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THE ASSAY OF DIPHTHERIA TOXIN

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SYNOPSIS

A precise assay of diphtheria toxin is described, based on the linear relationship between the diameter of the skin reaction to, and logarithm of the dose of, toxin. It eliminates the need for preliminary titrations, is economical, provides information about the slope of the log-dose response lines and, therefore, of the validity of the assay, and yields limits of error of potency from the internal evidence of the assay.

A study has been made of the effects of avidity, combining power, toxicity and buffering on the assay of diphtheria toxins against the International Standards for both Diphtheria Antitoxin and Schick-Test Toxin.

All the toxins assayed against the standard toxin, whatever their other properties might be, gave log-dose response lines of similar slope provided that they were diluted in buffered physiological saline. The assays were therefore valid.

These experiments were repeated concurrently in non-immune and in actively immunized guinea-pigs, and comparable figures for potency obtained in both groups. The result was not significantly affected by the avidity or combining power of the toxin.

However, non-avid toxins gave low values in Schick units when assayed, by the Römer & Sames technique, in terms of the International Standard for Diphtheria Antitoxin. The problem of the ultimate standard and the implications of these findings are discussed.

The introduction of the Schick test [14] made it possible to differentiate between persons susceptible and immune to diphtheria; those with sufficient antitoxin to neutralize one Schick dose of toxin, injected intradermally, are classified as Schick negative (immune), the remainder as Schick positive (non-immune). Control of diphtheria is based on identification and immunization of the latter. A standard Schick method, essential for the comparison of results, depends on the standardization of diphtheria (Schick) toxin.

The alternative method of detecting immunity to diphtheria is by the titration of circulating antitoxin, using the classic Römer & Sames

technique.[13] This method, in which the skin of a guinea-pig or rabbit is used to detect unneutralized toxin in antitoxin-toxin mixtures, also depends upon the use of a standard toxin.

The assay and selection of toxins for either purpose, that is to say, for use as a Schick test toxin or for the assay of antitoxin, is considered in this paper.

Current Requirements for Schick Toxin

Glenny [4] stated that the Schick test, the detection of immunity by intradermal injection of diphtheria toxin, was concerned both with toxicity of the toxin for the skin and with combining power,[a] i.e., the ability of the toxin to be neutralized by antitoxin. Toxins, like antitoxins, vary in " avidity "—a phenomenon closely related to, if not identical with, combining power. Avidity is measured by the ease with which mixtures of dilutions of antitoxin and toxin dissociate.[1]

Glenny contends that toxins with high combining power should be used for the Schick test. His arguments were accepted by the Permanent Commission on Biological Standardisation of the League of Nations.[6] They advised that:

" The Schick test dose, for injection in a volume of 0.2 c.c. shall be that quantity of toxin which, when mixed with 1/750th part or more of an international unit of diphtheria antitoxin and injected intracutaneously into a normal guinea-pig, causes no local reaction; but, when mixed with 1/1250th part or less of an international unit and similarly injected, causes a marked reaction of the type of a ' positive ' Schick reaction; *provided that* the toxin is such that 1/25th of the Schick test dose as above determined, without admixture with antitoxin, when injected intracutaneously into a normal guinea-pig, causes a definite local reaction of the type of a ' positive ' Schick reaction; but that 1/50th of the Schick test dose, similarly injected without admixture, causes no local reaction of this type."

In brief, the recommendation is that a Schick unit of toxin must be neutralized by 1/1000 unit of standard antitoxin, but wide limits are permissible. Indeed, they are so wide that their value for excluding non-avid toxin is limited.

The recommendations have been followed only by Great Britain and Canada. The *United States Pharmacopeia* (1950) accepts the traditional definition; it specifies that a Schick test dose is 1/50 of a minimum lethal dose (MLD) in 0.1 ml. But the MLD is defined as the smallest amount of toxin injected subcutaneously to kill 225-275 g of guinea-pigs, not less than 75% by the fourth day. These modifications are designed to increase

[a] Combining power can be defined as the ability of a given antitoxin and toxin to yield identical neutralization potencies on dilution, that is, the Lr/100, Lr/1000 and Lr/10 000 doses bear a simple mathematical relationship to one another when combining power is high and deviate from this relationship when it is low —i.e., *Toxin A* (high combining power): Lr/100=0.001; Lr/1000=0.0001; *Toxin B* (low combining power): Lr/100=0.001; Lr/1000=0.000 002. (The use of this definition is believed to be original.)

accuracy. The regulations of the *Pharmacopée française* (1949) and the *Pharmacopoeia of Japan* (1952) are similar. These authorities rely therefore only on tests for toxicity. But these methods are unsatisfactory. The response of guinea-pigs to diphtheria toxin is so variable that the use of the " animal unit " is inaccurate [2] (see Miles [11] for criticism of the " animal unit " as a basis for standardization). To define a Schick test dose as 1/50 of one MLD is extravagant in animals, unnecessarily inaccurate and, having no advantages that are not possessed by less cruel methods, is difficult to justify. The minimal reacting dose (MRD) is preferable to the MLD. It is economical; it eliminates animal variation because standard and unknown toxin can be assayed in the same animal; it causes little suffering.

Nevertheless, both the MLD and the MRD have the disadvantages that the end-point—death in the former, skin reaction in the latter—are influenced by many non-specific factors. The distinction between the minimal reacting dose and non-specific reaction to needle trauma and diluting fluid is frequently an esoteric one. However, there is little to justify the continued use for the assay of diphtheria toxin of an arbitrary end-point—the MRD—when a linear relationship exists between the diameter of the skin reaction and the log-dose of diphtheria toxin,[7, 10] and when an International Standard for Diphtheria Toxin is available. Measurement of distinct lesions is preferable to visual assessment of ill-defined minimal reactions. To deal with a similar problem, a precise, simple technique, economical in time and animals, was devised for the assay of tuberculin.[9] A comparable and equally satisfactory method for the assay of toxicity of Schick toxin is described in this paper. Moreover, by carrying out the assay of an unknown toxin against the International Standard for Schick-Test Toxin, in both immune and non-immune guinea-pigs, an indication of the " combining power " of the unknown toxin can be obtained under conditions analogous to those that apply to the Schick test in man.

The assay method described provides information about the slope of the log-dose response lines, yields limits of error of potency from the internal evidence of the assay, and detects loss of toxicity on dilution due either to inadequate buffering or to an unstable toxin.

Methods

Albino guinea-pigs of the Hampstead strain, weighing from 450 g to 750 g were used. In any one experiment the range in weights was less than 100 g. When necessary, they were immunized to diphtheria toxin by injection into the adductor muscles of the right leg of 1 Lf in 1 ml of physiological saline of a laboratory standard alum-precipitated diphtheria

toxoid Ba 536. After an interval of not less than one month, guinea-pigs so treated have circulating antitoxin that quantitatively and qualitatively resembles that of man.

Unless otherwise stated, dilutions of toxin were buffered with phosphate-bovine albumin, similar to that in which the International Standard for Schick-Test Toxin is dispensed (see Annex, page 549). Equally satisfactory results are obtained with borate buffer (see Annex).

All toxins were given in 0.2-ml volumes. The test toxins used were:

(1) The International Standard for Schick-Test Toxin.[a] Containing 900 International Units per ampoule; an avid toxin with good combining power.

(2) Toxin TP 2776. The current laboratory standard for diphtheria toxin, used in this Department. Toxicity, combining power and avidity, comparable with that of the International Standard, have remained constant since 1948.

(3) Toxin RX 3723. A highly avid toxin with good combining power, making it particularly satisfactory for use in the Römer & Sames technique.

(4) Toxin G 12/6. The laboratory standard toxin used by the Wright-Fleming Institute [15] both as a test toxin and for the preparation of toxoid. It proved to be an avid toxin with good combining power.

(5) Toxin 1239. For many years this was the laboratory standard toxin used in this laboratory. Its toxicity remains unchanged, but in 1948 it suddenly lost combining power (L/100 = 0.001 ml; L/1000 = 0.000 002 ml) and became non-avid.

(6) Toxin TP 2704. This toxin has always been non-avid, with poor combining power (L/100 = 0.02 ml; L/1000 = 0.0005 ml). It has remained stable for some years.

(7) Toxins TZ 3046 and TZ 3051. These might be termed average toxins with fair avidity and combining power. They differ slightly in their ratios of toxicity to combining power.

The details of the method of assay are given in full in the Annex. The minimal reacting dose (MRD) is defined in the 1955 Addendum to the *British Pharmacopoeia* as the " smallest quantity of toxin which, when injected intracutaneously into guinea-pigs causes a small characteristic reaction at the site of injection ". The MRD provides the end-point for the assay of antitoxin by the Römer & Sames technique; its definition is therefore a matter of importance, particularly in the assay of low-titre, low-avidity antitoxin such as is found in man, monkey and guinea-pig. In these species the slopes of neutralization curves are shallow and the " small characteristic reactions ", if judged at the 2-mm level, will give

[a] Unpublished working document WHO/BS/274

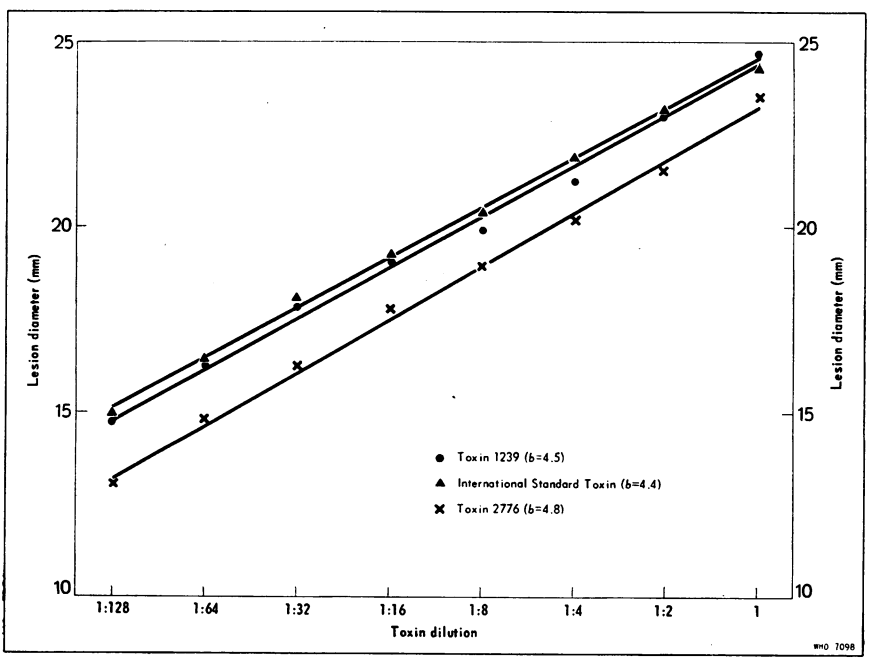
different values from those judged at the 8-10-mm level. For this reason, in this paper, the MRD is defined as the amount of toxin injected in a volume of 0.2 ml that produces a lesion, on a comparable site in the same animal, of the same diameter and intensity as that produced by the unneutralized toxin from a mixture containing one International Unit of Schick-test toxin and 0.001 unit of the International Standard for Diphtheria Antitoxin, injected in a volume of 0.2 ml. As the MRD has different meanings in different laboratories, it might be preferable to introduce the term " standard reacting dose (SRD) " to apply to the end-point so defined.

Results

Linearity of log-dose response lines for diphtheria toxin

The diameter of the lesion resulting from the intradermal injection of diphtheria toxin, in a constant volume of liquid, is directly proportional to the log of the dose.[7, 10] This finding was confirmed over a wide range of doses for the International Standard for Schick-Test Toxin, for the laboratory standard Toxin 2776 and for the non-avid Toxin 1239 (Fig. 1).

FIG. 1. PARALLELISM OF DOSAGE-RESPONSE LINES FOR INTERNATIONAL STANDARD DIPHTHERIA TOXIN, TOXIN 1239, AND TOXIN 2776

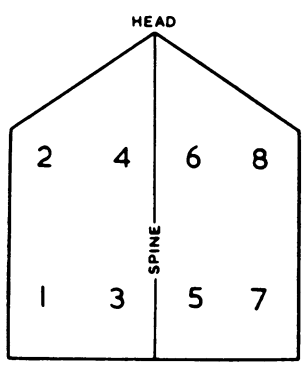


Eight doses, in graded twofold dilutions of each toxin, were arranged in a Latin square, rows being the eight sites of injection on each animal and columns the eight animals in the experiment (Table I). The analysis of variance for the experiment with the International Standard toxin is shown in Table II. The site variation was just significant at the $P = 0.05$ level, but was small compared with the variation between the response of different animals. The log-dose response line did not depart significantly from linearity. Similar results were obtained with Toxins 2776 and 1239, but in these experiments the site variation was not significant. The slopes (*b*) of the dosage-response lines are similar, and this has been so in our experience with a large number of experiments involving all the toxins named.

Site variation

In these experiments there was little variation in the sensitivity to toxin at different sites on the guinea-pig skin. In all cases these sites were on the flank, excluding the thin skin on the abdomen and that covering the limbs or directly influenced by their movement. As in work on sensitivity to tuberculin,[9] any variation in sensitivity between sites was always due to differences in sensitivity between the lateral and paravertebral area of each flank. There was no significant difference between sides or antero-posteriorly.

FIG. 2. GUINEA-PIG INJECTION SITES



In order to eliminate the site and animal variations from the error of the estimated potency, each of the four doses is injected (in duplicate) into every animal and at each of the eight possible sites over the whole group of eight animals (Fig. 2). Thus, potency and slope are both independent of animal and site variation. This method has been used for further experiments with diphtheria toxins possessing different biological characteristics.

TABLE I. RANDOMIZATION OF DOSES IN LATIN SQUARE

Guinea-pigs	Sites							
	1	2	3	4	5	6	7	8
1	S_H	S_H	T_L	T_H	T_L	S_L	T_H	S_L
2	T_H	T_L	T_L	S_L	S_H	S_H	S_L	T_H
3	S_L	S_H	T_H	T_L	S_L	T_L	T_H	S_H
4	T_L	T_H	S_L	S_H	S_L	T_H	S_H	T_L
5	S_H	T_L	S_L	T_L	T_H	S_H	S_L	T_H
6	T_L	S_L	S_H	S_H	T_H	T_H	T_L	S_L
7	T_H	T_H	S_H	S_L	S_H	S_L	T_L	T_L
8	S_L	S_L	T_H	T_H	T_L	T_L	S_H	S_H

S=Standard preparation; H=high dose; T=unknown test preparation; L=low dose

TABLE II. ANALYSIS OF VARIANCE IN EXPERIMENT DESIGNED TO DETERMINE VARIABILITY OF REACTION AT DIFFERENT SITES TO THE INTERNATIONAL STANDARD SCHICK-TEST TOXIN

Source of variation	Sum of squares	Degrees of freedom	Mean square	Variance ratio (F)	P
Between animals	106.58	7	15.23	16.92	< 0.001
Between sites	15.65	7	2.24	2.49	< 0.05
Between doses	599.46	7			
Linear regression	598.00	1	598.00	664.64	< 0.001
Deviations from regression	1.46	6	0.24	< 1	—
Residual	37.79	42	0.90		
Total	759.48	63			

Stability

Bacterial toxins are notoriously unstable. Slight changes in pH tend to cause degradation leading to loss of toxicity. For this reason, toxins are usually diluted with buffered diluents. In the concentrated form of a broth filtrate, changes in pH are slight, owing to the presence of buffering agents in the broth, but, on dilution with physiological saline or other unbuffered reagent, change in pH occurs. This may lead to a progressive loss of toxicity on dilution which may be slight, particularly if the toxin is a relatively stable one. Slight losses of toxicity on dilution cannot be readily detected by any current method of assay for Schick toxin. Buffered and unbuffered samples of Toxin 2776 were assayed in terms of the International Standard (see Fig. 3 and Table III). No additional buffer was added to that already present in the International Standard toxin. The dilutions were made with physiological saline.

In both experiments there was a significant difference ($P < 0.01$) between the response of different animals to toxin. When Toxin 2776 was not buffered, there was a highly significant ($P < 0.001$) variation between the response at different sites to toxin; the smallest lesions were those near the spine (a constant finding in these experiments but seldom significant even at the $P < 0.05$ level). But the most significant finding of all was that although Toxin 2776 buffered or unbuffered gave lesions of the same size at the higher concentration, unbuffered toxin caused small lesions at the

FIG. 3. COMPARISON OF DOSAGE-RESPONSE LINES FOR INTERNATIONAL STANDARD DIPHTHERIA TOXIN AND TOXIN 2776 WITH AND WITHOUT BUFFER

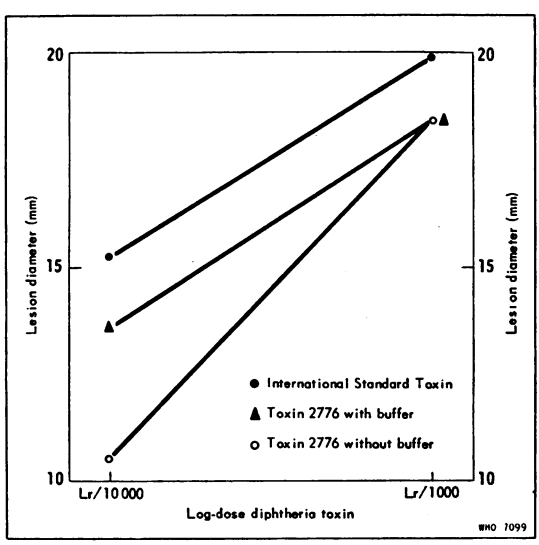


TABLE III. ANALYSIS OF VARIANCE OF ASSAY OF TOXIN TP 2776

A. Buffered *

Source of variation	Sum of squares	Degrees of freedom	Mean square	Variance ratio (F)	P
Between animals	15.78	7	2.25	3.09	< 0.01
Between sites	7.63	7	1.09	1.50	> 0.1
Between treatments	519.59	3			
Between substances	1.89	1	1.89	2.60	> 0.1
Common linear regression	517.56	1	517.56	711.35	< 0.01
Departure from parallelism	0.14	1	0.14	< 1	
Residual	33.47	46	0.73		
Total	576.47	63			

* Potency, 0.87; 5% limits of error expressed as a percentage of potency 84%-119.0 %

B. Unbuffered †

Source of variation	Sum of squares	Degrees of freedom	Mean square	Variance ratio (F)	P
Between animals	34.41	7	4.92	3.68	< 0.01
Between sites	42.03	7	6.00	4.50	< 0.001
Between treatments	827.37	3			
Between substances	159.39	1	159.39	119.36	< 0.001
Common linear regression	628.13	1	628.13	470.36	< 0.001
Departure from parallelism	39.85	1	39.85	29.84	< 0.001
Residual	61.43	46	1.34		
Total	965.24	63			

† Potency cannot be calculated in view of departure from parallelism.

lower concentration. The resulting, highly significant ($P < 0.001$), departure from parallelism thus invalidates the assay.

It is noteworthy that, judged by the traditional method of the MRD, this toxin would have been given a false potency and the invalidity of the assay would have passed unnoticed. Further experiments showed that borate buffer (see Annex) or the phosphate-bovine albumin buffer (see Annex) both maintained a constant slope of the toxin on dilution. More-

over, when the lesion diameter resulting from the injection of unbuffered Toxin 2776 was plotted against the log-dose of toxin, there was a significant departure from linearity ($P < 0.001$) in the lower dilutions. In all other experiments, dilutions of toxins were made in buffered saline. When this was done there was no significant departure from parallelism. In brief, toxins differing in biological characteristics produce log-dose response lines with slopes that do not differ significantly provided they are diluted with buffered diluent. An important deduction from this fact is that such toxins can be assayed in terms of toxicity of the International Standard for Schick-Test Toxin in the skin of the guinea-pig.

International Standard for Schick-Test Toxin versus International Standard for Diphtheria Antitoxin

The toxins listed were assayed, first in Schick units in terms of the International Standard for Schick-Test Toxin, using the method described in the Annex and then in Schick units in terms of the International Standard for Diphtheria Antitoxin. The potencies by each method are shown in Table IV.

Clearly two different properties of toxin are being assayed. The assay in terms of the standard toxin is a straight measure of toxicity; that in terms of the standard antitoxin measures the ability of the toxin to be neutralized by antitoxin.

In many cases the figures are so close that they can be considered not to differ. But Toxin 1239 gives widely different values. Our concern is not so much as to which is the ultimate standard, as with the problem of selecting toxins for the Schick test or for the assay of antitoxin.

The degree of difference in values, in terms of the two standards, for the Schick unit for each toxin shown in Table IV provides an accurate index of their avidity. Indeed, this simple technique provides a convenient means of judging the avidity of a toxin.

Toxins that give comparable values in terms of both standards will not only pass all current regulations for Schick toxins but also prove satisfactory for the assay of antitoxin by the Römer & Sames intradermal technique. Toxins that give different values in terms of the two standards will not serve for the Römer & Sames technique because they will prove to be non-avid. The problem to be considered next is whether they will prove satisfactory as Schick-test toxins.

The influence of avidity of toxin on neutralization in the skin of actively immunized guinea pigs

The most (2776, 3723 and 3051) and least (1239 and 2704) avid toxins available were assayed in terms of the International Standard for Schick-Test Toxin (Diphtheria) in non-immune and in immune guinea-pigs.

TABLE IV. SCHICK UNITS, IN ML, OF AVID AND NON-AVID TOXINS, ASSAYED AGAINST THE INTERNATIONAL STANDARD FOR SCHICK-TEST TOXIN AND AGAINST THE INTERNATIONAL STANDARD FOR DIPHTHERIA ANTITOXIN

Toxin	Schick Unit (ml)	
	Standard Toxin *	Standard Antitoxin
3046	0.000 008 (89.8-111.3)	0.000 006
3051	0.000 005 (84.0-119.0)	0.000 0075
G 12/6	0.000 01 (74.0-135.2)	0.000 009
2704	0.000 99 (53.2-188.1) **	0.000 5
1239	0.000 09 (83.1-120.4)	0.000 002
2776	0.000 03 (74.3-134.6)	0.000 02
3723	0.000 006 (77.1-129.7)	0.000 02

* These values are potencies with limits of error expressed as a percentage of potency.

** The wide limits of error with this particular toxin are a constant feature that recurred in repeat experiments. Such a range has never been found with another toxin.

The results (Table V) show comparable potencies whether the toxins were avid or not. The analogy with the Schick test in man is close, for guinea-pig and human diphtheria antitoxins both tend to be low in titre and avidity. It is likely, therefore, that avid or non-avid toxin would serve equally well for the Schick test in man.

TABLE V. COMPARISON OF POTENCIES OF AVID AND NON-AVID TOXINS IN TERMS OF INTERNATIONAL STANDARD FOR SCHICK-TEST TOXIN, IN NON-IMMUNE AND IN IMMUNE GUINEA-PIGS

Toxin	Potency in terms of International Standard for Schick-Test Toxin	
	non-immune	immune
2704	0.71 (74.3-134.6)	0.79 (89.0-112.3)
1239	0.41 (70.1-142.7)	0.44 (80.1-124.8)
2776	0.41 (68.9-145.1)	0.87 (84.0-119.0)
3723	7.98 (73.5-136.1)	5.66 (65.7-152.2)
3051	0.27 (60.8-164.4)	0.38 (79.4-125.9)

Discussion

There seems little justification for including current tests for " combining power " in the standardization of Schick toxin, for it would appear from the results quoted that they are not in themselves valuable. However, the experienced immunologist is cautious when it comes to recommending the standardization of toxins in terms of toxicity rather than in terms of neutralization by antitoxin. Ever since the time of Ehrlich,[2] toxin standards have been mistrusted because of their instability (see Miles [10]). However, the International Standard for Schick-Test Toxin is a remarkably stable preparation.[a] In this respect it is atypical. Its stability may be due, at least in part, to the fact that it is dispensed in buffer. The introduction of a standard to which a stabilizer has been added creates a precedent which might be considered to contravene an important assumption of standardization emphasized by Miles [11]—namely, that " the standard preparation contains neither impurities, having a specific activity resembling that of the active principle, nor substances which in any way modify the behaviour of the active principle ". Furthermore, buffered toxin is not identical with unbuffered or inadequately buffered toxin; the assay of the latter in terms of the former is therefore invalid on theoretical as well as on practical grounds (Fig. 3). There are therefore good reasons for using the International Standard for Schick-Test Toxin as a convenient reagent and research tool and retaining the International Standard for Diphtheria Antitoxin as the ultimate standard. By doing this, any sudden change in the properties of the standard toxin—such as occur with toxins (cf. Toxin 1239)—would be promptly detected. But, against this suggestion, is the fact that a toxin with poor combining power (non-avid), if standardized in terms of the antitoxin standard by the Römer & Sames technique (Table IV), would prove insufficiently toxic when used for the Schick test.

It is recommended that toxins should be standardized in terms of the International Standards for Schick-Test Toxin (by the technique described here) and for Diphtheria Antitoxin (by the Römer & Sames method) and that those that give identical values by both methods will serve either for the Schick test or for the Römer & Sames technique for the assay of diphtheria antitoxin. In addition, it is suggested that the minimum reacting dose (MRD), which provides the end-point for the Römer & Sames technique, should be defined in terms of the International Standards for Toxin and Antitoxin (see the section on methods). It might then be called the Standard Reacting Dose (SRD).

Our finding that avidity of toxin does not affect neutralization by antitoxin in the skin of the guinea-pig is in keeping with our earlier

[a] Unpublished working document WHO/BS/274

observation (quoted by Miles [12]). Miles [10] showed that the relationship between the response obtained from the inflammatory lesion-diameter at 24 hours and the log-dose of toxin in passively immunized guinea-pigs is linear; different log-dose response lines are substantially parallel; and the shift in log-dose response lines is proportional to the antitoxin content of the blood. By fitting the regression of response on log-dose, it is possible to detect relatively small, but significantly different, antitoxic immunity in different groups of animals. The same relations hold in actively immunized animals (see Hartley [5]); and a simple assay technique, essentially similar to the multiple Schick method,[5] has been devised.[7, 8] This method has the simplicity of toxin challenge as a test of immunity without the disadvantages, already stated, of using death as the end-point.

This modified multiple Schick method has advantages over all others for the assay of diphtheria antigens, in particular, in its independence of avidity.

Annex

DETAILS OF ASSAY METHOD

Eight albino guinea-pigs of at least 350 g in weight have both flanks depilated to provide space for eight reactions. It is better to use female animals since this diminishes the risk of skin damage by fighting.

Two doses of the International Standard for Schick-Test Toxin (Diphtheria) are used. The high dose is 1 unit, and the low dose 0.1 unit, in 0.2 ml of buffered physiological saline. The unknown toxin is diluted with buffered physiological saline to approximately the same strength as the Standard on the basis of Lf or Lr determinations (Lr/1000=1 Schick-test dose). If these are not available, a dilution of 1/500 and 1/5000 of most toxins will, almost invariably, provide a satisfactory assay as the level of toxicity is not critical. The high doses should be ten times the low doses and the latter should give lesion diameters of at least 10 mm.

Since the average slope in assays of this type is 4.5, the difference between the mean response to high and low doses is about 4.5 mm. The four doses—namely, standard toxin high dose ($^{S}_{H}$) and low dose ($^{S}_{L}$), test (unknown) toxin high dose ($^{T}_{H}$) and low dose ($^{T}_{L}$)—are arranged in a Latin square in such a way that, when all the animals have been injected, each dose has been injected in two of the animals at corresponding sites (Table I). The particular arrangement of the doses given at the sites on each animal in any one test is, apart from these restrictions, chosen at random.

Doses are injected intradermally in a volume of 0.2 ml and the diameter of the resulting lesions is measured with a transparent ruler after 24 hours. (The long and short diameters of elliptical lesions are measured and the

diameter is recorded as the square root of their product.) The potencies were estimated by the traditional method.[3]

Buffering Agents

The borate buffer used in this Department consists of:

Sodium borate (crystals)	2.58 g
Boric acid	2.33 g
Sodium chloride	2.75 g
Distilled water to	1000 ml

The phosphate-bovine albumin buffer [a] consists of:

Disodium hydrogen phosphate	2.374 g
Potassium dihydrogen phosphate	0.363 g
Bovine albumin	1.000 g
Distilled water to	1000 ml

Depilation

The depilating powder consists of:

Barium sulfide	250 g
Castile soap powder	50 g
Talcum powder	350 g
Flour	350 g
	1000 g

The whole is well mixed.

Fur is removed from both flanks with electric clippers. Sufficient water is added to the depilating powder to make a thick paste, which is spread firmly with a spatula over the clipped area. Ten minutes later every trace of paste is washed away with warm running water and the skin dried and adherent fur rubbed off gently with towels. The animals are used the same day.

ACKNOWLEDGEMENTS

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Toxin G 12/6 was kindly provided by Dr L. B. Holt, of the Wright-Fleming Institute for Microbiology, St. Mary's Hospital, London, England.

[a] Unpublished working document WHO/BS/274

RÉSUMÉ

Les sujets présentant une immunité à la diphtérie peuvent être distingués des sujets qui en sont dépourvus, au moyen de l'épreuve de Schick. Cette dernière décèle la présence dans le sérum humain d'antitoxine diphtérique en quantité suffisante pour neutraliser la dose d'épreuve de toxine. Pour établir une méthode standard d'application de ce test, nécessaire à la comparabilité des résultats, il faut disposer d'une toxine d'épreuve étalon. La méthode de Römer & Sames qui permet elle aussi de mettre en évidence l'immunité antidiphtérique, d'après la réaction cutanée du cobaye à un mélange toxine-antitoxine, requiert également une toxine étalon de référence. Le choix des toxines pour l'une et l'autre de ces épreuves fait l'objet de cet article.

Les auteurs décrivent un essai de la toxine diphtérique, basé sur le rapport linéaire entre le diamètre de la réaction cutanée et le logarithme de la dose de toxine. Cet essai permet d'éviter les titrations préliminaires, renseigne sur l'inclinaison de la courbe log dose-réponse et donne des limites d'erreur d'activité.

Le rôle des divers facteurs qui interviennent — toxicité, pouvoir de combinaison, avidité — a été étudié dans l'essai des toxines diphtériques par rapport à l'Etalon international de Sérum antidiphtérique et à l'Etalon international de Toxine diphtérique pour l'épreuve de Schick.

Toutes les toxines soumises à l'essai par rapport à la toxine étalon, quelles que puissent être leurs autres propriétés, ont donné des courbes log dose-réponse de même inclinaison, à condition qu'elles aient été diluées dans le soluté salin tamponné.

Les expériences ont été répétées sur des cobayes non immunisés et sur d'autres qui avaient subi une immunisation active. Des chiffres d'activité comparables ont été obtenus dans les deux groupes. Ni l'avidité, ni le pouvoir de combinaison de la toxine n'ont affecté sensiblement les résultats.

Les auteurs recommandent que les toxines soient standardisées par rapport à l'Etalon international de Toxine pour épreuve de Schick (d'après la méthode décrite dans cet article) et par rapport à l'Etalon international de Sérum antidiphtérique (d'après la méthode de Römer & Sames), et que celles qui donnent des résultats identiques par les deux méthodes soient employées soit pour l'épreuve de Schick, soit pour l'essai de l'antitoxine diphtérique. En outre, ils proposent que la dose réactive minimum, qui représente le point final dans la méthode de Römer & Sames, soit exprimée par rapport aux deux étalons internationaux précités et qu'elle soit appelée dose réactive étalon.

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