

## ROUTINE EXPERIENCE OF THE MOUSE-PROTECTION ASSAY OF PERTUSSIS VACCINE

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(With 2 Figures in the Text)

It has been pointed out by Miles (1954) that an immunization test necessarily involves 'a progressive interaction between two kinds of living organisms'. Variation thus arises in two ways, and it is often inordinately large. The mouse-protection assay for pertussis vaccine is no exception. Variation in viability of the mice and variation in virulence of the *Haemophilus pertussis* strains both contribute to the uncertainty. Irwin & Standfast (1955) have attempted to reduce this variation by litter-mate control. They concluded that what little advantage could be gained was more than offset by the extra work involved in animal management.

The test is a quantal one, since the mice either live or die. It has therefore usually been interpreted by considering the regression of probit percentage mortality on the logarithm of the dose. By this method it can be calculated from values quoted in the literature that to obtain an estimate of activity subject to a fiducial range ( $P=0.95$ ) 60–167%, at least 300 or possibly as many as 900 mice would be required. We are not aware of any publication showing that the test can be used for routine standardizations.

Available evidence indicates that the mouse-protection test (Kendrick, Eldering, Dixon & Misver, 1947) might be a useful indication of the possible antigenic value of the vaccine in children, and the M.R.C. report (1956) reveals the real value of the test. It is now widely used to determine the potency of vaccines for correlation of the mouse test with clinical results or as a routine test for production control. It is obvious that for the former purposes an estimate of activity of the greatest accuracy possible is required. The use of large numbers of mice is then fully justified. For routine manufacture, however, the object is different. A vaccine that is to be used as a general prophylactic must provide at least the required protection; but no harm will follow if excess protection is given. A minimum activity limit must be the aim and this is technically easier to establish.

### MATERIALS AND METHODS

These were described in detail by one of us (Ungar, 1952) and are here only briefly summarized. We have used Albino mice, weight 12–15 g. descended from strong  $A_2$  ancestors and continuously inbred in these laboratories ( $A_2G$ ) for thirty-five generations. Three groups of fifteen mice (regardless of sex) are used for each vaccine, and three for control. As a standard of comparison, the vaccine adopted as the British Standard Preparation is used; it contains  $20,000 \times 10^6$  organisms/ml. Each vaccine on test is diluted in 0.85% NaCl solution to contain 400, 2000 or

$10,000 \times 10^6$  bacterial cells per 1 ml., and 0.1 ml. of the diluted vaccine is injected intraperitoneally.

For challenge we have used *H. pertussis* (strain 214 E) maintained in our laboratory in a dry state. A fresh suspension is prepared by recovering the strain, from the dry state, on Bordet-Gengou medium incubated at 37° C. for 2 days. After incubation the growth is washed off with sterile saline and emulsified with the aid of glass beads. The suspension is adjusted turbidimetrically by means of a photometer to contain approximately  $5000 \times 10^6$  organisms/ml. The suspension is diluted in 50% broth saline to contain 5000, 500 or 50 organisms in 0.05 ml. All groups of mice treated with vaccine and one of the control groups are given the challenge dose of 5000 organisms per mouse in 0.05 ml. Of the remaining two control groups, one is given 500 organisms/mouse in 0.05 ml. and the other 50 organisms/mouse in 0.05 ml.

After injection of the challenge dose the mice are observed daily for 14 days. All deaths occurring before the end of 72 hr. are considered as being non-specific. Each mouse showing paralysis is counted as having died.

#### STATISTICAL ANALYSIS

The mouse test has been in use in these laboratories for several years. The period considered in this study was the year June 1954 to June 1955. Data for the dose-response curves were selected at random from a list of dates on which the assays were commenced. The analytical procedure has been that given by Finney (1947), and it was used without adjustment for the survival of unprotected mice. Such adjustment would require special justification.

#### *Regression of mortality of normal mice on dose of Haemophilus pertussis*

Fig. 1 is based on the total mortalities shown in Table 1 and shows a curve relating mortality and dose of *H. pertussis*. It is calculated on the total mortalities without provision for day-to-day changes in either the LD50 or the slope. The estimated survival rate from the challenge dose is 0.7% and agrees with the finding of one survivor in groups of fifteen mice in 10% of cases in the year's work.

Table 1. *Mortality among mice given different challenges of Haemophilus pertussis*

Dose (organisms/mouse)	Survivors/total	%
50	58/85	68
500	77/85	91
5000	88/88	100

#### *Standard curve for the protection test*

Fig. 2 is based on the total mortalities shown in Table 2. The mice were protected with doses of 40, 200 or 1000 million cells/mouse of the British Standard Preparation of pertussis vaccine and challenged with 5000 organisms/mouse. Adjustment for the day-to-day changes in the ImD50 leads to an estimate of slope of 1.54.

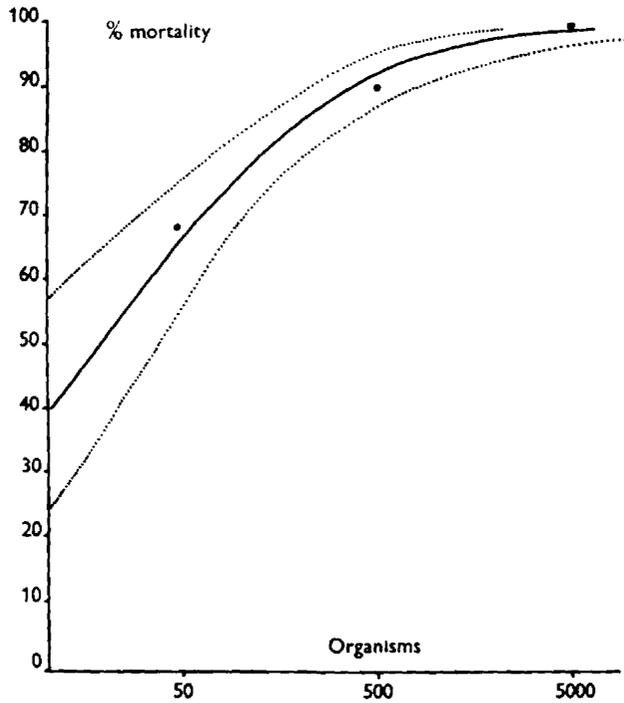


Fig. 1. Regression of mortality of normal mice on the intracerebral dose of *H. pertussis*. The solid line is the best-fitting probit response log dose line. The dotted lines are the upper and lower fiducial limits  $P = 0.95$  and the marked points the observed values.

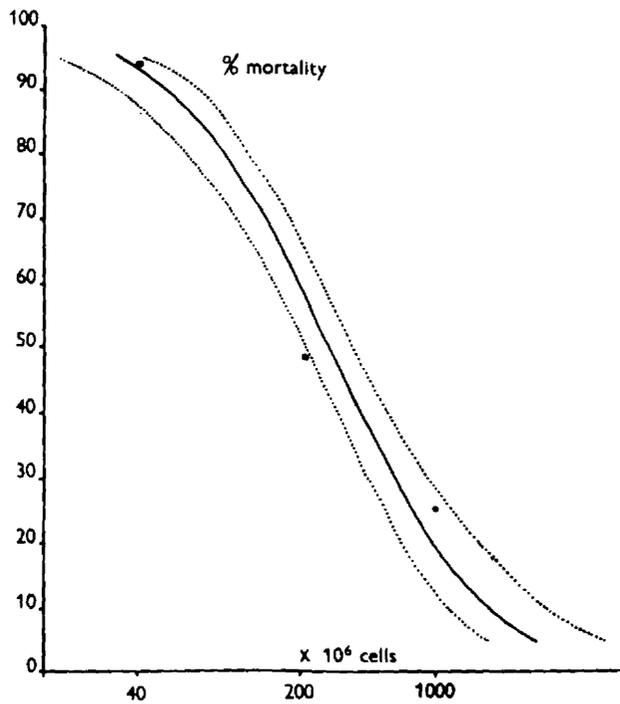


Fig. 2. Standard curve for the protection test. The mortality of mice challenged with *H. pertussis* plotted against the dose of pertussis vaccine. The solid line is the best-fitting probit response log dose line. The dotted lines are the upper and lower  $P = 0.95$  fiducial limits and the marked points the observed values.

Table 2. *Mortality among mice given different doses of Haemophilus pertussis vaccine in the protection test*

Dose (cells/mouse)	Survivors/total	%
40 × 10 <sup>6</sup>	5/85	6
200 × 10 <sup>6</sup>	41/80	51
1000 × 10 <sup>6</sup>	63/84	75

*Fiducial range of assays*

Twelve assays were selected at random from a list of dates in a period of 18 months, including the year to which the standard curves relate but not involving the same data. Table 3 shows the activities, fiducial ranges and slopes for the ten samples, one assayed in triplicate. Of these twelve assays, each using ninety mice

Table 3. *Relative activities, fiducial ranges and slopes of ten batches of pertussis vaccines*

Sample	Relative activity	Fiducial range ( <i>P</i> = 0.95)	Slope
37	0.63	0.24-1.4	1.66
39	1.1	0.44-2.8	1.89
41	5.3	1.4-50	1.09
42	1.2	0.15-21	0.85
44	0.63	0.28-1.4	2.09
BV	3.3	0.36-	0.61
B	0.55	0.18-1.5	1.42
B	0.43	0.18-0.96	1.80
B	2.1	0.35-21	1.06
T2	0.24	0.089-0.54	1.69
56	0.87	0.31-2.4	1.49
57	0.85	0.086-5.8	1.12

in a (3 + 3) dose design, six have lower limits greater than 33 % of the mean. The six remaining assays had lower regression slopes, giving wider fiducial ranges with consequent low reliability for the estimates of activity. The sample assayed in triplicate gave two good assays and a poor one. The combined results from these three are little influenced by the poor assay, as might be expected. The weighted estimate of slope from all twelve assays is 1.40, in reasonable agreement with the value quoted above for the British Standard Preparation. The combined estimate of slope from all sources is 1.47.

DISCUSSION

The dose-mortality curves of normal mice treated with *H. pertussis* show that the challenge dose has been capable, on the average, of killing 99.3 % of the mice. Making an adjustment for this 0.7 % survival would have almost no effect on the resultant estimates of ImD50 in the protection test, but not to do this involves continual wastage of all those assays in which at least one normal mouse survives the challenge dose.

The doses of vaccine 40, 200 and  $1000 \times 10^6$  were chosen at an earlier date. Though they are adequate, an increase of 50% would have had the advantage of making the middle dose nearer to the ED50.

The present tests were based on a design involving three doses of standard and three of test preparation. This has the advantage when activity is to be estimated that, should any 0 or 100% results occur, sufficient information for a reasonable assay still remains, whereas the more efficient (2+2) assay is liable to fail from the occurrence of a 0 or a 100% response. The (2+2) assay would no doubt lead to more accurate assays when the doses could be chosen with some degree of foreknowledge but, in practice, many assays might fail.

For a vaccine to be suitable for routine clinical use, it is essential to be sure that it can give the desired protection. Manifestly this cannot continue indefinitely to depend on clinical appraisal. If, as seems likely, a good correlation between clinical results and the mouse-protection test is established, it will then be possible to express activity in terms of the latter.

The manufacturer must produce a vaccine with a specified minimum activity. To achieve this he has to allow both for minor variations in manufacturing conditions and technique and also for the inherent imprecision of the biological assays. He must, therefore, aim at always producing vaccines of higher activity than the required minimum. The degree of extra activity depends on the extent to which the assays can be depended on as means of rejecting batches below the minimum. From the mean-response curves determined and the variability of the responses it can be shown that for vaccines which are in fact three times as active as the necessary minimum, one assay, or at the most two, on ninety mice should be sufficient to establish that minimum activity; with a factor as low as one and a half, assays involving the use of at least 1000 mice would be needed to achieve the same object.

#### SUMMARY

The mouse-protection test is discussed as a routine procedure. It has been shown that the assay is exceptionally subject to variation and that an average slope of 1.47 has been found over a year. With this slope and tests involving 90–180 mice it is possible to be reasonably sure that a vaccine actually 3 times as potent as needed passes a test which lays down that the lower fiducial limit ( $P=0.95$ ) should exceed the specified requirement. For a vaccine only 1.5 times as potent as needed, much more extensive laboratory work would be necessary.

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(MS. received for publication 16. II. 56)