may vary in virulence. Finally, the possible effect of changes in the diet of the animals must be kept in mind.

Summary

The mouse-protection test has been used to show that *H. pertussis* vaccine stored at 4° C. does not deteriorate appreciably and that there could be a half-life of 5.76 years (P=0.05) or, at worst (P=0.01), of 3.23 years.

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BRITISH STANDARD FOR PERTUSSIS VACCINE: ITS USE IN ROUTINE CONTROL OF COMMERCIAL VACCINES

BY

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Pertussis vaccine has been used in this country for a number of years on a fairly wide scale. Control of the vaccine for safety and potency has been carried out in the usual way through the Regulations made under the Therapeutic Substances Act and through the monograph on the vaccine in the *British Pharmacopoeia*. Up to the present time the control measures have not included any direct test for potency, and indirect methods depending upon opacity, etc., have been the only ones applied. In recent years several large-scale field trials of the protective power of pertussis vaccine have been organized by the Medical Research Council. All vaccines used in

these trials have been tested for potency by the mouse-protection method (W.H.O., 1953) and some have also been tested by an agglutinin test (Evans and Perkins, 1953, 1954).

It was generally believed that the former was a valid method of assessing the protective power of vaccine in children, and, in fact, vaccine used in other countries, notably the United States, has for some time been officially controlled by use of this method. There was, however, little direct evidence that the results obtained in the mouse-protection test were correlated with the protective power of the vaccine in children until the publication of a further report on the British field trials (Medical Research Council, 1956, referred to below as the M.R.C. report). In giving this evidence the authors of the report recommended that only vaccines so tested should be used in this country. It is the purpose of this paper to examine the practicability of this general recommendation.

Like all bioassay methods the mouse-protection test is essentially comparative, and depends upon the determination of the ratio between some dose of the batch under test and that dose of a standard preparation giving an equal protection. The need for a standard was recognized at an early stage of the M.R.C. trials, and a particular batch of vaccine was set aside for this purpose. Unfortunately, at a much later stage, when the analysis of the assays was completed, it became apparent that this standard batch, and many of the other vaccines, might, on storage in the fluid state for several years, have lost a good deal of their antigenic potency (M.R.C., 1956, p. 459). For any one vaccine the dose required to produce a given protective effect in mice appeared to increase at a fairly constant rate over a period of four years. If this trend was assumed to be due to a steady rate of deterioration of the vaccine, it was possible to allow for this and to estimate the actual potency of all vaccines used in the trial (M.R.C., 1956, p. 461). Nevertheless the apparent instability of fluid vaccine made it wholly unsuitable for use as a standard preparation, and in May, 1953, it was decided to investigate the possibility of using freeze-dried vaccine as a standard.

The M.R.C. Whooping-cough Committee, acting in conjunction with the Department of Biological Standards of the National Institute for Medical Research, set up a subcommittee* to organize these studies. Preliminary experiments showed that there was little practical difficulty in freeze-drying the vaccine, that there might be a loss of antigenic potency during drying, and that 6% dextran added to the suspending fluid of the vaccine might help to protect the antigen during the drying process.

Preparation of the British Standard

It was decided to make the British Standard, if possible, from a batch of vaccine which, in the fluid state, had been used in the field trial and found to have good protective power in children. Accordingly, in 1954, a vaccine (V 12) was selected and a supply of 4.3 litres was made available by the generosity of the Glaxo Laboratories. This vaccine had been manufactured in 1951, used in the field study during 1952, and was consequently nearly three years old at the time of drying. The vaccine was of the usual strength for field use—namely, with an estimated 20,000 million organisms per ml.—but, with the help of Dr. J. Ungar, it was concentrated 2½-fold—that is, to a concentration of 50,000 million organisms per ml.; this final concentrate also contained 6% dextran and 1 in 7,500 parts of thiomersal.

The concentrated solution was dispensed by accurate volumetric methods in 1-ml. amounts in ampoules. The ampoules were placed in a freeze-drying apparatus and the solution lyophilized. The ampoules were then filled with pure dry nitrogen and sealed. A sample of the original V 12 vaccine was retained for further study and was held as a fluid at 4° C.

Ampoules of the dried material were then selected at random for the tests to be described, and the remainder have been stored ever since at −10° C.

Effect of Drying on Potency of Standard

Ampoules of the standard were reconstituted with distilled water to make a solution containing 10,000 million organisms per ml. The reconstituted standard was then compared by the mouse-protection test with the starting material. The results in Table I were obtained in three

TABLE I.—*Estimates of Potency-ratio of Dried Vaccine Against Original V 12 Vaccine*

Laboratory	No. of Assays	Pooled Value of Log Potency Ratio (With 95% Fiducial Limits)
1	6	−0·041 (−0·223 to 0·141)
2	8	−0·508* (−0·808 ,, −0·207)
3	4	−0·396 (−0·780 ,, −0·011)
Pooled value for laboratories 1 and 3		−0·173 (−0·318 ,, −0·038)
Antilog = estimated potency		0·67 (0·48 ,, 0·94)

* Results from individual assays differ significantly at 1% level.

different laboratories—the Glaxo Laboratories, the Wellcome Research Laboratories, and the National Institutes of Health, Bethesda, U.S.A. The results from the eight assays performed in Laboratory 2 were discrepant from each other, and the pooled result from this laboratory differed significantly from the other two. The best procedure seemed to be to omit these particular results. The pooled results from the other two laboratories gave an estimated log potency after drying of −0.173, with a standard error of 0.074. Thus (taking limits of twice the standard error) the antigenicity appears to have dropped during drying by between 6 and 52%. Loss of potency during drying, provided its degree is known with fair accuracy, is not a serious problem, since due allowance can be made for it in deciding what potency, in terms of the standard, should be required for fresh batches.

Stability of Standard

Of much greater importance than the loss of potency during drying is the stability of the standard. Steps were accordingly taken to check as far as possible that no further loss of potency need be expected, once the standard had been dried.

Methods of checking the stability of standard preparations are necessarily all indirect, and the various possibilities have been discussed by Jerne and Perry (1956). The method chosen for this study was the accelerated degradation test. Randomly selected ampoules of the standard were divided into three groups which were stored at −10°, 22°, and 37° C. respectively. At the end of two months' storage, one-half of the ampoules in each group was removed from the storage boxes; the rest of the ampoules were left in storage at these temperatures for 24 months in all.

Each of these sets of ampoules was tested in the same way—namely, by comparing, in mouse-protection tests, the potency of those stored at 22° and 37° C. with those stored at −10° C. The assays were done in three separate laboratories on the two-month samples and in two laboratories on the 24-month samples. The laboratories taking part were the Glaxo Laboratories, the Wellcome Research Laboratories, and the Lister Institute. The results of all the assays were calculated by standard probit analysis, sometimes modified to allow for survivors in the control groups of unvaccinated mice.

*The members of the subcommittee were: Dr. P. Armitage, Dr. W. C. Cockburn, Dr. D. G. Evans, Dr. J. O. Irwin, Dr. J. Knowelden, Dr. W. L. M. Perry (chairman), and Mr. A. F. B. Standfast.

The results of the two-month assays are given in Table II, and those of the 24-month assays in Table III.

TABLE II.—*Estimates of Potency-ratio of Standard Vaccine Stored for 2 Months at 22° and 37° C., in Terms of That Stored at −10° C.*

Laboratory	No. of Assays	Pooled Value of Log Potency-ratio (With 95% Fiducial Limits)	
		22° v. −10°	37° v. −10°
1	4	0·132 (−0·091 to 0·354)	0·162 (−0·063 to 0·387)
2	4	0·003* (−0·862 ,, 0·868)	−0·065* (−1·116 ,, 0·986)
4	7	−0·019 (−0·308 ,, 0·271)	−0·092† (−0·611 ,, 0·426)
Pooled value for three laboratories		0·069 (−0·098 ,, 0·236)	0·090 (−0·099 ,, 0·279)
Antilog = estimated potency		*1·17 (0·80 ,, 1·72)*	*1·23 (0·80 ,, 1·90)*

* Results for individual assays differ significantly at 0·1% level.
† Results for individual assays differ significantly at 5% level.

TABLE III.—*Estimates of Potency-ratio of Standard Vaccine Stored for 24 Months at 22° and 37° C. in Terms of That Stored at −10° C.*

Laboratory	No. of Assays	Pooled Value of Log Potency-ratio (With 95% Fiducial Limits)	
		22° v. −10°	37° v. −10°
1	6	0·138 (−0·047 to 0·323)	−0·201* (−0·824 to 0·421)
4	8	0·012 (−0·163 ,, 0·188)	−0·017 (−0·190 ,, 0·156)
Pooled value for both laboratories		0·072 (−0·056 ,, 0·199)	−0·049 (−0·206 ,, 0·109)
Antilog = estimated potency		*1·18 (0·88 ,, 1·58)*	*0·89 (0·62 ,, 1·29)*

* Results for individual assays differ significantly at 5% level.

There seems to be some inconsistency between repeated tests in the same laboratory (Labs. 2 and 4, Table II; and Lab. 1, Table III). This heterogeneity is allowed for when results from different laboratories are combined. The results from all laboratories then agree satisfactorily when tested by the χ^2 method, and the pooled estimates in all cases are not significantly different from unity, the fiducial range in all cases including unity. For the two-month assays both estimates are greater than unity.

The tests provide, therefore, no evidence that storage at 22° or 37° C. for up to 24 months caused a greater loss of potency than storage at −10° C. for the same length of time. The limits of error are wide enough to permit the data to be consistent with a loss of up to 20% of the potency in two months. That this rate of loss did not continue for 24 months is quite clear, but there might have been a loss of up to 40% in the longer period. But if there had been any appreciable loss at −10° C., the loss at 37° C. over 24 months would have been enormous. It therefore seems reasonable to suppose that when stored at −10° C. the standard is stable enough to allow its legitimate use without correction for loss of potency.

Use of Standard

Instructions to Users.—The ampoule of dried standard should be carefully opened and exactly 5 ml. of distilled water added. Great care should be taken to ensure that the dried powder suspends completely before use. The suspension thus made is estimated to contain 10,000 million organisms per ml.

Routine Use of Standard in Control of Commercial Vaccines.—Final decisions on the methods used for the control of commercially prepared pertussis vaccines lie in the hands of the authorities responsible for drafting Regulations under the Therapeutic Substances Act and for monographs of the *British Pharmacopoeia.* Our present purpose is merely to outline the purpose and practicability of such control. The control measures, whatever they may be, must take account of two things: (1) the need to ensure that material is, on the average, at least as potent as vaccine known to have been effective in the field trials; and (2) the need to ensure that as few batches as possible of material as low in potency as vaccine known to have been ineffective in the field trial ever pass the test.

Relation of Potency of Standard to Field Results

The question of the relationship between the results of field trials and those of mouse-protection tests raises the problem of estimating the deterioration (if any) of fluid vaccines, and we shall first make the same assumption as in the M.R.C. report—that all fluid vaccines considered there lost potency at a rate of 0.2 log units a year over the period 1947–51, during which the laboratory tests reported there were performed.

In Table XII of the M.R.C. report, all vaccines were calibrated by the mouse-protection test in terms of vaccine G 61 as tested in the early months of 1947. If all the vaccines used in the field trials are divided into two groups —those with a log potency (in terms of G 61 in 1947) above and below −0.25 (which we call R)—we find that those with a potency greater than R had home-exposure attack rates varying from 4% to 30%; those with a potency less than R had attack rates varying from 29% to 87%. This means that the value R is a fairly good index of discrimination between apparently acceptable and apparently unacceptable vaccine.

We must next try to determine the value of R in terms of the potency of the standard, which means trying to evaluate the log potency of the standard relative to vaccine G 61 in 1647.0.* If we call this m, then $m = x - y + z$, where

x = log potency of standard in terms of V 12 in 1954.75 (when tested together—see above);

y = amount of deterioration (in logs) of V 12 between 1952.4 and 1954.75;

z = log potency of V 12 in 1952.4 (when tested in the field) in terms of G 61 in 1947.0.

The estimated value of z is obtained from the data included in the M.R.C. report (Table XII), and is −0.150 ± 0.147. The estimated value of x is that quoted in a previous section of this paper—namely, the result of the test done to determine whether there was any loss of potency of the material during drying (Table I); the figure is −0.173 ± 0.074. To estimate y we have examined the trend in the dose required to protect 50% of mice (ImD_{50}) in a number of experiments with V 12. The estimated change in log potency over the relevant period of 2.35 years is 0.045 ± 0.148. Since an *increase* in potency is unlikely to have occurred it will be safest to take a figure of 0 ± 0.148.

Hence $m = -0.173 - 0 - 0.150 = -0.323$;

Standard error of $m = \sqrt{((0.074)^2 + (0.148)^2 + (0.147)^2)}$ + 0.221;

95% fiducial limits for m are $-0.323 \pm (1.96)(0.221) = -0.756$ and 0.110.

We can, on this basis, express the desirable level, R, as a multiple of the standard (Table IV). The lower limit for m, of course, determines the *upper* limit for the "desirable" level.

TABLE IV.—*Desirable Level for Potency in Terms of Standard, With 95% Fiducial Limits, on Assumption that Vaccine Referred to in M.R.C. Report Deteriorated at Rate of* 0.2 *log a Year*

Assumed Value of m (Logs, in Terms of G 61 in 1947·0)	Desirable Level, R = −0·25, as a Multiple of Standard, i.e. (−0·25 −m)	
	Log Scale	Antilog
Upper limit, 0·110	Lower limit, −0·36	0·44
Estimate, −0·323	Estimate, 0·07	1·2
Lower limit, −0·756	Upper limit, 0·50	3·2

The weakest link in the above argument seems to be the assumption that during the period 1947–51 the potency of the vaccines declined at the same rate, 0.2 log a year. This seemed to the authors of the M.R.C. report to be

*The decimal indicates a proportion of the way through a whole year—e.g., 1952.4 means 0.4 of the way through 1952.

a reasonable assumption to make, since the $ImD_{50}s$ of a number of vaccines increased fairly consistently at this rate. However, an examination which we have carried out of more recent results of mouse-protection tests performed in laboratories on these and other vaccines suggests little or no loss of potency over a period of several years, a conclusion confirmed by Kendrick *et al.* (1955). It therefore seems quite possible that the previously observed trends in $ImD_{50}s$ were due to temporary fluctuations in the behaviour of the mice rather than to deterioration of the vaccines. We shall now consider an alternative (and presumably extreme) assumption that fluid vaccines do not deteriorate at all. This assumption could not invalidate the general conclusion of the M.R.C. report that field and mouse results are related (as may be seen by plotting the home-exposure attack rates from Table XII of the M.R.C. report against the uncorrected log potency-ratios in terms of G 61 given in Table IX of that report). But it does affect the relative potencies of different vaccines.

In Table IX of the M.R.C. report, uncorrected potencies are expressed in terms of G 61. A reasonable value for R′, the desirable level in terms of G 61, appears to be 0.25. Vaccines with a potency greater than R′ had home-exposure attack rates varying from 4% to 29% ; those with a potency less than R′ had attack rates varying from 30% to 87%. It will be seen that R′ provides almost exactly the same discrimination between vaccines as did the level R on the previous assumption. If we denote by m' the value of R′ in terms of the standard, then $m' = x + y'$, where

x=log potency of standard in terms of V 12 (as before);

y'=log potency of V 12 in terms of G 61.

As before, $x = -0.173 \pm 0.074$. The estimated value of y' (cf. Table IX of the M.R.C. report) is 0.928 ± 0.147.

Hence $m = -0.173 + 0.928 = 0.755$;

Standard error of $m = \surd\ (0.074)^2 + (0.147)^2) = 0.165$;

95% fiducial limits for m are $0.755 \pm (0.96)\ (0.165) =$ 0.432 and 1.078.

We can now express the desirable level, R′, as a multiple of the standard (Table V).

TABLE V.—*Desirable Level for Potency in Terms of Standards, With 95% Fiducial Limits, on Assumption that Vaccines Referred to in M.R.C. Report did not Deteriorate*

Assumed Value of m' (Logs in Terms of G 61)	Desirable Level, R′=0·25, as a Multiple of Standard, i.e. (0·25−m')	
	Log Scale	Antilog
Upper limit, 1·078	Lower limit, −0·83	0·15
Estimate, 0·755	Estimate, − 0·50	0·32
Lower limit, 0·432	Upper limit, −0·18	0·66

A comparison of Tables IV and V shows that the two different assumptions about the possible deterioration of the vaccines referred to in the M.R.C. report considerably affect the estimate of the "desirable" potency in terms of the standard. The two estimates are 0.3 and 1.2, and the upper and lower limits given in these tables show that either estimate might well be inaccurate by a factor of two in either direction. Nevertheless, it is upon these figures that any control requirements must be based. There is no way of determining which of the two estimates is correct, since they depend upon alternative interpretations of past experiments which cannot be repeated. The choice must therefore be arbitrary, and we do not wish to embarrass the legal authorities who will make it. Consequently, we propose to discuss the implications of both choices.

Precision of Assay Methods

Having decided upon the level of potency which it is desirable to ensure, we must then discover whether the available assay methods are precise enough to enable us to attain such a level with any degree of certainty and regularity. The precision of the mouse-protection test is not great, and depends to a very large extent upon the slope of the dosage-response line.

The stability tests of the standard, described above, provided an opportunity of determining again the value of this slope, under conditions of reasonable accuracy. The mean values for the three laboratories were 1.1, 1.3, and 1.4 (probits/log dose). Irwin and Standfast (1957) report a slope of 0.8 in assays performed some years ago; the more recent values indicate an improved precision since then, and go far toward making it possible to introduce laboratory control of the potency of the vaccine.

Previous assays have usually been carried out at three-dose levels of vaccine, using 15 mice per dose (a total of 90 plus uninoculated controls). In Table VI we show the limits of error of an assay of this design when the slope has the values 0.8, 1.1, and 1.4 respectively. When the slope is 0.8, a total of eight complete tests (720 mice) are required to obtain limits of error of roughly 50% to 200%. When the slope is 1.4 comparable limits of error are obtained with only two tests (180 mice).

TABLE VI.—*Limits of Accuracy Achieved in Tests with Three Fivefold Doses on Each of Two Vaccines, with* 15 *Animals per Dose. The* 95% *Fiducial Limits are Expressed as Percentages of the Estimated Potency*

	Slope 0·8	Slope 1·1	Slope 1·4
% Survivals:			
(*a*)	(29, 50, 71) (29, 50, 71)	(22, 50, 78) (22, 50, 78)	(16, 50, 84) (16, 50, 84)
(*b*)	(20, 39, 61) (39, 61, 80)	(12, 35, 65) (35, 65, 88)	(7, 31, 69) (31, 69, 93)
1 test:			
(*a*) ..	15– 689	37–369	36–281
(*b*) ..	21–2,423	31–643	38–387
2 tests:			
(*a*) ..	30– 334	42–238	50–201
(*b*) ..	33– 568	43–313	50–238
4 tests:			
(*a*) ..	45– 225	55–182	62–162
(*b*) ..	45– 295	55–211	61–178
8 tests:			
(*a*)	57– 175	66–152	71–140
(*b*)	56– 204	65–166	67–148

The survival rates have been chosen so that their probits are exactly linearly related to the log dose. Those marked (*a*) are the same for each vaccine, and are symmetrically placed about 50%. These give the narrowest possible fiducial range for a given probit slope. Those marked (*b*) correspond to a fivefold difference in potency between the two vaccines.

It seems possible that some slight saving of animals might be effected by using a (2 plus 2) dose design with groups of 20 mice per dose (80 plus uninoculated controls per test). Table VII gives the limits of error for this design with the same values as before for the slope; these are very similar to the limits obtained using the three-dose design.

TABLE VII.—*Limits of Accuracy Achieved in Tests With Two Fivefold Doses on Each of Two Vaccines, With* 20 *Animals Per Dose. The* 95% *Fiducial Limits are Expressed as Percentages of the Estimated Potency*

	Slope 0·8	Slope 1·1	Slope 1·4
% Survivals:			
(*a*) ..	(39, 61) (39, 61)	(35, 65) (35, 65)	(31, 69) (31, 69)
(*b*) ..	(29, 50) (50, 71)	(22, 50) (50, 78)	(16, 50) (50, 84)
1 test:			
(*a*) ..	0– 109	18– 565	31– 320
(*b*) ..	0– ∞	30–1,535	37–1,393
2 tests:			
(*a*) ..	20– 492	38– 265	48– 208
(*b*) ..	31–7,614	41– 774	48– 391
4 tests:			
(*a*) ..	40– 252	53– 188	61– 164
(*b*) ..	42– 677	51– 315	58– 228
8 tests:			
(*a*) ..	55– 183	65– 154	71– 141
(*b*) ..	52– 299	61– 205	67– 170

See note to Table VI.

From the practical point of view, therefore, it is reasonable to expect a lower fiducial limit of error of 50% of the estimated potency in an assay which is not too cumbersome for routine use.

Recommendations for Routine Control

A lower fiducial limit (L.F.L.) with P = 0.95 implies that, unless a 1 in 20 chance has come off, the real potency

is greater than the value of the L.F.L. Thus, if we require that the L.F.L. should exceed the minimum potency we wish to secure, the "desirable" potency, we shall be fairly safe in assuming that accepted batches are of satisfactory quality.

However, we do not know the "desirable" potency exactly, and we shall err on the safe side if we use the upper fiducial limits (U.F.L.) given in Tables IV and V: (*a*) assuming no deterioration (Table V), the "desirable" potency has a U.F.L. of 0.66 times the standard; and (*b*) assuming deterioration to have occurred (Table IV), the "desirable" potency has a U.F.L. of 3.2 times the standard.

If we now require that the L.F.L. of the *estimated* potency in a routine assay shall exceed one of these levels —namely, 0.66 or 3.2 times the standard—we shall have a high probability that the *true* potency of the vaccine exceeds the *true* value of the "desirable" level.

Furthermore, if we require that the L.F.L. in a routine assay shall be greater than 50% of the estimated potency, then we must set the requirement for estimated potency at about 1.3 and 6.4 times the standard. These are the two extremes. The choice might fall on either, or on some figure intermediate between them. As we have seen, whatever the choice may be, it must be arbitrary, and it must lie with the legal authorities.

We can, however, conclude that a measure of control is now practicable, and we would accordingly endorse the recommendation to this effect in the M.R.C. report.

The experimental work upon which this paper is based was carried out by Dr. A. E. Francis, of the Wellcome Research Laboratories, Beckenham, Kent, U.K.; Dr. M. Pittman, of the National Institutes of Health, Bethesda 14, Maryland, U.S.A.; Dr. A. F. B. Standfast, of the Lister Institute, Elstree, U.K.; and Dr. J. Ungar, of the Glaxo Laboratories, Greenford, Middlesex, U.K. We are greatly indebted to them for undertaking the work and for allowing us to quote from it.

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SENSITIZATION TO P.A.S., STREPTOMYCIN, AND ISONIAZID

BY

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During the latter part of 1952 several articles relating to the simultaneous development of sensitivity to P.A.S. and streptomycin were published in the *British Medical Journal* (Cuthbert, 1952; Julian, 1952; Jeffery *et al.*, 1952; Macpherson, 1952). The cases described were the first of this nature to be reported.

I have seen no previous record of a patient who proved more or less simultaneously hypersensitive to P.A.S. and isoniazid and possibly also to streptomycin.

Case Report

A man aged 62 was admitted to hospital on April 22, 1955, and found to have bilateral pulmonary tuberculosis. On May 17 a course of isoniazid, 300 mg., plus P.A.S., 15 g., daily was begun. On June 16 he developed a rash which was ascribed to P.A.S. sensitivity, and the administration of this drug was discontinued. The isoniazid was continued. By June 19 the rash had greatly diminished in intensity, and streptomycin, 1 g. daily, was substituted for the P.A.S.

The patient was transferred to the County Sanatorium, Oban, on June 22. As the rash was again increasing in extent and intensity drug treatment was discontinued. By June 27 there occurred oedema of the eyelids, exfoliative rash on the face and upper part of the trunk, swelling of the forearms associated with papular rash, and punctate erythema over the lower part of the trunk.

Desquamation of the whole face, head, upper trunk, and upper limbs occurred during the ensuing three weeks.

On July 28 a test dose of one 50-mg. tablet of isoniazid was given at 10 a.m., as it was assumed that this was the least likely of the three drugs to have caused the sensitization. No reaction followed, and another tablet was given at 6 p.m. At 9.30 p.m. he complained of headache. His temperature was 100.2° F. (37.9° C.) and he had a generalized erythematous rash. During the next few days he developed oedema of the right eyelid and a papular rash on the right forearm.

On August 4 desensitization to isoniazid was begun: 12.5 mg. was given orally, the dose being increased by 12.5 mg. daily until he was receiving 200 mg.

As recrudescence and considerable worsening of the initial rash had occurred coincidentally with the beginning of streptomycin therapy, it was thought wiser to proceed with desensitization to this drug rather than risk a further recurrence of the sensitivity reaction. While the course of isoniazid was continued, 20 mg. of streptomycin in 0.06 ml. was injected intradermally on August 20 without upset. However, the dose of 40 mg. on the second day resulted in an indurated and erythematous reaction which persisted for 36 hours round the site of injection. The same dose was repeated the following day. Thereafter the daily dose was increased slowly to 1 g.

By November 27 he had completed a 90-g. course of streptomycin. Isoniazid was continued and desensitization to P.A.S. was begun, using sodium P.A.S. in the strength of 6 g. per fl. oz. (21 g. per 100 ml.). A test dose of ½ dr. (1.75 ml.) at 9 a.m. produced no reaction. Further ½-dr. (1.75-ml.) doses were given at intervals of 2½ hours, until 9.30 p.m. During the next few days the single dose was increased to ¾, 1, 1½, and 2 dr. (2.6, 3.5, 5.2, and 7 ml.), and on December 3 to 3 dr. (10.5 ml.) six times daily.

On December 4 his temperature rose to 99° F. (37.2° C.) the respiratory rate increased, the face became flushed, and a punctate erythema appeared on the trunk, upper arms, and legs, and a papulo-erythematous rash on the forearms. P.A.S. was discontinued. On December 6 he was still moderately breathless. In view of the case reported by Cuthbert (1954) an *x*-ray film of the chest was taken, but there was no evidence of Löffler's syndrome. However, there was an eosinophilia of 11%.

Because the pulmonary condition merited long-term drug treatment a further attempt at desensitization was begun on January 20, 1956, and proved successful. On this occasion the daily dose was increased more slowly.

Discussion

Allied Chemical Compounds.—Mayer (1928) noted that sensitization to substances containing an aminophenol group may be caused by that group and not by the whole molecule. He showed that the skin transforms these substances into their derivatives and that it is to these that the reaction occurs. This is more common when the amine radical is in the para position. Jeffery *et al.* (1952) reported a case of P.A.S. sensitization which developed an acute generalized skin rash when 0.5 g. of "sulphatriad" was given, and another in which patch tests were markedly positive to 1% dilutions of P.A.S., *para*-aminobenzoic acid, sulphanilamide, procaine, *para*-phenylenediamine, *meta*-aminophenol, and *para*-aminophenol.

Antibiotics.—There appears to be some slight antigenic similarity among the members of the antibiotic group. Bedford (1951) reported that a patient known to be sensitive to penicillin developed a violent reaction to chlortetracycline.