VOLUME 47, NO. 2 JUNE 1949

QUALITATIVE DIFFERENCES AMONG TOXINS AND TOXOIDS

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Although the potency of toxins and toxoids, as measured by the Lf dose, may constitute some rough measure of antigenic efficiency, qualitative differences are frequently so great that a weak toxin may be a better antigen than a very strong one. The terms 'potency', 'quantity' and 'strength' refer to the total content of toxin + toxoid, measured most readily by flocculation, and generally referred to as 'combining power' (for antitoxin). 'Quality' refers to efficiency in immunizing, or antigenic power. The expression 'antigenic unit' is unfortunate when applied to the quantitative measurement of antigens because it suggests a measure of quality or antigenic efficiency.

Most of our experience has been acquired with diphtheria toxins and toxoids and, unless otherwise specified, statements refer to these antigens, although there is no reason to suppose that they are not of more general application. In our experience, times of flocculation of freshly prepared toxins and toxoids give no indication of antigenic efficiency: we have handled toxoids which proved to be poor antigens, whose time of flocculation was average immediately after preparation but increased enormously on storing. Other batches of toxoid retain the same flocculation time for many years.

The production of good and bad antigens frequently seems to occur over fairly well-defined periods of time. Extremes of good and bad rarely occur during the same period and the greatest variation usually occurs in the transition from a good to a bad period and vice versa. Failure to produce good antigens may sometimes be traced to some change in the strain, the medium or some factor such as the water supply. More often, however, the reason for failure is not evident and during a transition period, and indeed at any time, batches of comparable potency but very different antigenic efficiency may be produced under apparently identical conditions. Again, some organisms may at times produce toxins which are well up to the average potency when freshly prepared, but which deteriorate rapidly on storing. This deterioration may be manifested as loss of total antigenic content, or as loss of toxicity without appreciable loss in total combining power due to so-called natural toxoiding. These phenomena, and particularly the former, may also be connected with antigenic quality. We also have a strong impression that horses hyperimmunized with certain batches of diphtheria toxoid tend to produce non-avid antitoxin.

The immunologist requires toxins and toxoids which will efficiently stimulate antitoxin production, both in man for the purpose of prophylaxis, and in horses for the preparation of high-value antitoxin for therapeutic purposes. In prophylactic immunization against diphtheria, two or more well-spaced injections are given, and the material used is frequently toxoid treated in some manner so as to remove much of the non-specific broth material. In this country the prophylactic is usually in the form of a suspension of a relatively insoluble precipitate, which remains at the site of injection for some weeks and acts as a constant stimulus to antitoxin production. In our experience with animals, soluble purified toxins and toxoids often fail to produce good immunity, probably because of their rapid elimination from the body.

In the hyperimmunization of horses, however, crude toxins and toxoids are generally used, and increasing doses of these are given over a period of several weeks. For the production of diphtheria, staphylococcus and perfringens antitoxins, we use horses which already have some circulating antitoxin present, produced as a result of natural infection. Such horses give a rapid response to injections of the appropriate toxin or toxoid and no delay is necessary for the establishment of basal immunity as in tetanus and certain other types of immunization (Barr & Glenny, 1945).

We find that horses immunized with some batches of toxins and toxoids respond well in the early stages, but reach a low maximum value after a few weeks, which may either be maintained for some time or may soon slowly fall. The low value and failure to respond in the later stages of immunization may well be due to interference caused by response to other antigens: these antigens include bacterial proteins and possibly non-specific protein material in the broth. Horses possessing no normal antibodies to such antigens would give no immediate response to them and some weeks elapse before their immunizing action became effective. A horse with normal antitoxin may, on the other hand, possess basal immunity also to other specific antigens, and the response to immunization, as measured by the development of antitoxin, might

then be poor from the start. The effect of normal antitoxic value upon response to immunization with filtrates containing more than one toxin, has already been described by us in collaboration with other colleagues (Glenny, Barr, Jones, Dalling & Ross, 1933).

It is sufficient to quote one example of a horse immunized with *Cl. welchii* type B (lamb dysentery) toxin. This horse possessed some natural immunity to the main toxin, but none to the subsidiary toxin prior to immunization. Curves demonstrating the development of the two antitoxins showed that no measurable α antitoxin was produced during the first 30 days of immunization. Throughout the course, the phases in the response to α lagged behind those of β by 15–18 days, and when the β antitoxic response reached its maximum, the curve for α was still rising.

When using filtrates of this type which contain well-defined toxins, it is a simple matter to follow the antitoxic response to the various components of the filtrate. With other types such as diphtheria, however, in which only the classical toxin is easily recognized, we have a more difficult problem. The existence of more than one zone of flocculation provides evidence of the presence of other antigens in such filtrates, but frequently much tedious work is necessary in order to find them. Additional evidence is provided by the production of severe swellings in some horses after injection of certain batches of toxin or toxoid. Swellings are not invariably detrimental to antitoxic response, and are indeed at times beneficial. In such cases it seems probable that the specific toxin or toxoid may have become fixed to the tissues or combined with circulating antitoxin in such a way as to dissociate at a favourable rate, and so act as a continuous stimulus. With some filtrates, however, severe and persistent swellings are produced and antitoxic response after the first 2 or 3 weeks, or even earlier, is very poor. This may be due to the setting up of an allergic state in the animal by the injection of such antigens as bacterial protein.

While poor response to hyperimmunization may be ascribed to the presence of considerable quantities of 'unwanted' antigens in some filtrates, others may fail for reasons as yet not understood. One possibility is too rapid elimination of the antigen from the body. We know from experience that response may be influenced by some physical condition such as adsorption or some kind of combination of toxins, because the antigenic efficiency of toxins and toxoids is greatly increased by precipitation with alum or adsorption by certain other substances: there is also less difference in antigenic efficiency between the alum precipitates of a good and bad toxoid than between the toxoids themselves. In a similar manner, untreated toxoids, after injection, may be temporarily fixed to antitoxin in the tissues, or to some non-specific substance in the culture medium, and the exact nature of this fixation may determine the efficiency of the material as an antigen. The whole question of qualitative differences between batches may be connected in some way with the extraordinary phenomenon that well-immunized animals continue to respond to relatively small doses of toxoid which would be completely swamped out by the total circulating antitoxin. It may be that a good antigen reaches the site of antibody production in such a form that it is not swamped out or that the rate of dissociation of the toxin-antitoxin complex varies greatly with different batches of toxin and is of great importance.

Not only have we found very considerable differences in antigenic efficiency among batches of both toxins and toxoids used for the hyperimmunization of horses, but many toxoids used for this purpose have also been tested in guinea-pigs, and there, too, striking differences have been encountered. For guinea-pig experiments and for human immunization it is necessary to use toxoid, because the injection of immunizing doses of unmodified toxin may produce very severe symptoms or cause death of a non-immune animal. The use of toxoid rather than toxin, however, introduces another factor, namely the action of formalin. It is known that an excess of formalin acting over a long period may damage and even destroy an antigen, and there may well be other factors in its action of which we are not aware. The action of formalin on crude toxin is almost certainly not confined to rendering non-toxic the specific toxin in filtrates. Bacterial protein and non-specific substances in the broth may undergo chemical changes which cause them to become more antigenic, and so the relative amounts of such substances present in different filtrates may be of greater importance after toxoiding than in the unformalized state.

In experiments on antigenic efficiency there is another factor to be considered, and this is the variation in response among individuals in a group of animals. It is now well known that the response of guinea-pigs to immunization is greatly influenced by their health and diet and the conditions under which they are kept both prior to and during an experiment. For the purpose of comparison of antigens it is clearly desirable to use guinea-pigs from a single source, bred and kept under good and standardized conditions. If this be done, the inherent differences in immunizability of the animals should be the only variable factor remaining. In work on horses, the actual normal antitoxic value prior to immunization has some effect on final value (Barr & Glenny, 1945), but here again we find some animals with good basal immunity giving a poor response to immunization with antigens which have given excellent results in a number of other horses. Such failure may be due to the relative proportions of different proteins in the

blood, or to some other unknown constitutional peculiarity of the animal. We have encountered horses which have given poor results to immunization with three or more distinct types of toxin.

For the purpose of comparing the antigenic efficiency of different batches of diphtheria toxoid, we have used two main methods. First, the hyperimmunization of horses for the production of therapeutic sera, and secondly, determination of the response of guinea-pigs to two fixed doses of antigen given at an interval of 28 days: the second method is comparable with the immunization of man.

results shown here and in all tables showing horse values are those obtained in the preliminary course of injections: for this course, a value of 800 units can well be accepted as the lowest figure for a successful immunization, although in later courses, using toxin plus alum, values up to and occasionally exceeding 3000 units are obtained.

Table 1 shows that eight of the nine horses injected with TMP 2334 were successfully immunized within 5 weeks, three of this number reaching unusually high values within 3 weeks. Of seven horses injected with a slightly more potent toxoid TMP

Table 1. *Showing the antitoxic values in units per ml. of weekly serum samples from nine horses hyperimmunized with diphtheria toxoid TMP* 2334 (56 *Lf/ml.*)

Horse	1	2	3	4	5	6	7	8	9
Normal antitoxic value	0·2	0·2	0·1	0·1	0·04	0·04	0·02	0·02	0·001
Value after 1 week	200	10	200	25	10	150	25	150	10
Value after 2 weeks	800	200	800	300	25	250	150	1200	300
Value after 3 weeks	1700	600	2000	500	300	1000	120	1700	1000
Value after 4 weeks	2150	1000	2500	600	600	1000	400	1950	1000
Value after 5 weeks	—	1700	—	800	1400	1350	500	—	—

Table 2. *Showing the antitoxic values in units per ml. of weekly serum samples from seven horses hyperimmunized with diphtheria toxoid TMP* 2423 (62 *Lf/ml.*)

Horse	10	11	12	13	14	15	16
Normal antitoxic value	0·5	0·2	0·04	0·04	0·02	0·01	0·01
Value after 1 week	100	25	10	2	100	25	50
Value after 2 weeks	100	100	25	25	250	50	100
Value after 3 weeks	100	300	140	140	400	120	400
Value after 4 weeks	100	700	200	250	500	150	400
Value after 5 weeks	400	800	300	350	800	400	600
Value after 6 weeks	600	800	250	400	1000	600	700
Value after 7 weeks	700	700	300	600	—	700	700
Value after 8 weeks	—	—	300	500	—	500	—

Table 3. *Showing the antitoxic values in units per ml. of weekly serum samples from five horses hyperimmunized with diphtheria toxoid TMP* 2525 (41 *Lf/ml.*)

Horse	17	18	19	20	21
Normal antitoxic value	0·2	0·1	0·04	0·02	0·002
Value after 1 week	2	2	10	10	2
Value after 2 weeks	25	25	200	50	25
Value after 3 weeks	50	300	600	300	80
Value after 4 weeks	50	350	500	300	100
Value after 5 weeks	50	400	300	300	300
Value after 6 weeks	100	400	300	300	250
Value after 7 weeks	100	400	300	300	200
Value after 8 weeks	100	—	—	400	—
Value after 9 weeks	100	—	—	400	—
Value after 10 weeks	100	—	—	—	—

Table 4. *Showing the antitoxic values in units per ml. of weekly serum samples from four horses hyperimmunized with diphtheria toxoid TMX* 1302 (21 *Lf/ml.*)

Horse	22	23	24	25
Normal antitoxic value	0·2	0·1	0·1	0·1
Value after 1 week	10	10	10	2
Value after 2 weeks	50	25	10	10
Value after 3 weeks	50	25	50	25
Value after 4 weeks	100	50	100	100
Value after 5 weeks	200	150	200	200
Value after 6 weeks	250	150	250	400
Value after 7 weeks	500	200	450	500
Value after 8 weeks	650	350	500	500
Value after 9 weeks	1000	1000	700	—

Tables 1–4 give the results obtained with four diphtheria toxoids used for the hyperimmunization of horses, showing the antitoxic value developed by each horse during successive weeks of injection. The

2423 (Table 2) only two reached a value of 800 units, both after 5 weeks: of these, one showed a drop in titre at 7 weeks, while of the remainder, two failing to reach 800 units showed a fall in value at 8 weeks, two

https://doi.org/10.1017/S0022172400014352 Published online by Cambridge University Press

reached 700 units in 7 weeks (immunization was terminated) and the fifth horse achieved only 300 units after 8 weeks' immunization. All five horses injected with TMP 2525 (see Table 3) failed to produce antitoxin of useful value, and the preliminary course with this toxoid had to be abandoned. The low values reached, and the slow deterioration in titre of two of the horses, strongly suggest interference with response, caused by the presence of antigens other than specific toxoid. Table 4 shows that of four horses immunized with TMX 1302, two were successful and two unsuccessful. The fact that 8 weeks' immunization were necessary to produce useful values, can probably be accounted for by the low potency of the toxoid, which was prepared many years ago, using a different type of medium from that used for the other three toxoids, all of which were made under identical conditions of strain, type of medium and length of growth. Consideration of Tables 1–4 brings to light the following facts: (1) a low-value toxoid (TMX 1302, Table 4) may be a better antigen than a high-value toxoid (TMP 2423 and TMP 2525, Tables 2 and 3); and (2) great differences in antigenic efficiency exist between batches of approximately the same potency (TMP 2334 and TMP 2423, Tables 1 and 2). These differences cannot be accounted for by known factors such as type of medium or normal antitoxic value of horses. The variations in response of horses immunized with TMP 2334 are probably due to differences in immunizability or in the distribution of specific normal antibodies in the horses before immunization.

A study of the tables reveals the difficulty of forecasting the ultimate response of horses, from consideration of the values reached at any given time, and the impossibility of judging the antigenic efficiency of a batch of toxoid until several weeks have elapsed. As an example, horse 2 in Table 1 and horse 19 in Table 3 gave identical values for the first 3 weeks, but whereas the former continued to increase in titre and reached 1700 units in 5 weeks, the latter had already reached its maximum value, and a fall in titre was observed thereafter. Again, one horse injected with TMP 2334 (Table 1) reached only 25 units after 2 weeks' immunization, but its ultimate value was higher than those of four of the remaining eight horses in the group. It appears to us, however, from general experience, that a very good quality toxoid can be recognized after 3 weeks' immunization, but in order to distinguish between good, medium or bad toxoids, at least 4 weeks are necessary: even 6 weeks may not be long enough for slow toxoids.

Table 5 gives the collected values of horses after 6 weeks' immunization with diphtheria toxoids, at intervals over a period of 14 years: a large number of toxoids and more than 550 horses were involved during the 5 years reviewed, and the table clearly shows the fluctuations which may occur over long periods of time.

In 1931, approximately half the horses reached 800 units in 6 weeks, but from 1931 to 1937 values fell considerably, although the potency of the toxoids used was increasing. From 1938 to 1941 an improvement occurred, which was out of all proportion to the increase in strength of toxoids. During 1945, one toxoid TMP 2437 appeared to be producing better results than others in use at the same time. A comparison, given in the last two columns of Table 5, shows that a much greater proportion of higher values (over 1000 units) was obtained from horses immunized with this toxoid than with other batches: this fact cannot be accounted for by differences in potency of toxoids.

Table 5. *Showing the antitoxic values of horses after 6 weeks' immunization with diphtheria toxoids*

Year	1931	1935	1937	1941	1945	
Toxoid	—	—	—	—	TMP 2437	Others
Percentage no. of horses with values:						
Over 400 units	80	62	47	92	100	90
Over 500 units	73	57	34	84	90	84
Over 600 units	64	49	25	73	80	72
Over 800 units	49	28	9	52	65	34
Over 1000 units	34	18	2	34	45	19
Over 1200 units	15	9	1	20	25	16
Over 1500 units	6	2	0	10	10	3
Over 2000 units	1	0	0	2	5	0
No. of horses	123	163	103	115	20	32
Titre of toxoids	20–30	30–40	30–40	35–45	45	45–50

It should be mentioned here that the period of poor immunization (about 1935–8) coincided with a spell of severe swellings in horses. The relation between local reactions and antitoxin production is not clear. General experience suggests that the best response occurs when there are moderate reactions and that severe reactions usually, but not invariably, reduce antitoxic response. As we have already suggested,

https://doi.org/10.1017/S0022172400014352 Published online by Cambridge University Press

such reactions may be allergic in nature and due to unwanted specific antigens in the toxoid: interference of this kind may have played a major part in the failure of horses to produce high-value antitoxin at this time.

We also have evidence of qualitative differences in *Cl. welchii* type A toxins used for the hyperimmunization of horses. For the production of this antitoxin we use horses which already possess some natural immunity, and such animals can be injected from the start with unmodified toxin without danger of undue reactions.

Table 6. *Showing the antitoxic values of horses after 6 weeks' immunization with* Cl. welchii *toxins*

Year	1943	1944	1944
Broth	A	A	B
Percentage no. of horses with values:			
Over 100 units	99	91	85
Over 150 units	96	86	69
Over 200 units	93	80	57
Over 300 units	83	61	39
Over 400 units	71	43	26
Over 500 units	58	20	18
Over 600 units	41	12	11
Over 800 units	28	3	0
Over 1000 units	17	0	0
No. of horses	213	65	61

Table 6 gives the collected results of preliminary immunizations during the years 1943 and 1944, involving many batches of toxin and more than 300 horses: during 1944, two distinct types of medium were used in toxin production. The antitoxic values recorded are those obtained after 6 weeks' immunization, and show that deterioration in quality of toxins occurred from 1943 to 1944, using broth A and also that broth B was inferior to broth A. Taking 400 units as the lower limit of successful immunization there is a striking difference between the three groups. One probable contributory cause of the comparative failure of broth B toxins is that while the titre of the main toxin α is as high or higher than that in broth A filtrates, broth B appears to favour the production of the subsidiary haemolytic toxin θ. Values obtained during 1945 with both types of broth, showed that deterioration in quality was still occurring.

Differences in antigenic efficiency among batches of diphtheria toxoids, as judged by the response of guinea-pigs to two injections, are even more striking. In our experiments the second injection of toxoid was given 28 days after the first, the animals bled 10 days later, and the individual sera tested for antitoxic content. For the purpose of comparison it is necessary to use a fixed critical dose (in terms of Lf doses) such that many failures to respond will occur when poor quality toxoids are injected and a good secondary res ials injected with good quality toxoids. a fair scatter in responses should occur with average quality batches.

Some years ago we tested a large number of toxoids in this manner, using 2 Lf doses for each injection, and encountered great differences between them. More recently, however, when following the same procedure, differences have not been great and most animals have shown a good secondary response. This is almost certainly due to a general improvement in the quality of toxoids, because the dose (2 Lf doses) was originally worked out at a transition period from bad to good quality toxoids, the majority of batches being very poor antigens. It may also be due in part to the fact that most toxoids tested recently have been freshly prepared, and storage may have an adverse effect on the quality of some batches.

The results obtained in guinea-pigs are too numerous to record in full, and a number have been published collectively elsewhere (Barr & Glenny, 1947).

Table 7. *Showing the response of guinea-pigs to two injections, each 2 Lf doses, of diphtheria toxoid, given at an interval of 28 days*

	Percentage no. of guinea-pigs with antitoxic values over				
Toxoid	0·001	0·01	0·1	1·0	5 units
TMR 1292	37	23	19	2	0
TMX 1302	98	96	93	49	9

A full comparison between two particular toxoids is given in Table 7. These batches were tested concurrently on four separate occasions, using a total of about 100 guinea-pigs, in order to neutralize differences due to condition of animals and possible seasonal variation in response. The collected results given in Table 7 show that whereas over 60 % of the guinea-pigs injected with TMR 1292 failed to produce any detectable antitoxin (0·001 unit) nearly 50 % of those injected with TMX 1302 produced more than 1 unit per ml.

It is evident that differences in antigenic efficiency can be demonstrated by the two methods we have described, that is by hyperimmunization of horses and by the injection of two small doses at fixed intervals, into guinea-pigs. Table 8 gives a comparison between results obtained with toxoids tested by the two methods, and shows that those batches which were poor antigens in guinea-pigs were of no use for immunization of horses. There is a fair correlation throughout, but among those which gave good results in guinea-pigs there are some failures in horses. These anomalies may be accounted for by the existence of three factors in horse immunization

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Table 8. *Showing the response of horses to hyperimmuniz[illegible]phtheria toxoids of varying antigenic efficiency in guinea-pigs*

Geometric mean of antitoxic response in guinea-pigs (units/ml.)	Percentage no. of horses reaching antitoxic values over					
	400	500	800	1000	1500	2000 units
Under 0·01	29	29	5	0	0	0
0·01–0·049	50	40	15	5	5	0
0·05–0·099	70	59	41	23	0	0
0·1–0·5	82	70	42	33	14	4
Over 0·5	80	65	39	39	19	13

which do not enter into work with guinea-pigs. One of these is the state of normal immunity of the animals before injection—whereas many horses have natural immunity to diphtheria, we do not find this state in guinea-pigs, though it should be noted that some workers in other countries have the impression that it may exist. Another factor is the continuous injection of horses twice or thrice weekly over a long period, whereby reactions may be produced which may assist or interfere with immunity response. The presence of 'unwanted antigen' also is less likely to affect the response in guinea-pigs than that in horses, unless such antigens are present in relatively large amounts, because two small doses of a weak antigen are insufficient to produce a secondary response.

It is possible that the response of guinea-pigs to a single injection of toxoid might prove to be a useful method of comparing the quality of different batches. In work already done on these lines, we have so far failed to find a satisfactory dose which will detect differences so well as the two-injection method. Results of work already done on the response of guinea-pigs to one injection of toxoid or of A.P.T. suggest that two distinct levels of dosage should be sought. One of these should be sufficiently low that poor antigens would produce very little immunity, whereas good batches would produce good immunity within 4 weeks of injection: it is to be expected that results obtained using such a dose would give a good correlation with those obtained from the guinea-pig two-injection method. The other level of dosage should be sufficiently high that with certain toxoids a damaging effect would come into play, due to the presence in the dose of sufficient bacterial protein or other specific or non-specific antigen, to exert an inhibitory effect on the true antitoxic response. The results obtained using such a dose might be expected to show agreement with those obtained in the hyper-immunization of horses.

It has been established that the affinity of toxoid for antitoxin is less than that of toxin. It appeared possible to us that in addition to degrees of avidity for toxin in different batches of antitoxin, there might also be degrees of affinity of toxins and toxoids for antitoxin. Some preliminary work suggests that this is so, and that affinity for antitoxin is related to antigenic efficiency. The relative affinities of different batches of toxoid were measured by determining the Lr dose of a mixture composed of 9 Lf doses of toxoid and 1 Lr dose of a fixed toxin. From the results it is possible to calculate the proportions of toxoid combined and free, when all the toxin is just neutralized by antitoxin. Examination of about fifty batches of toxoid against one particular serum showed that the amounts of toxoid in combination as determined by this method ranged from 10 to 47% of the total present in the mixtures. These figures we provisionally designate the 'affinity coefficient' of the toxoid, and values are given in Table 9 for seven chosen

Table 9. *Showing the affinity for antitoxin of different batches of diphtheria toxoid*

Toxoid	Value of affinity coefficient against antitoxin		
	A (slightly non-avid)	B (average)	C (avid)
1	39	47	58
2	30	30	50
3	29	29	47
4	25	29	38
5	19	22	27
6	15	18	24
7	8	10	13

toxoids, for which the affinity coefficient was determined against three sera of different degrees of avidity. This table shows that the same general sequence occurred from toxoid 1 to 7, whatever serum was used, but that in general the affinity coefficient for any given toxoid was higher the more avid the serum against which it was determined. This means that more toxoid enters into combination with avid than with non-avid antitoxin, which is in accordance with the unpublished observations of Glenny and Stevens on tetanus.

Table 10 shows that there is a relation between the affinity coefficient of toxoids and their antigenic

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Table 10. *Comparing the response of guinea-pigs to two injections of 2 Lf doses of diphtheria toxoids with the affinity coefficient of the toxoids*

Affinity coefficient of toxoids	Percentage no. of guinea-pigs with antitoxic values above				
	0·001	0·01	0·1	1	10 units
Over 30	93	92	82	48	8
15–30	82	72	56	13	0
Under 15	48	39	33	5	0

efficiency, as measured by response of guinea-pigs which we have described earlier. For the sake of brevity the toxoids have been divided into three groups according to the value of their affinity coefficients as measured against serum B. It is evident that those toxoids which had relatively high affinity for antitoxin also produced good immunity in guinea-pigs. The same relationship existed also between affinity coefficients of toxoids and their efficiency in the hyperimmunization of horses as shown in Table 11. It must again be stressed that

Table 11. *Comparing the response of horses to hyperimmunization with diphtheria toxoids, with the affinity coefficient of the toxoids*

Affinity coefficient of toxoids	Percentage no. of horses reaching antitoxic values above					
	400	500	800	1000	1500	2000 units
Over 30	89	82	55	44	19	7
20–29	86	82	50	32	9	0
Under 20	68	63	37	16	5	0

these data were accumulated between the years 1937 and 1941, during which time, as shown in Table 5, a transition was occurring from very poor to good quality toxoids. Extremes of good and bad quality were therefore encountered over this period, whereas at times when the batches produced are uniform in character, little difference can be seen in the affinity coefficients. The correlation shown in Tables 10 and 11 between the antigenic efficiency of toxoids and their affinity for antitoxin, appears to be of theoretical importance, and it might appear logical to conclude that combination of injected antigen with circulating antibody is a necessary condition for the further production of antitoxin, and that firmness of combination leads to a longer retention of antigen in the animal undergoing immunization. It might be argued, therefore, that while differences in quality would be manifested in antigens injected into partially immune animals, it should not be evident when injected into non-immune animals in whose circulation there is no antitoxin to bind the antigen. There is, however, abundant evidence that for every toxoid there is a threshold dose, below which a poor response or none is obtained and this dose varies from one batch to another. Further, in a limited number of experiments, we have compared the responses of guinea-pigs to two injections of good or bad toxoids, the doses having been arranged as follows:

Group	First injection doses	Second injection doses
1	2 Lf of good toxoid	2 Lf of good toxoid
2	2 Lf of good toxoid	2 Lf of bad toxoid
3	2 Lf of bad toxoid	2 Lf of good toxoid
4	2 Lf of bad toxoid	2 Lf of bad toxoid

Very considerable differences in response were obtained between the groups, those from groups 1 and 2 being of the same order, with group 2 not quite as good as group 1. Groups 3 and 4, in that order, were definitely inferior, just over one-third of the guinea-pigs in group 3 producing over 0·1 unit per ml. as against all the animals in group 1. This shows once again the importance of a good first dose in prophylaxis, and also shows that the failure to immunize with a bad toxoid is not entirely due to poor affinity for antitoxin, because good basal immunity could not have been established by the first injections given to groups 3 and 4. It therefore appears probable that those toxoids which show good affinity for, or firm combination with, antitoxin, also combine strongly with, or are readily adsorbed by the body tissues. This suggests that toxoids, as present in crude formalinized culture filtrates, may not be molecularly homogeneous. As shown by us (Barr & Glenny, 1931*a*, *b*) strongly avid and slightly non-avid antitoxic fractions can be salted out from a single serum, and it appears possible that crude toxoids may likewise contain molecules possessing varying degrees of affinity for antitoxin. Though all these molecules might have the same basic structure, slight steric differences might exist which assisted or hindered firm combination with antitoxin or tissues or favoured the production of antitoxin. On the basis of this supposition, the quality of a toxin or toxoid would be dependent upon the proportion of molecules which could readily and firmly be fixed to antitoxin.

SUMMARY

1. Considerable differences in antigenic efficiency are encountered among batches of (1) diphtheria toxoid, and (2) *Cl. welchii* (perfringens) toxins prepared under standardized conditions. These differences were revealed in the results of hyperimmunization of horses.

2. Such differences in antigenic efficiency in batches of diphtheria toxoid were also detected by means of injections into guinea-pigs. Two injections, each of 2 Lf doses, were given at an interval of 28 days, and the response measured by means of antitoxin titrations on bleedings taken 10 days later.

https://doi.org/10.1017/S0022172400014352 Published online by Cambridge University Press

3. A general correlation was found between antigenic efficiency as measured by these two methods.

4. A method is described for comparing the affinity for antitoxin, of batches of diphtheria toxoid, by determining the test dose of mixtures containing 9 Lf doses of toxoid and 1 Lr dose of a fixed toxin. The greater the avidity of the antitoxin, the more toxoid entered into combination with it.

5. A corr[illegible] found to exist between affinity for [illegible]d antigenic efficiency of toxoid, as measured both by the response of horses to hyperimmunization and the response of guinea-pigs to two spaced injections.

6. It is tentatively suggested that crude toxoids may contain molecules possessing varying degrees of affinity for antitoxin, due to slight steric structural differences.

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(*MS. received for publication* 3. I. 49.—Ed.)