ON THE BIOLOGICAL ASSAY OF TOXICITY OF PERTUSSIS VACCINE USING MICE*

I. SELECTION OF RESPONSE METAMETER AND REPRODUCIBILITY OF RELATIVE TOXICITY

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The present regulation on Pertussis Vaccine in Japan requires the following method for the "safety test": Each of two guinea pigs should be inoculated intraperitoneally with 5.0 cc and 5 mice 0.5 cc of a test vaccine. The animals should survive for a specified period without any pathological signs due to the vaccine, and their body weight should regain to its starting level within a specified time. This method is essentially equal to those used in many other countries.*

This type of method belongs to the so-called "minimum requirement" type in which an absolute value of toxicity is defined. The definition of an absolute toxicity in terms of animal reactions (animal units) is unsatisfactory, because the animal unit is highly variable due not only to the variation of animals, but also to that in the mean response of stocks of animals in different laboratories and at different times. Even if the assay conditions are controlled as strictly as possible, it is not possible that the experimental conditions are quite the same or different only in minor factors, therefore another source of variation occurs. Thus, when the toxicity of a vaccine is sufficiently far from the specified level, no matter whether it is above or below the level there will be no trouble. It is obvious, however, that the results of assay are variable when the difference in toxicities between a test preparation and the specified level is within the range of variation of the response in the system, as we have often experienced in the routine assay for toxicity of commercial pertussis vaccines.

In contrast to the failure to make accurate measurements in terms of animal reactions, much greater success has been attained by the introduction of a method estimat-

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This method of "safety test" detects any contaminating toxic agents as well as the toxic effects of the vaccine itself. We shall here confine ourselves to the latter only.

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ing relative potency, in which estimation of an unknown preparation is made by comparison with a standard (or a reference) preparation. The standards in a stable form, which was introduced by Ehrlich, made this comparative methods possible.

Besides, by the method of minimum requirement type neither quantitative determination of toxicity nor statistical analysis of the accuracy of the results are possible.

For these reasons we investigated the comparative method in order not only to establish a more reliable method in the "safety test" of pertussis vaccine, but also to reinvestigate the relationship between the toxicity of pertussis vaccine and the magnitude of reactions by the vaccine in human beings.

As there had never appeared reports concerning biological assay for toxicity of pertussis vaccine by the comparative method, the first step of this study was to select the response of animals or its metameter (Bacharach *et al.*, 1942) biologically reasonable and suitable for the statistical analysis. Since the final step of the study should be the comparison of results between human beings and test animals, it would be desirable to select such a response as fever, which is common and measurable both in human beings and in such an experimental animal as the rabbit. However it was considered impractical to select fever as the response because of the difficulty in using adequately large numbers of rabbits for the routine work. We have long been making assays measuring the body weight changes of animals according to the current regulation. Measuring the body weight is relatively easy in practice and a large number of mice are available for the test under more or less controlled condition. Therefore, the assay method by the body weight change in mice was first investigated.

If we find no significant correlation between the magnitude of reaction in human beings and the toxicity of vaccines estimated by the method proposed, then we will have to further search for other animal responses suitable for the purpose.

This report is concerned with the investigations on the response metameters represented by the body weight changes in mice suitable for the purpose and on the possibility of the biological assay of toxicity of pertussis vaccine.

MATERIALS AND METHODS

Vaccines: The reference vaccines were prepared from the phase I pertussis bacilli, strain S4, grown on Bordet-Gengou medium, killed with ethyl-mercuri-thio-salicylate at 4°C., suspended in 5% dextran, and freeze-dried.* Test vaccines, which were mostly commercial products submitted for the national assay, had been prepared with various strains of the phase I, grown either on Bordet Gengou media or on charcoal media.

Animals: In most cases mice of both sexes of randombred ddY were used, and sometimes randombred gpc, CF#1 or ddO or inbred SMA, A/He, or DBA/2 strains were used. At the age of about 5 weeks, a vaccine was inoculated. Five mice were caged together, fed with pellet diet and water, and weighed daily for at least 7 days before the inoculation. Those showing regular increase in weight were selected for test animals. Macroscopical investigations and microbiological examinations were carried out on the animals which died during the observation period and the surviving mice were killed and examined for the interpretation of conditions of the animals after the experiment.

Allotment of Animals: Five or six selected animals were again allotted to a cage group for one dose of a vaccine. Mice receiving different doses were never put together, because the weakening of the mice which received a higher dose would become more eminent when they are mixed with the ones which received a lower dose acting more vigorously, and thus would result in a bias in response. One dose was applied to two or more cages in order to analyse

* The production of the reference vaccines was performed by Dr. J. Kaneko at Hikari Plant, Takeda Chemical Industries.

any disturbing effect concerning cages. The allotment of mice to cages and of the cages to positions on shelves were all randomized.

Statistical analysis: The symbols and procedures in statistical analysis of the data accorded to Finney (1952 b). All significance tests were made at the probability level $p=0.05$ and all fiducial limits were determined for this probability unless otherwise stated. The criteria for fundamental conditions for valid biological assay were referred to Jerne and Wood (1949) and Finney (1952 b).

EXPERIMENTS

1. Selection of Measure or Its Metameter

A. Possible measures of the body weight change or their metameters to be investigated:

When inoculated intraperitoneally with pertussis vaccine, the body weight of a mouse generally decreases first and then increases to reach and exceeds the body weight at the time of inoculation (initial body weight), as schematically shown in Fig. 1.

Fig. 1. Schema of body weight change of mice after inoculation of pertussis vaccine

Let the weight of a mouse on the day of inoculation be A, and on each of following days B₁, B₂, ..., B_n. Analyses of the observations with a number of animals suggested that the increasing phase was practically linear (Fig. 2). A secondary decrease or little increase of body weight is occasionally observed, probably from accidents, such as diseases. The line AD shows a level of the initial weight A. Let the day when the weight exceeds the initial level be C, and the point where the line formed with body weights in the increasing phase and the initial level cross be C'. The length

Fig. 2-1. Body weight recovery line of mice.

Circles show mean BWD of mice of each strain.

Fig. 2-2. Body weight recovery lines of several strains of mice.

Strain of mice	Range of starting body weight	Regression function ($y = \text{BWD}$, $t = \text{time in days}$)
gpc	19.75–23.0 g	$y = 0.98t - 2.62$
SMA	18.25–23.0	$y = 0.73t - 2.33$
ddY	18.25–20.5	$y = 0.59t - 1.39$
DBA/2	15.50–18.0	$y = 0.53t - 3.05$
A/He	16.75–20.5	$y = 0.33t - 1.75$

AE is the time in days to the minimum weight or the maximal decrease. From the schema, the following measures of the body weight change should be investigated to select the response suitable for the purpose.

i) *Body weight difference* (BWD): Difference of the weight on the i th day, or B_i , and the starting weight A , $BWD = A - B_i$, or *Body weight ratio* (BWR): ratio of B_i to A , $BWR = B_i/A \times 100$. Ratio of B_i to A is small value and is magnified by 100 in convenience to the calculation.

ii) *Maximum difference* ($D_{\max}$) or *minimum ratio* ($R_{\min}$): Difference or ratio of the minimum weight ($B_{\min}$) and A , $D_{\max} = A - B_{\min}$, or $R_{\min} = B_{\min}/A$.

iii) *The day of maximum body weight decrease* ($T_{\min}$): The day showing $B_{\min}$, $T_{\min} = AE$.

iv) *The day of weight regain* (T_R): The number of days needed for regain to A . There are two kinds of method obtaining the value,

(a) Direct value: number of days on which the weight regained to A or exceeded that, $T_R = AC$ (in the Fig., $AC = 7$), and

(b) Indirect value: The length of abscissa in days to the crossing point of line AD and the increase line, $T_R = AC'$ (in the Fig., $AC' = 6.2$).

v) *Integrated body weight differences* (S_D): Ideally, (a) the area surrounded by the line AD and the response curve, or, practically, (b) the sum of body weight differences until the regain,

$$S_D = \sum (A - B_i),$$

where $B_i \leq A$.

B. Normality of response metameters:

The above-mentioned measures of body weight change or their metametric transformations were tested first for the normality in their frequency distribution.

One half cc of a vaccine containing 15×10^9 cells/cc was inoculated intraperitoneally into each of 112 mice, and their body weights were measured for seven days. The normality of each of the measures was examined by plotting their integrated percentage frequency on the normal-probability paper. The results could be summarized as follows:

The measure $T_{\min}$ should be discarded because only 3 values at most were available, when the measuring was made once a day.

In case of BWD or BWR, the transformed value $y = 3\sqrt{A - B_i}$ or $y = 3\sqrt{B_i/A}$ showed little departure from the normality, but with the measure itself, $y = A - B_i$ or $y = B_i/A$ only slight departure from the normality was observed. In respect that the calculation is much simpler the latter would be convenient without any practical objection.

Though either BWD or BWR on any day from the 1st to the 7th seemed to be available in respect of the normality, a tendency to decrease in the slope on the normal-probability paper was observed with the increase of days, indicating the increased variance with progress of days. This tendency was clearer with the strain gpc than with ddY (Fig. 3). It seems to be, therefore, favorable to use earlier response in order to get a higher accuracy of tests (Fig. 4). And BWR seems to be more convenient than BWD as its variance is comparable between species, strains and ages of test animals, irrespective of difference in body weight.

In respect of the normality there were little to choose among all following measures or their metameters examined,

Fig. 3-1. Test for normality of frequency distribution of BWD on normal probability paper.

Fig. 3-2. Test for normality of frequency distribution of BWR on normal probability paper.

Fig. 4. Comparison of variance between strains and measures.

$$y = \log(1 + D_{\max}) = \log(1 + A - B_{\min}), \quad y = R_{\min} = B_{\min}/A,$$

$$y = \log AC \text{ and } y = \log AC', \text{ and}$$

$$y = \log S_D.$$

As to the measures requiring logarithmic transformation, there remains a problem in treatment of the animals showing no diminution of the body weight after the inoculation of vaccine.

It could be concluded hence that many metameters, of which measuring and calculations are easier, might be chosen arbitrarily.

C. Linearity of dose response line and homoscedasticity :

The next investigations were carried out on the linearity of dose-response lines and the homoscedasticity of the measures or their metameters above.

One half cc each of two-fold dilutions of a pertussis vaccine was inoculated intraperitoneally into mice and the animals were observed for seven days. Each of the

Fig. 5-1. Test for normality of frequency distribution of T_R on normal probability paper.

Fig. 5-2. Test for normality of frequency distribution of S_D on normal probability paper.

above-mentioned measures or their metameters was tested for the linearity of dose-response line and homoscedasticity. The linearity was tested by the least-square method, and the homoscedasticity by the Bartlett's, Cochran's, or Hartley's method.

No significant departure from linearity of regression between the log dose and the response was observed whichever measure was used, as the example shown in Fig. 6. It was suggested therefore that any of the measures mentioned above was available as the response for the assay by the parallel line method within the range of doses tested.

Fig. 6. Linearity of dosage-response line (Response=BWR).

Analysis of variance for the data of Fig. 6

Variation	Sum of square	d. f.	Mean square
Regression	1276.90	1	1276.90**
Linearity	9.63	1	9.63
Error	2774.05	57	48.67
Total	4060.58	59	

** Significant at 1% level.

Here some more considerations would be needed. With pertussis vaccines, the linearity was usually seen only within the range of 4-8 times doses. With higher doses giving lethal effect the mean weight of surviving animals showed a tendency to depart from linearity. With doses lower than a threshold level the mean response was not on the regression line. Therefore, the assay should be performed with a relatively narrow range of doses, or the portion of doses giving mean response fairly close to a straight line should be employed for the analysis.

In most cases, any significant evidence against the homoscedasticity was absent, and the variances of each experiment were nearly the same. In some cases, however, unusually large variances were observed in one or two dose irrespective of the magni-

tude of dose, or a correlation between doses and variances certainly existed (Table 1). In the portion of these irregular cases pneumonia or some other pathological changes were demonstrated in most of animals, though microbiological examinations failed to detect any pathogenic agent.

Table 1. Examples of variance per dose of BWR₃

Preparation	Date	Number of mice	Dose	Variance	Strain of mice
			$\times 10^3$ cells/cc		
D-1	1957-3-20	5	15	10.50	ddY
375C	6-26	"	30	25.75	"
	"	"	7.5	2.25	"
	"	"	1.88	1.25	"
375C	7-9	"	30	103.25	"
	"	"	15	69.25	"
	"	"	7.5	11.75	"
	"	"	3.75	7.00	"
356	9-11	"	30	3.75	"
	"	"	15	3.00	"
	"	"	7.5	3.50	"
	"	"	3.75	2.75	"
	"	"	1.88	6.25	"
374C	9-11	"	30	4.75	"
	"	"	15	5.50	"
	"	"	7.5	2.75	"
	"	"	3.75	5.25	"
	"	"	1.88	6.25	"
374C	9-17	"	30	2.75	"
	"	"	15	11.25	"
	"	"	7.5	21.00	"
	"	"	3.25	9.00	"
D-III	10-11	22	15	4.75	"
	"	"	15	5.00	"
374C	9-25	30	7.5	7.34	"
O-VI	1961-1-24	20	30	77.8	"
	"	"	15	6.3	"
	"	"	7.5	9.7	"
OS1	1-24	10	85	12.2	"
	"	"	60	23.3	"
	"	"	42	8.1	"
	"	20	30	17.1	"
	"	"	15	15.5	"
	"	"	7.5	15.1	"

It was considered, therefore, that the homoscedasticity was usually realized with any of the measures mentioned above, and that, if the contrary was observed, there should exist some factors disturbing the response. It is suggested that the homoscedasticity test would be an indicator of the conditions of test animals.

From the investigations presented above it was concluded that the BWR on the 3rd day should be selected as the most practical response with which further investigations should be planned. The reasons were as follows: It was easy to measure and to calculate; the present regulation requires the observation of body weight of the animals on the 3rd day; any delay of observation would cause possible increase in disturbing effects; the ratio is more convenient than the differences as to compare with other species of animals or conditions, because the unit of the measure is eliminated.

D. Parallelism of dose-response lines of various kinds of preparations :

The fundamental assumption for a valid biological assay is that of the similarity of effective constituent(s) of the standard and the test preparations. This assumption can be tested statistically by examining the identity in form of the regressions between the dose and the response. In assays in which some function of the response is linearly related to the log of the dose, like in the present system, the parallelism of the dose-response lines is examined. With any of the above-mentioned measures, no significant departure from the parallelism of regressions of various kinds of pertussis vaccine was observed, as in Fig. 7.

Fig. 7. Parallelism of dosage-response lines and estimate of relative toxicity ($y=BWD$, BWR , $\log T_R$ and $\log S_D$)
Strain of mice: ddY, 5 mice per dose.

Estimates of relative toxicity and fiducial limits

Metameter	Relative toxicity*	Fiducial limits
BWD_2	0.33	0.13, 0.56
BWD_3	0.25	0.08, 0.44
BWR_2	0.31	0.12, 0.54
BWR_3	0.22	0.08, 0.41
$\log T_R$	0.37	0.21, 0.54
$\log S_D$	0.38	0.28, 0.79

* Relative toxicities of 374C to 356.

Analysis of variance for the data of Fig. 7.

Metameter	Nature of variation	SS	df	MS
BWD ₂	Preparations	3.6261	1	3.6261**
	Regression	5.6818	1	5.6818**
	Parallelism	0.0251	1	0.0251
	Linearity	0.0337	2	0.0169
	Error	4.4251	24	0.1844
	Total	13.7918	29	
BWD ₃	Preparations	4.6761	1	4.6761**
	Regression	4.6023	1	4.6023**
	Parallelism	0.0546	1	0.0546
	Linearity	0.0837	2	0.0419
	Error	3.5000	24	0.1458
	Total	12.9167	29	
BWR ₂	Preparations	91.27	1	91.27**
	Regression	133.10	1	133.10**
	Parallelism	1.76	1	1.76
	Linearity	0.84	2	0.42
	Error	105.20	24	4.38
	Total	332.17	29	
BWR ₃	Preparations	126.15	1	126.15**
	Regression	112.01	1	112.01**
	Parallelism	3.84	1	3.84
	Linearity	8.30	2	4.15
	Error	85.20	24	3.55
	Total	335.50	29	
log T _R	Preparations	0.2144	1	0.2144**
	Regression	0.2735	1	0.2735**
	Parallelism	0.0097	1	0.0097
	Linearity	0.0063	2	0.0063
	Error	0.1395	24	0.0058
	Total	0.6434	29	
log S _D	Preparations	0.1567	1	0.1367**
	Regression	0.3481	1	0.3481**
	Parallelism	0.0126	1	0.0126
	Linearity	0.0233	1	0.0233
	Error	0.2498	20	0.0125
	Total	2.6170	24	

** Significant at 1% level.

Table 2. Precision of estimates in terms of λ

Measure	$ \lambda = s/b$	
	1957-9-11	1961-12-7
BWD ₃	0.303	0.265
BWR ₃	0.286	0.206
S _D (a)	0.202	0.295
	(b) 0.195	
T _R indirect	0.196	0.298
	direct 0.337	

E. Precision of estimates :

Precision of estimates is a factor to be considered in making a choice of the measures or their metameters, though it is not the fundamental condition for a valid assay. In this respect comparison in terms of $\lambda=s/b$ (Gaddum, 1933) between the above-mentioned measures or their metameters was made. As shown in Table 2, where the values of λ calculated with each of the above-mentioned measures or their metameters for the data of two experiments were summarized, no certain correlation between the measure and the value of λ was demonstrated.

2. Reference Preparation for Toxicity Test of Pertussis Vaccine

Conditions necessary for a standard or a reference preparation are stability in specified potency as well as similarity in effective constituent(s) to the preparations to be tested. In addition it is also desirable for reference pertussis vaccine for toxicity test to have a relatively high toxicity so as to enable to get a regression line over as wide a range of dose as possible by inoculation with 0.5 cc or less.

After several trials, we have selected the method to suspend the killed bacilli in 5% dextran and to freeze-dry in ampules as indicated for the International Standard Preparation for Pertussis Vaccine (Potency Test) (Expert Committee, 1955). Each ampule contained 600×10^9 bacilli. The content is easily reconstituted in saline and

Each point plotted is the concentration of the vaccine giving a value BWR=100 calculated from the dose response line of each experiment.

Fig. 8. Stability of toxicity of the reference vaccine OVI.

stable at least for 2 years at 5–10°C, though giving variations in toxicity from time to time without any tendency of decrease, as in Fig. 8. After storage at 37°C for 3 months, it showed no decrease in toxicity (Fig. 9). No significant departure from the parallelism of dose-response lines between the reference and commercially manufactured vaccines has been demonstrated, as in Fig. 10.

These results seem to support the use of the proposed reference preparation in the toxicity test of pertussis vaccine by the parallel line assay.

3. Reproducibility of Relative Toxicity

The next steps of the study concerned reproducibility of the results by repeating

Strain of mice : ddY
Number of mice : 10 per dose

Fig.9. Stability of toxicity of the reference vaccine.

Nature of variation	SS	df	MS
Preparations	4.2	1	4.2
Regression	1155.6	1	1155.6**
Parallelism	0.7	1	0.7
Linearity	16.6	2	8.3
Between doses	1177.6	5	
Error	847.8	54	15.7
Total	2025.4	59	

** Significant at 1% level.

the experiments of estimating relative toxicities on several test vaccines.

In this work, two reference vaccines, OVI and OVII, manufactured by the same method at different times, were used. These two were proven to have an approximately equal toxicity as in Table 3.

The relative toxicity R was obtained by the equation (1) (Burn *et al.*, 1950; Finney, 1952) :

$$M = \log R = \bar{x}_s - \bar{x}_T - \frac{\bar{y}_s - \bar{y}_T}{b} \dots\dots\dots (1)$$

where M is the estimate of log toxicity, $\bar{x}_s$ and $\bar{x}_T$ are mean log doses of the standard and the test preparations respectively, $\bar{y}_s$ and $\bar{y}_T$ are mean responses of the standard and the test preparations respectively and b regression coefficient of y on x.

And the variance of it by the equation (2) :

$$V(M) = \frac{s^2}{(1-g)^2 b^2} \left[(1-g) \left\{ \frac{1}{N_s} - \frac{1}{N_T} \right\} + \frac{(M - \bar{x}_s + \bar{x}_T)^2}{\sum S_{xx}} \right] \dots\dots (2)$$

Fig. 10. Parallelism of dosage-response lines between the reference vaccine and commercial vaccines.

Nature of variation	SS	df	MS
Preparations	51.6	1	51.6*
Regression	819.2	1	819.2**
Parallelism	4.7	1	4.7
Linearity	18.2	3	6.1
Between doses	893.7	6	
Error	1230.4	98	12.56
Total	2124.1	104	

* Significant at 5% level.

** Significant at 1% level.

where $g = \frac{s^2 t^2}{b^2 \sum S_{xx}}$, s^2 is the estimated variance, t t-deviate with $N_s + N_T - 2$ degrees of freedom, N_s and N_T are total numbers of subjects for the standard and the test preparations respectively, and $\sum S_{xx}$ total sum of squares of deviations for x .

When this "g-criterion" was less than 0.05, $V(M)$ was calculated by (3):

$$V(M) = \frac{s^2}{b^2} \left\{ \frac{1}{N_s} + \frac{1}{N_T} + \frac{(M - \bar{x}_s + \bar{x}_T)^2}{\sum S_{xx}} \right\} \dots \dots \dots (3)$$

The repeating of tests were performed either in one laboratory at different times or in separate laboratories. Representative results are summarized in Table 4.

DISCUSSIONS

Since toxicity of pertussis vaccine is measurable only by responses of experimental animals, the toxicity expressed in terms of an absolute value would be of no use, because the value is inevitably variable from time to time in one laboratory or from

Strain of mice: ddY

Number of mice: 12 mice per dose

Nature of variation	SS	df	MS
Preparations	0.9	1	0.9
Regression	989.7	1	989.7**
Parallelism	23.6	1	23.6
Linearity	14.6	1	14.6
Between doses	1028.8	4	
Error	918.1	51	18.0
Total	1946.9	55	

** Significant at 1% level.

laboratory to laboratory, no matter how carefully the experimental conditions are controlled.

The method proposed to overcome these difficulties is to express the toxicity by the value relative to a reference having a known and stable toxicity. The method of statistical analysis for the evaluation of data from a comparative assay was given by a number of authors (Emmens, 1948; Burn, Finney and Goodwin, 1950; Finney, 1952; Bliss, 1952; Gaddum, 1953a & 1953b, and so on). This method inevitably requires for its strict validity a number of assumptions, which were discussed in the above literatures. The paper of Jerne and Wood (1949) is especially valuable.

We have studied a possibility to adopt the biological methods for reproducible, quantitative assay of toxicity of pertussis vaccine, considering the fundamental conditions for a valid assay. With any of the measures or their transformed metameters of the body weight change in mice there have been no strong evidences against either fundamental validity or statistical validity. Among these measures a measure was tentatively proposed as the appropriate response for the assay purpose, because of its simplicity in measurement and calculation, and its relationship to the current regulation.

Table 3. Comparison of toxicity of the reference vaccines OVI and OVII

Exper. No.	Date	Relative* toxicity	Fiducial limits	Strain of mice	Laboratory
1	1961-6-28	1.01	0.75, 1.37	ddY	NIH
2	" 11-21	1.02	0.82, 1.30	"	"
3		0.83	0.64, 1.04	CF#1	Tak**
4		0.76	0.56, 0.95	gpc	"

* Toxicity of OVII relative to OVI.

** Carried out by Dr. J. Kaneko at Hikari Plant, Takeda Chemical Industries.

Table 4. Reproducibility of estimates of relative toxicity and their fiducial limits

Exper. No.	Reference vaccine	Test preparation	Date	Relative* ¹⁾ toxicity	Fiducial limits	Strain of mice	Laboratory	Number of doses		Number of mice per dose
								S	T	
1-1	VI	OS1	1961- 1-24	0.26	0.20, 0.33	ddY	NIH	3	5	20* ³⁾
1-2	VI		-11-21	0.29	0.21, 0.39	"	"	5	2	12
1-3	VII		-12- 7	0.29	0.22, 0.40	"	"	3	4	12
2-1	VI	OS2	1961- 6-28	0.42	0.34, 0.52	ddY	NIH	2	4	20* ³⁾
2-2	VI		-11-21	0.42	0.31, 0.57	"	"	5	2	12
2-3	VII		-12- 7	0.38	0.28, 0.50	"	"	3	5	12
2-4	VI			(0.48)* ²⁾	(0.34, 0.66)	CF1	Tak* ⁴⁾	1	1	10
3-1	VI	D13	1961-11-21	0.63	0.48, 0.87	ddY	NIH	5	3	12
3-2	VII		-12- 7	0.36	0.24, 0.50	"	"	3	2	12
4-1	VI	D14	1961-11-21	0.40	0.28, 0.54	ddY	NIH	5	2	12
4-2	VII		-12- 7	0.46	0.32, 0.66	"	"	3	2	12
5-1	VI	9217	1961-11-21	0.54	0.40, 0.75	ddY	NIH	5	2	12
5-2	VII		-12- 7	0.57	0.41, 0.88	"	"	3	2	12
6-1	VI	40	1960- 1-28	0.70	0.44, 1.04	ddY	Bik* ⁵⁾	3	3	10
6-2	VI		- 2- 4	0.41	0.15, 0.70	ddO	"	3	2	10
7-1	VI	W	1960- 1-27	0.72	0.23, 1.52	ddY	Tos* ⁶⁾	3	2	10
7-2	VI		- 2-26	0.87	0.64, 1.11	"	"	3	2	10

*1) The value was not corrected assuming that the toxicities of the two reference vaccines were the same.

*2) The value was estimated using the regression coefficient in a separate experiment with the same strain of mice.

*3) For 3 doses from the highest of the test preparation, 10 mice were inoculated.

*4) See in Table 3.

*5) Carried out by Dr. S. Morinaga at the Research Institute for Microbial Diseases, Osaka University.

*6) Carried out by Dr. H. Hashimoto at Toshiba Institute of Biological Science.

The reproducibility of the estimates was fairly sufficient.

The data, however, seem to be insufficient to expect that the criteria for the hypothesis of similarity of the effective constituent are always fulfilled even with a vaccine prepared by quite a different manufacturing method, because our material in question is composed of many complicated constituents, the nature of which we have never recognized yet. It seems always better to try to make the assay design able to test the criteria.

The most important, however, is to inquire the direct relation of the thus expressed toxicity of pertussis vaccine to the reactions in human beings. The research concerning this point is now under way by the Research Committee including us. After this study it is expected that the toxicity level applicable to human beings without particular injury shall be able to express in terms of the toxicity of the reference preparation.

Further study to minimize the variances and enlarge the slope of dose-response lines by selecting species, strains, sexes or ages as well as conditions of feeding and keeping of animals and other experimental methods, etc. may improve still more the precision of the assay, which is under progress.

SUMMARY

The authors studied a possibility to adopt the biological assay method, based on the comparative method, for the determination of the toxicity of pertussis vaccine. The body weight change in mice after inoculating vaccines was investigated, considering the fundamental hypothesis on which a validity of the assay depends.

Some conditions for manufacturing the reference vaccine, indispensable in the biological assay, were also studied. There have been no strong evidence against the possibility, and the practicability of the parallel line assay method was obtained. Brief discussions were also made about further problems and the way to solve them.

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