SOME PRACTICAL APPLICATIONS OF IMMUNOLOGICAL PRINCIPLES

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(With 5 Figures in the Text)

All methods of immunization, whether of laboratory animals, horses or man, are based on the fact that previous experience of an antigenic stimulus profoundly modifies the response of the animal to further stimulation with the antigen. As Glenny & Südmersen (1921) state:

(i) In animals possessing no normal antitoxin, a single injection of toxin, either 'attenuated' or under cover of antitoxin, whether injected previously or at the same time or present in the form of passive immunity maternally transmitted, is followed by a latent period of about 3 weeks and the maximum immunity is reached in about 8 weeks. (Primary stimulus and response—see first curve on Fig. 2.)

(ii) In immune animals, whether naturally immune or artificially immunized, a single injection of toxin or toxin-antitoxin mixture is followed by a latent period of 4 days, and the maximum immunity is reached in about 10 days: the great and rapid immunity response to the secondary stimulus offers a striking contrast to the small and gradual response to the primary stimulus. (Secondary stimulus and response—see second curve on Fig. 2.)

(iii) In partially immune animals the response to an injection of toxin is in magnitude and rapidity of character intermediate between the responses following a primary and a secondary stimulus.

These points were established for diphtheria immunization in guinea-pigs, rabbits, sheep, goats, horses and man; unpublished work showed similar results in immunization with the toxins of *Cl. tetani*, *Cl. welchii*, *Cl. oedematiens* and *Cl. septicum* (see Glenny, 1925). Thus the main principles have a wide if not universal application both as regards species of animal and type of toxin. Immunization whether of man or animals depends on the same fundamental principles; neglect of them is likely to lead to failures which, particularly in the immunization of children, may bring the practice of prophylaxis against infectious diseases into disrepute.

Since the original publication (Glenny & Südmersen, 1921), further work has been done in relation to size of dose and interval between doses. The latent period is shortened as the size of the primary stimulus is increased. Normal rabbits injected with several hundred Lf doses of concentrated diphtheria toxoid have been shown to develop circulating antitoxin by the 10th day after the primary stimulus. It is possible that a massive dose of antigen can act in the same manner as a secondary stimulus. This view is supported by the rapid response in production of precipitin in rabbits from 5 to 8 days after an injection of 1 c.c. of horse serum: the mass of antigen is far greater than any which has been used in experiments with bacterial toxins and toxoids.

An intermediate response follows a second injection of antigen given too soon after the primary stimulus, or after a primary stimulus of insufficient strength. We have found that guinea-pigs, injected twice with 1/100th human dose (0·1 Lf) of A.P.T. at an interval of a month, give variable results, a secondary response occurring in some animals and an intermediate response in others. If the dose is increased from 2- to 5-fold, good basal immunity is established by the primary stimulus and all animals give a secondary response to the second injection. So long as the first injection, or course of injections, is adequate, the size of the second injection has little influence on the degree of response. The Schick dose (0·001 Lf) of toxin will give a recognizable response in guinea-pigs and rabbits (see Glenny & Allen, 1922) and in children (see Copeman, O'Brien, Eagleton & Glenny, 1922) with well-established basal immunity, so long as the amount of antitoxin circulating at the time of the Schick test is not so great as to render the dose ineffective. If, however, the primary stimulus is inadequate, the intermediate response following a second injection is improved with increased dosage. Fear of causing reactions has led to the use of a small dose, 0·2 c.c. (10 Lf) of A.P.T., followed by a larger dose, 0·3 or 0·5 c.c., for the immunization of children against diphtheria. A proportion of children do not become Schick-negative until 2–3 months after the second injection. This is due to the fact that while some children are sufficiently immunized by the first dose to give a secondary response to the second injection, others give only an intermediate response, inferior both in magnitude and rapidity of development. The

latter class should always be given a further injection after about 6 months, because it should be the aim, in the immunization of children, to obtain a good secondary response: this could also be ensured by increasing the initial injection to 100 Lf, if the dose could be tolerated without undue reaction.

Although a secondary response is usually obtained from an animal reinjected 3–4 weeks after a primary

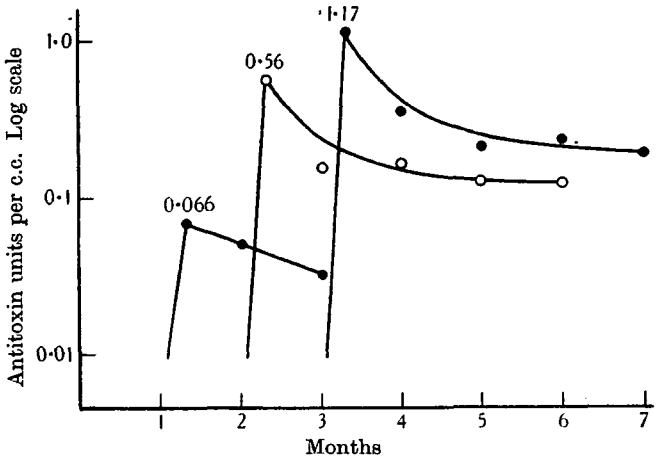

Fig. 1. Showing the geometric means of the antitoxic values of the serum of three groups of fifteen guinea-pigs given two doses of 0·005 c.c. (0·25 Lf) of A.P.T. at intervals of 1, 2 and 3 months respectively.

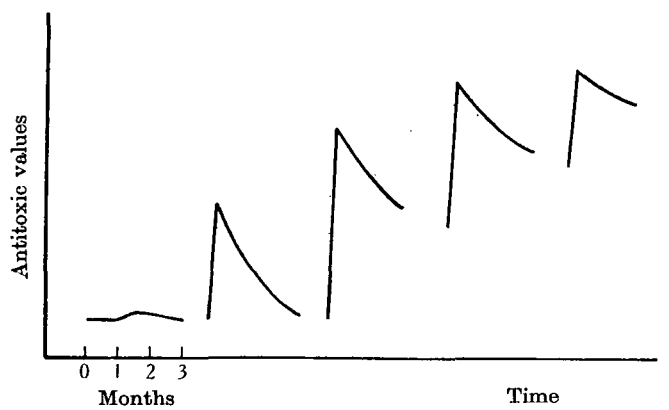

Fig. 2. Showing the general scheme of antitoxic response to a series of stimuli over a long period.

stimulus, a better response is obtained if the interval between the two injections be increased. Results of an experiment illustrating this point are given in Fig. 1. These results agree with other experiments on horses and rabbits injected with tetanus toxin-antitoxin mixtures, and with the conclusion of Marvell & Parish (1940) that in the immunization of man with tetanus toxoid wider spacing of the doses than 6 weeks gives better immunity. While this is true in the majority of cases, it does not hold when an insufficient primary stimulus is given. We have found, using A.P.T. in guinea-pigs, that with very small dosage a worse response is obtained if the interval is 3 months than 1 month.

The power of response or potential immunity of an animal appears to be a function not only of the extent of the stimulation received but also of the time elapsing since that stimulation. After a primary stimulus of adequate size the power of response to a subsequent injection continues to increase long after the amount of circulating antitoxin is declining. When two injections are given at an interval of 1 month or more the amount of antitoxin produced as a result of the second injection bears a closer relation to the size of the primary stimulus than to that of the secondary, but it is probable that the subsequent development of the power to respond may be improved by the use of a larger secondary stimulus. With successive stimuli given at well-spaced intervals antitoxin production increases to a limited extent, but the rate of loss of circulating antitoxin decreases (see Fig. 2). We suggest that this is due to continued production rather than to a decreased rate of elimination.

Although the general principles of primary and secondary stimulation have been worked out by means of single injections in animals, the same general principles apply if in place of 'a single injection of toxin' an animal receives a series of small stimuli each in itself too small to exert any obvious effect. It is our belief that natural immunization usually results from a great number of such small stimuli. The degree of immunity resulting is a function of both time and dose; eventually the full basal immunity is established and subsequent small stimuli give secondary responses. The number of Schick-negative children in a relatively homogeneous population is a function of the average dose-time integration and therefore is an indication of the ease with which the positive reactors can be artificially immunized. A child can only be regarded as successfully immunized if two separate results have been reached: (1) sufficient circulating antitoxin to combat initial production of toxin after infection, (2) a well-established potential immunity so that there is a rapid production of antitoxin to protect against continued production of toxin. Once this potential immunity is well established small stimuli naturally encountered may ensure the continued presence of sufficient antitoxin. The work on guinea-pigs mentioned above suggests that a dose of 20–50 Lf should produce such well-established immunity in children. We are assuming that comparable results can be obtained in young children and guinea-pigs, using a ratio of dosage approximately that of body weight, which can be taken as from 40 to 100 to 1. It must be remembered

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that the Schick test measures antitoxic content at a given time without reference to potential immunity unless considered in relation to the time interval since immunization. A naturally Schick-negative child has well-established immunity and will respond rapidly to small stimulation. A negative Schick reaction recorded 10–14 days after a second injection of antigen shows that the child has reached a similar stage of actual and potential immunity. A negative reaction several months after a course of immunization may be given by children who have only given an intermediate response to the last injection. Such children may now respond fairly well to subsequent stimuli, but their power of response will be less than that of those who have already shown a secondary response. In the meantime their antitoxic content will fall more rapidly and reach a dangerously low level in the absence of any natural stimulation.

The production of antitoxin for therapeutic purposes involves, in many cases, the immunization of horses which are completely non-immune. Many horses have certain antitoxins (e.g. diphtheria, *Cl. welchii*, *Staphylococcus*) circulating in their blood, developed as a result of natural infection, but certain other antitoxins are seldom if ever present. A horse becoming infected with tetanus probably always succumbs to the disease, and, if treated with large doses of antitoxin, fails to develop active immunity. Buxton & Glenny (1921) found no tetanus antitoxin in 500 normal horses. Of recent years however, since the use of tetanus toxoid or A.P.T., a small proportion of incoming horses has been found with circulating tetanus antitoxin. Römer (1908) has shown that cows frequently have naturally acquired tetanus antitoxin. In some cases (e.g. dysentery) lack of opportunity to acquire immunity probably accounts for the fact that no normal horses have circulating specific antitoxin. The principles of primary and secondary stimulation are used in the hyperimmunization of non-immune animals for the production of therapeutic sera. A primary stimulus, or short course of five or six injections, is given, followed by a rest. While a rest of 4 weeks is sufficient to produce a secondary response to another injection, it is an inadequate interval between the primary stimulus and hyperimmunization. Hyperimmunization, involving two or three injections per week of strong toxin or toxoid, if started too soon after preliminary injections, results in the production of antitoxin of low value and poor quality; a resting period of some months is necessary. Any injections given during this period are not only wasteful, but usually harmful, because they may provoke a tendency, overcome only after months or years of immunization, for the horse to produce non-avid or poor-quality antitoxin.

The best material to use as a primary stimulus for the short course of preliminary injections is A.P.T. (alum-precipitated toxoid). A dose of toxin large enough to act as a primary stimulus would kill a non-immune animal. Before toxoids were known, toxin-antitoxin mixtures were used, the toxicity of these being gradually increased as immunity developed. Toxoid was used later and was in turn displaced by A.P.T. The advantage of A.P.T. as a primary stimulus is obvious: the antigen is in relatively insoluble form, and remains at the site of injection for several months, gradually dissolving in the tissue fluids and acting as a continuous stimulus. Possibly the same effect would result from an initial large dose of toxoid followed by a long course of injections of very small doses, but the use of A.P.T. avoids the labour of such a procedure. The response to one such injection is very considerably greater than that to a single injection of toxoid of the same strength: A.P.T. probably acts in the same manner as small very frequent natural infections, and is therefore outstandingly effective in producing good basal immunity. When 3 or more months have elapsed since the preliminary injections were given, hyperimmunization may be started, and regular injections of unmodified toxin given. Ramon (1931) produced tetanus antitoxin of very high value by the hyperimmunization of horses which had been given two doses of tetanus 'anatoxin' some time previously. The figures given by Glenny, Pope, Waddington & Wallace (1925), who first introduced the method of rest, show the average value in international units for ten horses hyperimmunized without rest to be 310; for fourteen horses rested from 1 to 3 months it was 825 units. From horses rested 2–3 months we have obtained an average of 1090 units, 4–6 months 1340 units and over 6 months 1490 units per c.c. A more detailed summary of the effect of length of resting period upon antitoxic response is shown (for another group of tetanus immunizations) in Table 1.

Using the methods described above, the average values of some of the antitoxins produced by us at the present time are as follows:

	i.u.
Tetanus	1850
Cl. oedematiens	1500
Cl. septicum	825
Shiga dysentery	3400

Workers in other laboratories to whom we have communicated our methods have obtained similar results.

In the production of some other antitoxins it is possible to start with animals which already have some immunity, acquired as a result of natural infection. This may be detected by the presence of antitoxin circulating in the blood (e.g. diphtheria, *Cl. welchii* α and β, *Staphylococcus*), or, in cases where testing for low values presents difficulties, a bleeding

Table 1. *Showing the influence of rest after preliminary immunization upon the hyperimmunization of horses for the production of tetanus antitoxin*

Length of rest. Months	Total no. of horses	No. of horses producing (i.u. per c.c.)				
		Under 600	600–1000	1000–1500	1500–2000	Over 2000
Under 3	17	3	4	6	1	3
3–6	27	2	4	8	5	8
6–12	23	0	2	9	6	6
Over 12	6	0	0	0	4	2

taken 10 days after an injection of the appropriate toxoid or A.P.T. will contain a more easily measurable level of antitoxin developed as the secondary response to injection if the animal is already potentially immune (e.g. *Cl. septicum*).

All horses have natural *Staphylococcus* α-antitoxin and the frequency distribution of the logs of antitoxic values given in Fig. 3 shows fair approximation to the normal frequency curve ($\chi^2 = 9{\cdot}712$, $n = 5$, $P = 0{\cdot}087$). This suggests that all horses have had many infections, are well grounded in immunity and form a single population. The scatter is relatively

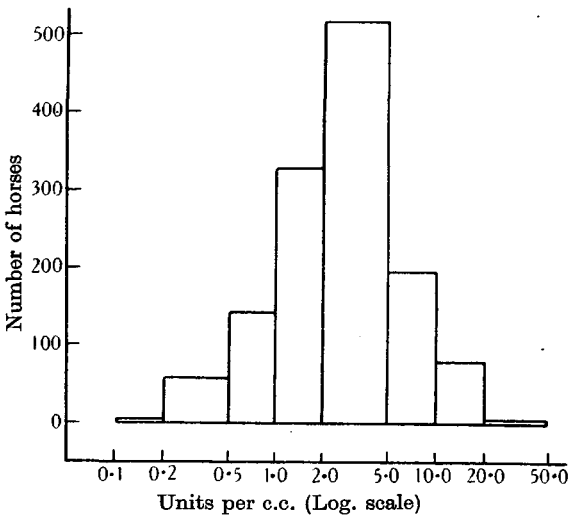

Fig. 3. Showing the distribution of *Staphylococcus* α-antitoxin in the serum of 1338 normal horses.

small, and the variation in value is probably due to differences in capacity to respond and possibly in the interval since last infection. Diphtheria and *Cl. welchii* α give a different picture (see Figs. 4, 5). In the first place, only a fraction of horses examined have any detectable antitoxin circulating, and also the distribution of values extends over a considerable range, and is far removed from the theoretical normal curves. The probable explanation of these differences is that natural infections with *C. diphtheriae* and *Cl. welchii* are not comparable with those produced by the *Staphylococcus*. The latter is an extremely common infecting agent in man and many animals, and is, moreover, an inhabitant of the skin. It is probable that horses receive frequent, almost daily, stimulation to produce antitoxin, in the process of grooming. Immunity of horses to

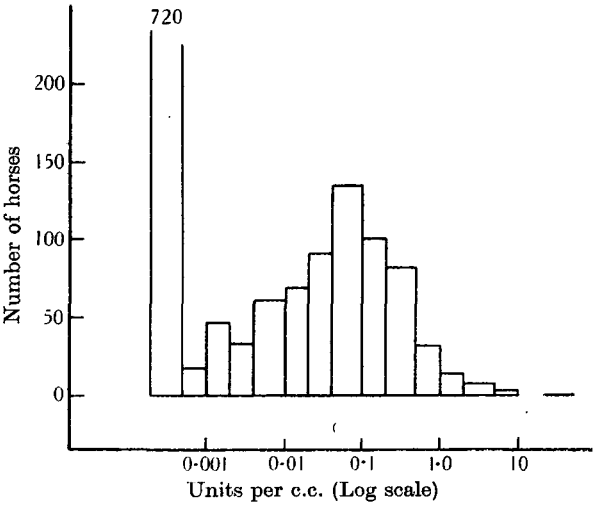

Fig. 4. Showing the distribution of diphtheria antitoxin in the serum of 1418 normal horses.

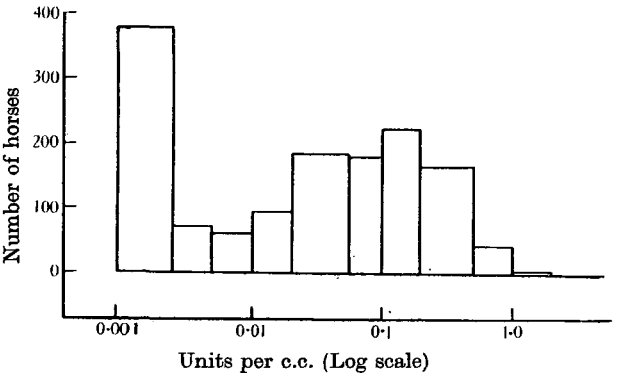

Fig. 5. Showing the distribution of *Cl. welchii* α-(*perfringens*)-antitoxin in the serum of 1430 normal horses.

diphtheria is also acquired through infection in scratches on the skin, but this infection is usually transmitted by carriers. Horses in country districts therefore do not have the same opportunities of

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developing immunity as horses in towns, because of the limited contact with man. As regards *Cl. welchii*, the manner of infection in the development of natural immunity is unknown, but as the organism is an inhabitant of soil, human contact is unlikely to play any part. It seems possible that country horses may have the better chance to develop antitoxin from natural infection, and also that differences in the bacterial flora of soil may be met with from one locality to another. Our experience has shown that horses from different sources give very different immunity figures. During the years 1935–7 we had the opportunity of studying three distinct populations of horses; these consisted of about 1400 English civilian horses (town and country), about 250 English army horses and 400 horses from Poland. There was but little difference between the *Staphylococcus* antitoxic values of the three groups, but the differences between the diphtheria and *Cl. welchii* values were most marked. The Polish horses showed a very low immunity rate to *Cl. welchii* and a very high one to diphtheria, and the English army horses were higher in both antitoxins than the English civilian ones. Table 2 gives figures for immunity rates to diphtheria in these three populations. The existence of immunity in horses without any circulating antitoxin was detected by the occurrence of a secondary response 10 days after an injection of A.P.T. Of the English civilian horses, 66·3 % had no circulating antitoxin, and 33·7 % had detectable antitoxin; 45·1 % were non-immune, and 21·2 % were immune though they had no circulating antitoxin. The total percentage of immune horses in this group was therefore 54·9 %, and the corresponding figure for army horses was 80·2 % and for Polish horses 91·0 %.

Table 2. *Showing the percentage number of horses from different sources divided according to their diphtheria antitoxic content and their power of rapid response to antigenic stimulus*

	Percentage number of horses				
	Without antitoxin. Non-immun. *a*	Without antitoxin. Immun. *b*	With antitoxin. Immun. *c*	Summary: No antitoxin *a+b*	Summary: Immun. *b+c*
English civilian horses	45·1	21·2	33·7	66·3	54·9
English army horses	19·8	8·8	71·4	28·6	80·2
Polish horses	9·0	16·4	74·6	25·4	91·0

The presence of normal antitoxin is of the greatest possible importance in serum production, because by using a horse with some basal immunity the long delays necessary in the preparation of non-immune horses are avoided. It is possible to start hyper-immunization of such horses at once, and it is not necessary to inject completely non-toxic material as must be done with non-immune animals. The significance of actual antitoxic values of normal horses is a matter of some importance. The relation between normal antitoxic content and immunological history can be seen from a consideration of Fig. 2. Horses with little early contact with diphtheria may have a fairly high content of antitoxin for a short time only; it follows that the chances of finding a horse with a good antitoxic content but poor basal immunity are relatively small. It is not possible to allot any figures to such a general representation as that given in Fig. 2, nor can any one such scheme represent all the variations in history that must occur among horses. Consider a horse with no detectable antitoxin: such a horse might have had any one of four distinct immunological histories. It might never have been infected and so be non-immune; it might be at the stage of the latent period following a primary stimulus; it might have responded at some earlier time to a primary stimulus, but its antitoxic value would have fallen owing to absence of further infection; or finally it might have had many past infections but none for a considerable time. An animal which has had many stimulations may maintain an appreciable antitoxic level over a period of several years, so that very few horses without circulating antitoxin would fall into the last group, and the probability is that the majority have had few or no infections. This reasoning also applies to all horses with low normal diphtheria antitoxic values (below about 0·01 unit): all are immune, but most have probably not received many or frequent stimulations. As the observed value becomes higher, it becomes increasingly probable that the horse has had multiple infections, because the falling off in antitoxic value is less marked after each response to stimulation. We do not know what level could be reached as the peak of a primary response to natural infection, but it is unlikely to exceed 0·01 unit and is probably lower. Work still in progress suggests that a very large number of natural infections is necessary to produce a primary response. It appears reasonable to suppose that a normal value of 0·2 unit could not be the result of a primary stimulus, but there is a chance that such a value might represent the peak of a single

secondary response. It is unlikely that many horses with high normal values fall into this class, for if they did, bleedings taken a short interval before any injections have been given should show well-marked changes in antitoxin content; it is our custom to test two bleedings from each horse at an interval of from 3 to 7 days, and such changes have seldom been found. In the majority of cases, therefore, it appears that low normal values must be the result of few infections and high normal values the result of many infections. The question arises as to whether any particular lower limit of normal value should be set in the choice of horses for hyperimmunization. Table 3 shows the percentage numbers of horses, grouped according to normal value, which reached 800 units or more of diphtheria antitoxin. Great differences occur, for whereas only 28 % of those starting below 0·01 unit reached 800 units, 60–70 % of those between 0·01 and 0·1, and 80 % of those at 0·2 or higher, were

Table 3. *Showing the relation between the diphtheria antitoxic content of normal horses and the results of hyperimmunization*

Normal value (units per c.c.)	Total no. of horses	Successful immunizations (800 upwards units)	
		Percentage no. of horses	Average value (units per c.c.)
Under 0·01	25	28	1485
0·01	59	65	1314
0·02	97	60	1422
0·04	147	67	1253
0·1	118	68	1266
0·2	115	80	1289
Over 0·2	82	82	1504

successful. The last column in the table gives the average value of the successful horses in each group: this figure does not increase with increasing normal value, but remains sensibly the same, the difference between the highest and lowest values being about 20 %. The conclusions to be drawn from the table are first, that a horse of any normal value is capable of producing equally high-value antitoxin, and second, that the proportion of those which actually do so is much greater in the high normal-value groups than the low. The 28 % of horses of low normal value which reached 800 units or more were probably horses whose normal value had fallen because of lack of recent infection, and might also include good responders from groups less well grounded in immunity. The 18 % of high normal-value horses failing to reach 800 units would be the bad responders of a well-grounded population and horses near the peak of a secondary response at the time of the normal-value test, thus giving an 'apparent normal value' removed from the stable level.

There is another important matter upon which normal values have a bearing: this is avidity, or quality of antitoxin. Antitoxin varies in quality, and bleedings from some horses are of better quality than those from others. A good-quality or avid antitoxin combines rapidly and firmly with toxin to form a stable combination, but a poor-quality or non-avid antitoxin combines so loosely that dilution is sufficient to free toxin from the complex. Differences in avidity have been demonstrated for many types of antitoxins, and can in some cases be correlated with the relation between the antitoxic value obtained by the flocculation test and the apparent value obtained in animal tests, the ratio between which is referred to as the 'serum ratio'. Glenny & Llewellyn-Jones (1931) showed that the serum ratio was a measure of the avidity of diphtheria antitoxin; this was also demonstrated for tetanus antitoxin by Glenny & Stevens (unpublished). The *in vivo* and *in vitro* values of the majority of samples of diptheria antitoxin agree to within 10 %, but non-avid sera give *in vivo* results which vary according to conditions of testing and which are lower than those obtained by flocculation, owing to dissociation occurring on dilution after injection. The serum ratio is therefore used as a routine measure of avidity. Table 4 shows the proportion of horses producing diphtheria antitoxin of different grades of avidity, grouped according to the normal value. Only 0·9 % of horses starting with normal values of 0·2 unit or more produced very non-avid antitoxin compared with 17·4 % of those starting with 0·001 unit or less. Slightly better quality but definitely non-avid antitoxin was produced by 11·6 % of horses with the high normal values compared with 30·4 % of those with the lowest normal values. Antitoxin with a serum ratio between 0·85 and 0·94 is described as possibly non-avid because some of these ratios would fall within the limit of error of routine testing. At the other end of the scale we find that 14·3 % of horses starting with 0·2 unit, 7·8 % of those with from 0·02 to 0·1, 3·4 % of those with 0·002 to 0·01, and none starting with lower normal values produced antitoxin with a serum ratio of 1·15 or more.

A comparison between the figures given in Tables 3 and 4 suggests a relation between quantity and quality of antitoxin produced: this relation is shown in Table 5, which gives the serum ratio of a number of batches of tetanus antitoxin divided according to the antitoxic strength of the serum and to the length of rest between primary stimulation and hyperimmunization. Horses given little rest, i.e. those whose hyperimmunization started before basal immunity was well established, produced mostly non-avid antitoxin, although some were of high value. Given longer rest some horses produced non-avid low-value antitoxin and others high-value

Tables 3 and 4 show that horses with little or much natural diphtheria antitoxin react to immunization in a manner similar to that of horses immunized against tetanus toxin after a short or long rest. In the absence of all knowledge of the interval since a horse received its initial diphtheria stimulus, we can conclude, on consideration of Fig. 2, that considerable time must have elapsed since the majority of horses with high normal antitoxin received their first stimulation. Thus it can be definitely accepted that time is essential for the establishment of good basal immunity. The results recorded for tetanus hyperimmunization

Table 4. *Showing the relation between the diphtheria antitoxic content of normal horses and the avidity of the antitoxin produced as a result of hyperimmunization*

		Percentage no. of horses with normal antitoxin			
Serum ratio	Degree of avidity	0·001 or less	0·002 to 0·01	0·02 to 0·1	0·2 or more
Under 0·75	Very non-avid	17·4	7·5	2·3	0·9
0·75–0·84	Non-avid	30·4	18·5	14·5	11·6
0·85–0·94	Possibly non-avid	34·8	37·8	25·6	22·2
0·95–1·04	Average	14·5	23·6	37·0	35·1
1·05–1·14	Possibly avid	2·9	9·2	12·8	15·9
1·15–1·24	Avid	0·0	1·7	5·8	5·5
1·25 or more	Very avid	0·0	1·7	2·0	8·8
Total no. of horses		69	119	484	328

We use the expression 'avid' here to indicate antitoxin with a high serum ratio. Such antitoxin may not be of greater avidity in the sense of firmness of combination than that with a serum ratio of unity marked in the table as 'average'.

Table 5. *Showing the influence of rest after preliminary immunization upon both the quality and the quantity of tetanus antitoxin subsequently produced by hyperimmunization in horses*

Length of rest.	Average serum ratio of bleedings reaching (i.u. per c.c.)					
Months	Under 400	400–600	600–1000	1000–1500	1500–2000	Over 2000
Under 3	0·73	0·74	0·78	0·90	0·93	0·86
3–6	0·64	0·77	0·79	1·07	1·13	1·33
6–9	—	—	0·80	1·10	1·18	1·32
9–12	—	0·82	1·08	1·18	1·19	—
Over 12	—	—	—	1·12	1·23	1·23

avid antitoxin. When the resting period was extended to 12 months only high-value avid antitoxin was produced.

After preliminary stimulation the potential immunity of an animal increases independently of its antitoxin content. Fig. 1 shows that guinea-pigs produce more diphtheria antitoxin after a second antigenic stimulus given 3 months after a single primary stimulus than those given two similar doses at an interval of 2 months or 1. Tables 1 and 5 show that horses given a primary stimulation many months before the commencement of the main immunization produce more tetanus antitoxin and of better quality than those without so long a rest period.

show that excessive stimulation before potential immunity is well established is not only effort wasted, but harmful in that it appears to damage the antitoxin-producing mechanism of an animal. It is probable that small stimuli such as those received in natural infection do not have a similar harmful effect, but it is possible that harm may be caused to the progress of immunization of children if two or more injections of diphtheria prophylactic are given at short intervals.

It is now generally recognized, at least for diphtheria toxoid, that different batches identical in strength may differ widely in immunizing power. The actual quantity (measured by antitoxic titre) and quality (measured by serum ratio) of antitoxin

produced in horses depend upon the strength and some as yet undetermined quality of the toxins and toxoids used for immunization. False conclusions might be drawn if analyses of results were made over long periods, when the policy of starting tetanus immunizations with shorter rests or of using horses for diphtheria immunization with a low content of natural antitoxin, coincided by chance with a period of poor toxin production. All the analyses of horse immunization given in this paper cover periods when little variation could be detected in either strength or quality of the toxins or toxoids used.

We mention in passing that most tetanus antitoxic sera prepared before the method of rest was established were non-avid, and international standards prepared from such sera were equally non-avid. Unpublished work by Glenny & Stevens shows that the apparent value of a non-avid antitoxin depends upon the constitution of the test toxin. Higher apparent values are obtained if the toxoid/toxin ratio is high. It follows, therefore, that in the past tetanus antitoxins were tested by comparison with variable standards, and different results were recorded by different workers testing the same antitoxins but using different test toxins.

SUMMARY

General immunological principles appear to be of wide if not univeral application, and can be applied equally to the hyperimmunization of horses for the production of therapeutic sera, or to human immunization against diphtheria and tetanus. A wider knowledge of the results obtained in animals, chiefly guinea-pigs, rabbits and horses, can help in a better understanding of all that is involved in the immunization of children against diphtheria.

The response of an animal to an antigenic stimulus depends almost entirely on its previous experience of that antigen. If it has had no previous experience, antibody is produced slowly and in small amounts. If it has had previous experience, a change occurs in its antibody-producing mechanism which enables it on further stimulation to produce antibody more rapidly and in larger amounts. To produce this effect the early experience must be adequate and followed by a sufficient period of rest. It is shown that the normal antitoxic values of animals can give considerable information on their previous experience of antigen; that this information can be made use of in the hyperimmunization of horses; and that inadequate primary stimuli and failure to provide adequate rest may lead to the production of antitoxins of low value and poor quality.

Two things are necessary for defence against toxins: circulating antitoxin and a high reactivity of the antibody-producing mechanism (potential immunity). Methods of immunization should be chosen so as to provide both these essentials.

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