IMMUNOLOGICAL CONSIDERATIONS IN THE PREVENTION OF DIPHTHERIA

BY

MOLLIE BARR, M.Sc., A.R.I.C.,

AND

A. T. GLENNY, F.R.S.

The Wellcome Research Laboratories, Langley Court, Beckenham, Kent

Before the days of extensive immunization against diphtheria, the majority of adults living in towns were actively immune; their blood contained antitoxin and their cells were able to produce more antitoxin, probably within a few days of infection. Newborn babies were also immune, but theirs was a passive immunity transmitted from their mothers, and their cells were therefore not able to produce antitoxin rapidly. The passive immunity was gradually lost and active immunity could only be acquired by the haphazard and dangerous way of contact with living toxigenic organisms. Artificial immunization is neither haphazard nor dangerous, but it has reduced the duration of immunity by lessening the chances of natural immunization. In the past the many cases of diphtheria among young children caused subclinical infections in others, establishing active immunity in some previously non-immune and boosting titres of those already immune. There was some degree of truth in the idea then prevalent "once immune always immune." Since the advent of mass immunization we can now only say "once well immunized always potentially immune." Potential immunity does not necessarily include the presence of circulating antitoxin, but implies the ability to produce some antitoxin within a few days (usually five to ten) in response to stimulation by a boosting dose of prophylactic or by toxin produced as a result of infection. We are strongly of the opinion that circulating antitoxin as well as good potential immunity is necessary for complete protection against diphtheria.
Many people do not fully realize the changed immunity status of the population and its implications, although attention has been drawn to the matter in recent papers and correspondence. In this connexion we refer to a sentence from a paper by Hertley (1949). "The present position of almost assured protection can so deteriorate that, with an inadequately immunized juvenile population and an adult population in which immunity has declined, an extremely serious position may develop." A further comment by one of us (Barr, 1950) reads: "Any conclusions as to the suitability of the Schick-negative state as a criterion for safety against clinical diphtheria, can only have been drawn at a time when epidemics were occurring, and the antitoxic values of the population were receiving intermittent boosts and were actually considerably above the Schick level." Reference to the work of Dudley, May and O'Flynn (1934) shows the very high values that existed in a closed community in which diphtheria was endemic.

THE SCHICK LEVEL

The "Schick level" of circulating antitoxin was originally regarded as being 0·03 unit per ml., and persons who were Schick positive were assumed to have titres below this level and Schick-negative subjects to have higher titres. The evidence on this point was not very convincing. Several of the papers published were by workers who obviously had had little or no experience in testing antitoxic values, and frequently titrations were made on blood withdrawn seven days after the Schick test. Since the advent of more extensive and more precise titrations of antitoxic values in human sera it has been generally recognized that the Schick level is considerably lower than was originally supposed. Thus Dudley et al. (1934) concluded that the critical level was about 0·01 unit per ml. The general evidence available now shows that although a few persons with as little as 0·001 unit per ml. may fail to give a positive reaction and a few with 0·01 unit may react, the majority with 0·002 unit are Schick positive and the majority with 0·004 unit are negative. It is clear then that persons who are only just Schick-negative have very low titres of circulating antitoxin: there are many persons with such titres today.

It is possible that different batches of toxin conforming to Schick's original definition varied so much in composition that greatly different levels of antitoxin have been detected by their use. The original definition was that a Schick dose contained one-fiftieth of a guinea-pig fatal dose of a matured toxin. A guinea-pig fatal dose is not an exact measurement, because guinea-pigs are far more susceptible to toxin in the winter than in the summer. "Matured toxin" is a vague term and may cover wide variation in toxoid content, affecting the amount of antitoxin needed to neutralize any dose fixed by reference to measurement of toxicity alone. The British definition of a Schick dose (recommended by The Wellcome Laboratories) is that it should be just neutralized by 0·001 unit of antitoxin, the toxin and antitoxin being mixed together and injected intracutaneously into a guinea-pig. The toxicity is defined between limits that ensure enough toxin to produce a good but not
excessive reaction in susceptible persons. A warning should be given against comparing figures for Schick positive or negative rates in other countries with those in Great Britain, because the British definition is not universally accepted: in the U.S.A. the dose is still defined solely in terms of toxicity and it is possible that the use of a dose in 0·1 ml. instead of 0·2 ml. (used in this country) may give a slightly different result.

The Schick test can only reveal the presence or absence of a small amount of antitoxin, without any indication of whether the individual has just passed the test or has a margin of 10, 100 or 1,000 times the minimum necessary amount of antitoxin. In any community the number of individuals negative to the test gives no indication as to their probable antitoxic values. Recent titrations on over 2,000 random samples, made by us in collaboration with various colleagues, have shown that a very large scatter of antitoxic values exists among the adult population. These values range from the lowest detectable level 0·001 unit to 20–50 units per ml., though values exceeding 5 units are comparatively rare. A Schick-negative person could therefore be expected to possess any antitoxic titre between 0·004 and 5 units per ml. or possibly higher. We are of the opinion that before artificial immunization became general, when the population relied upon natural infection to stimulate immunity, the great majority of Schick-negative persons had high titres of circulating antitoxin and most Schick-positive persons had none: in other words few persons were only just Schick negative. A different state of affairs prevails today. Recently published figures by Hartley and his colleagues (1950) show that considerable numbers among the child population of Tyneside and Dundee, though they had received courses of prophylactic injections, had titres round and only just above the Schick level 0·004 unit per ml. It appears from this report that of 199 cases of clinical diphtheria in immunized children in Dundee, nearly half had antitoxic values just above the Schick level (0·004, 0·01 and 0·02 unit per ml.) at the time of sampling. It is possible, however, that these titres were lower at the time at which infection actually occurred, and may have risen in response to toxin produced in the early stages of the disease before the blood samples were taken.

In the past it was generally thought that Schick-negative persons very seldom contracted diphtheria. The experiences in more recent epidemics both in this country and in Denmark and other European countries have shown that this is probably now untrue. At all events many "immunized" persons have contracted the disease, and although it is seldom or never fatal to them, severe cases have occurred among them (Hartley et al., 1950). Parish and Wright (1938) thought the Schick level was too low "to ensure safety," and Hartley (1949) stated: "It is quite likely that it may be necessary to estimate higher antitoxic concentrations in human populations than that hitherto indicated by the Schick test." Such higher concentrations could be detected by an adjustment of the toxoid content of the test toxin without increasing its toxicity. It would be necessary to introduce some form of purified toxoid in order to avoid severe "non-specific" reactions.
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The Schick Test and the Pseudo-reaction

The Schick test consists of the intradermal injection of 0.2 ml. of Schick toxin into the left forearm and 0.2 ml. of control fluid into the right forearm. The control fluid in use in this country is a portion of the same Schick toxin dilution which has been heated to 80° C. for half an hour to destroy the toxin. In Canada a dilution of diphtheria toxoid is used as Schick control. Although the heating destroys the actual toxin, certain other metabolic products such as bacterial protein are little changed, and give rise to a pseudo-reaction in some persons. It is generally accepted that a pseudo-reaction indicates that the person in question possesses antibodies to some metabolic product of the organism usually distinct from the specific toxin. The diphtheria pseudo-reaction is thus of the same allergic type as the tuberculin reaction, because it does not occur in a normal unsensitized person: the interaction of antigen and circulating antibody gives rise to the reaction. Schick toxin itself, however, causes a reaction in a normal non-immune person, or in one immune but without sufficient circulating antitoxin to neutralize the dose: thus a reaction to the toxin indicates that any antitoxin that may be present is below a certain amount, but it does not necessarily imply the absence of immunity.

Fortunately the two types of reaction can usually be distinguished by the relative rapidity of development and disappearance of the pseudo-reaction in contrast to the persistence of the positive Schick reaction: the pseudo-reaction is usually less intense than a positive Schick, but is raised. On the first and second days after injection it is frequently difficult to decide whether reactions are positive and pseudo or negative and pseudo, but at the end of a week the pseudo-reaction has usually disappeared while a positive Schick reaction may be very marked. Except in very young children the control test should always be done, and a positive control reaction is a warning to read the toxin reaction with care on more than one occasion. A severe pseudo-reaction usually denotes a high antitoxic content even though the toxin may produce a larger reaction than the control.

A positive and pseudo state is relatively uncommon; and it seems questionable to us whether it is worth while to immunize such persons, because they are liable to develop severe reactions after an injection of prophylactic, and the antibody which they already possess may constitute some useful form of defence against infection.

According to Dudley (1929) pseudo-reactions are found most frequently among diphtheria convalescents, recently recovered cases, carriers, and inhabitants of places where diphtheria is or has recently been prevalent (in that order). We do not know how frequently they occur in artificially immunized persons.

Active Immunization: Some General Considerations

Although some active immunization was performed in this country as long ago as 1921, it is only during the last ten years that it has become a general procedure here.
The aim of active immunization should be the laying down of sound potential immunity, the production of good antitoxic titres and the maintenance of some antitoxin in the circulation for many years after the course of injections of prophylactic.

The principles of immunization were all originally worked out in animals and two of the most important ones are as follows.

(1) The Primary and Secondary response phenomenon (Glenny and Sødmersen, 1921): In animals which have had no contact with infection and no previous injections of prophylactic, a single injection of prophylactic is followed by a latent period of about three weeks, and the maximum immunity is reached in about eight weeks. In immune animals, whether naturally immune or artificially immunized, a single injection of prophylactic is followed by a latent period of about four days and the maximum immunity is reached in about ten days: this great and rapid immunity response offers a striking contrast to the small and gradual response to the primary stimulus.

(2) The effect of interval between injections (Barr and Glenny, 1945): Although a secondary response (i.e., a rapid production of antitoxin) is usually obtained from an animal reinjected three to four weeks after a primary stimulus, a better response (higher antitoxic value) is obtained if the interval between the two injections is increased. While this is true in the majority of cases, it does not hold when a very small first dose is given.

The times of appearance and of maximum concentration of antitoxin given above vary according to the dose injected. With small doses no antitoxic response may follow a single injection of prophylactic, but good potential immunity may be developed, as a result of which a high antitoxic titre may appear in the circulation after a second injection. With a very large single dose, antitoxin may appear as early as twelve days after injection in a non-immune subject.

The procedure in common use at the present time in human prophylaxis is the injection of a certain dose (0.2, 0.3 or 0.5 ml. of A.P.T.) followed a month later by a similar injection, usually 0.5 ml. It is generally considered that the intramuscular route is preferable to the subcutaneous route both as regards the immunity produced and the smaller liability to reactions. This procedure is adopted for all persons, whether young babies, school children or adults, but there is a tendency to inject smaller doses into adults than into young children because of their greater liability to develop reactions.

From the immunological standpoint dosage should be considered in relation to body-weight. We are strongly of the opinion that at present infants are the only persons who are satisfactorily immunized against diphtheria; the information gained by work on babies should be used in the consideration of procedures proposed for older persons.

IMMUNIZATION OF BABIES

It has long been known that babies born of mothers immune to diphtheria inherit antitoxin which is transmitted to them in utero. This antitoxin is
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It has also been known for many years that a negative Schick reaction in very young babies does not necessarily signify the presence of circulating antitoxin. Within the last few years quantitative estimations of antitoxic content in cord blood samples have been made in Sweden by Vahlquist and in this country by us in collaboration with Dr. K. J. Randall, Pathologist at Farnborough Hospital. By testing blood samples taken from the babies at chosen times we found that, like other passively conferred antitoxin, the maternal antitoxin was lost at a steady logarithmic rate. Knowing the cord blood value, it is possible to calculate at what age the baby's antitoxic titre will reach any given level.

In experiments on immunization we found that if a first dose of 0.5 ml. of A.P.T. were given when the baby still possessed more than 0.04 unit per ml. of maternal antitoxin, immunization was unsatisfactory. A similar conclusion was reached by Vahlquist (1949). This amount of circulating antitoxin was enough to interfere seriously with the action of the A.P.T. When the maternal antitoxin had fallen to 0.04 unit per ml. or less at the time of the first injection, immunization was satisfactory. In assessing the success of immunization, we titrated the antitoxic values of blood samples taken from the babies about two months after the second injection of A.P.T.; this was given, on an average, eight to nine weeks after the first injection. Fig. 1 shows the distribution of values of samples from 43 babies, all of whom had less than 0.02 unit per ml. of residual antitoxin and whose ages ranged from 6 to 26 weeks at the time of the first injection of A.P.T. These results are taken from one of our publications (1950) and satisfy our criterion of successful immunization, which is that the logs of the values should be normally distributed over a small range.

In any population, some individuals respond to immunization better and others worse than the majority, but differences are small when the course of

![Fig. 1.—The distribution of antitoxic values in babies two months after the second injection of A.P.T.](image-url)
immunization is good; this is shown by the small number of groups a to e in fig. 1. If smaller doses had been given or the interval between them made shorter, we should expect that most babies in groups d and e would have responded almost as well, but those in a and b would have produced much less antitoxin and some might have been below the Schick level. The poor responders of a population account for the failure to obtain 100 per cent success in large-scale work on immunization, and are mainly responsible for the later Schick relapse rate, because many of them only attain antitoxic values about two to five times the Schick level soon after immunization. With smaller doses we should expect to obtain an irregular distribution of values showing a tendency for the good and bad responders to separate into two populations. Fig. 2 shows the effect of dosage upon response in guinea-pigs;

![Graph](http://example.com/graph.png)

**Fig. 2.**—The effect of dosage upon antitoxic response.

A, distribution of values in guinea-pigs after 2 doses, each 1 Lf of A.P.T.

B, distribution of values obtained after 2 doses, each 1 Lf (0.02 ml.) of A.P.T. and resembles the distribution shown for well-immunized babies in fig. 1. Fig. 2b shows the much greater scatter of values obtained when only one-tenth of the dosage was used: in addition to the greater range of values in the main distribution there is a second population of very poor responders, some of which failed to produce any detectable antitoxin. Though the actual values reached two months after immunization may give some idea as to the relative times that antitoxin will remain in the circulation, the rate of loss of actively produced antitoxin depends on the opposing factors of loss in general metabolism and continued production which may go on for years after the last injection of prophylactic. This continued production is probably less in bad responders than in good responders.
It is interesting to note the relation of the actual values in fig. 1 to that which would be detected in the Schick test. The four babies with the lowest titres had about ten times as much circulating antitoxin as that required to make them just Schick negative. Very low standards of successful immunization are measured by the Schick test if it is done a few months after the last injection; most children who are only just Schick negative two months after immunization would be expected to relapse to the Schick-positive state six to twelve months later. It should be the aim for a course of prophylactic injections to produce such good immunity that all persons receiving it should be Schick negative for some years afterwards. Unfortunately there are indications that, in the immunization of adults, the Schick conversion rates may be sufficiently low that a Schick test could be considered desirable, or even necessary about two months after the last injection of the course, so that those persons who are still Schick positive can be detected and receive further injections before any benefit derived from the course is lost. We can only conclude from the results of our early work on babies that satisfactory immunity had been established by the preliminary course of injections and we do not yet know at what stage a boosting dose should be given.

In the course of the work we reached the conclusion, on small numbers of babies, that higher titres were achieved in those first injected before the age of 6 months, if the second dose of A.P.T. were given ten to eighteen weeks rather than six to nine weeks after the first. This was not so in older babies and there was a suggestion of poorer response among them. We thought it possible that this effect was due to the reduced first dose in relation to weight. At the present time it is officially recommended that babies should receive their first dose of prophylactic at the age of 8 months. This recommendation is based on the belief that little or no maternally-conferred antitoxin would remain at that age. The second injection is recommended to be given one month later and the dosage used varies, some workers giving two doses of 0·5 ml. and others 0·3 ml. followed by 0·5 ml. The injection of a small dose followed by a larger one is immunologically unsound and has presumably arisen as a result of the procedure frequently used in adult immunization. Because of the greater liability of adults to experience reactions after prophylactic injections, the use of a small first dose in them is justified. Babies, on the other hand, tolerate a dose of 0·5 ml. of A.P.T. without any reaction and there appears to us to be no reason to justify the use of a smaller dose. The larger the first dose the better the potential immunity produced, so that a greater and more rapid response will follow a second injection.

We are of the opinion that better results would be obtained in nearly all babies if the first injection were given at the age of 3 months. Over 80 per cent of babies have less than 0·04 unit per ml. of maternal antitoxin at this age and could therefore be satisfactorily immunized by two doses, each of 0·5 ml. of A.P.T. We suggested that all babies, except those born of potentially immune mothers further immunized during pregnancy, could be successfully immunized by three injections, given at the age of 3, 6 and 18 months. The advantages of
early immunization are the possibility of giving a larger dose (in relation to body-weight) which, in conjunction with an interval of three months before the second injection, would bring about the establishment of better potential immunity and increased antitoxin-production. Reactions are rare or unknown in very young babies and no adverse emotional disturbances are shown by them when they are injected.

IMMUNIZATION OF SCHOOL CHILDREN AND ADULTS

During the early part of the immunization campaign started during the recent war, many thousands of school children were immunized against diphtheria. The prophylactic most commonly used was A.P.T. and the dosage officially recommended was 0·1 ml. followed four weeks later by 0·3 ml.

The immunological state of school children and adults differs from that of babies, because they have no passive antitoxin to interfere with the action of prophylactics, but they may have acquired some active immunity through chance contact with infection. This active immunity might not include the presence of circulating antitoxin, but would confer upon the person possessing it a power or response superior to that of a person possessing no such potential immunity. This probably explains the frequent finding of an unsymmetrical distribution of antitoxic values among adults with comparable immunization histories; it is not unusual to obtain a set of values distributed round a certain level and another smaller set distributed round a much higher level. The latter group may well represent the values of persons who either had some potential immunity before a course of prophylactic injections, or received further stimulation from natural infection after immunization.

There is but little information on the efficacy of immunization in school children apart from Schick conversion rates. These were determined on a fairly extensive scale in the early stages of the campaign and it was found that while many batches of A.P.T. gave Schick conversion rates of over 95 per cent, a few gave very much lower figures. This may have been due to the use of phenol in the preparation of A.P.T. by some manufacturers who also set themselves far too low a standard of potency in regard to both flocculating equivalents and immunizing efficiency in guinea-pigs. At that time the authorities had not formulated new regulations governing the potency of a prophylactic, such as A.P.T., which was to be used in two doses instead of three. The unsatisfactory figures for Schick conversion rates, obtained after the use of these insufficiently potent preparations (referred to in one of the medical journals as “a.p.t.”!), caused the authorities to institute uniformity and a higher standard of antigenic efficiency among batches of A.P.T. At the same time we were asked to publish full details of the methods of preparation and testing of the A.P.T. prepared at the Wellcome Laboratories (Barr, Pope, Glenny and Linggood, 1941).

The Schick conversion rates were mostly determined early, two to four months after the last injection of the course, and the test of satisfactory
immunization was therefore not very stringent. These relatively early tests were useful, however, in revealing, in good time, the failures we have described. They also serve as a basis of comparison for the efficacy of recent immunization procedures in school children and adults. Fulton and colleagues (1942) obtained a Schick conversion rate of 97.8 per cent among school children at Oxford, following two injections, 0.1 ml. and 0.3 ml. of A.P.T. This figure was contrasted by Vollum and Wilson (1947) with their own finding of 86.1 per cent. Schick conversion rate among 36 nurses three months after the second of two injections, 0.2 ml. and 0.5 ml. of A.P.T. prepared at the same laboratories as the A.P.T. used by Fulton et al. When three injections, each 1.0 ml. of T.A.F. were given at fortnightly intervals, the Schick conversion rate among 32 nurses was only 68.7 per cent. Vollum and Wilson, commenting on this lower rate in adults as compared with children in whom a smaller dosage was used, stated that the result serves to confirm the general experience that adults are more difficult to immunize than children.

So long as there is a chance of natural immunity being acquired as a result of subclinical infection, it is reasonable to suppose that the older groups of non-immune persons contain a larger proportion of refractory individuals than the younger age-groups. Adults would have had a longer period than small children during which natural immunity might have been acquired, and it is probable that some of them received natural stimulation at some earlier time, but, being poor responders, had failed to acquire any useful immunity. In this particular comparison, the dosage in relation to body-weight would be about the same for the children and the nurses, but if a first dose of 0.5 ml. of A.P.T. is needed to give satisfactory immunity in a young baby, less than half this dose could hardly be expected to develop good potential immunity in the average adult. The biggest problem in immunization of adults against diphtheria arises from the fear of producing reactions in them after large injections of prophylactic. Two injections separated by a short interval might be a way of increasing the primary stimulus; consequently the old-fashioned practice of giving weekly injections of prophylactic may not have been as unsound as it appears, although three weekly injections would not be expected to be very beneficial. Two doses given at an interval of a week would have the same immunological effect as a single dose in a non-immune person, and a third injection could be given three to four weeks or longer after the second to act as a secondary stimulus. A great deal of work could profitably be done on adult immunization, and indeed some such work has been done in Denmark by the immunologists at the State Serum Institute; they concluded that three injections were needed to obtain a high degree of success. Since it is so much easier to lay down good immunity in infancy, it is hoped that eventually full immunization of adults will not be necessary, and only an occasional boosting dose be needed. Young babies can tolerate much larger doses than adults, and so there is no difficulty in establishing good basal immunity in them; when basal immunity is good, subsequent boosting doses can be small.
**Boosting Doses**

The generally recognized function of a boosting dose is to stimulate antitoxin production in persons whose titres have fallen some years after a course of immunization. It appears very probable, however, that in diphtheria immunization the boosting dose has to fulfil a much wider purpose by also reinforcing the inadequate potential immunity established by the preliminary course of injections. This was the case in one of us (M. B.) who, after a first boosting dose of A.P.T. gave a very slow antitoxic response, reaching a low maximum value two months after injection, and steadily falling thereafter so that the value was once again below the Schick level nine months after the so-called boosting dose. Somewhat better, but by no means good, results were obtained after a second boosting dose: in neither instance did antitoxin appear in the circulation by the seventh day after injection. This slow response may occur frequently in persons in whom the preliminary course of injections fails to produce good potential immunity, and the low maximum titre achieved may not be reached until four to eight weeks after the dose: such persons need a complete re-immunization rather than a boosting dose. In persons with good potential immunity (who frequently, however, also possess circulating antitoxin) antibody-production is sometimes initiated as early as the fourth or fifth day after a boosting dose.

The importance of boosting doses cannot be overestimated, since many of those in need of them are apt to be slow to respond to stimulation and thus may not produce antitoxin in sufficient quantity or with sufficient speed to enable them to resist infection. While a relatively small dose can boost the antitoxic titre satisfactorily, a large dose may be very much more efficient, in reinforcing potential immunity, and in maintaining an adequate level of antitoxic content for a long period. Some work on the effect of type and dosage of prophylactic used in boosting doses for young adults is now in progress at King’s College Hospital, where Dr. A. C. Cunliffe is working in collaboration with one of us (M. B.). It will be several years before any final conclusions can be drawn in this work because of the need to examine blood samples over a long period in order to follow the duration of immunity. It is hoped that in course of time it may be possible to institute a simple test that can be used to detect those persons who need re-immunization rather than a single boosting dose, and the information obtained in this work should throw light upon the degree of efficiency of recent methods of basic immunization. It should be mentioned that in any research on human immunization much valuable information is lost if the earlier immunization histories are not available: they are obviously essential for working out the optimum number and spacing of boosting doses. This subject, so far as we know, has never received serious attention. That higher and more durable antitoxic titres are produced in man after a third injection of tetanus toxoid was shown by Evans (1943). We ourselves have shown (1947) that there is a definite relation between the percentage of tetanus or diphtheria antitoxin remaining in the blood of hyper-immunized horses a year after the last injection, and the number of immuniza-
tions the horses have undergone. We are of the opinion that active immunization against tetanus is a much simpler proposition than active immunization against diphtheria, at any rate in adults. Better immunity is established in the preliminary course of injections in tetanus immunization, and frequent boosting doses can be given with much less fear of unpleasant reactions than is the case in diphtheria prophylaxis. It is therefore a relatively simple matter to maintain a high degree of tetanus immunity by the giving of boosting doses at definite intervals. At present, however, it appears to be inadvisable to give boosting doses of diphtheria prophylactic unless they are needed. It is in this connexion that the Schick test is of the greatest value. Any immunized persons who have relapsed to the Schick-positive state should receive a boosting dose unless they have also given a marked reaction to the Schick control fluid. The "positive and pseudo" state, as we mentioned earlier, is fortunately comparatively rare.

At the present time it is recommended that children should be given a boosting dose (usually A.P.T.) at the age of 5 years on starting at school. The reason for the choice of this particular time is obvious: children from the age of 5 onwards will be going out into the world and may be exposed to infection. There is no evidence as to whether this time is suitable from the immunological standpoint and much work would be needed in order to investigate the point. Nevertheless such work should be done, because ill-spaced boosting doses produce but little lasting benefit in persons who were not well immunized beforehand. Unfortunately any investigation on this subject could not include the use of the Schick test, because of its well-known boosting effect in some persons. Cases have been recorded of Schick-positive subjects with 0·002 unit per ml. of circulating antitoxin acquiring titres of 0·1 unit per ml. three to four weeks later, this antitoxin having been produced as a result of stimulation by the Schick toxin. Thus frequent Schick testing of the same persons, if carried out in order to determine how long after immunization they relapsed to the Schick-positive state, would give misleading results. In practice however, in routine work, the Schick test can serve a very useful purpose not only in detecting those that need a boosting dose but in helping to maintain antitoxic titres by providing small frequent boosts. A Schick-negative result does not indicate how long an individual can safely be left before a boosting dose should be given, but the stimulating effect of the test itself may extend the time.

**The Future Aims of Immunization**

We are of the opinion that circulating antitoxin as well as good potential immunity is necessary for complete protection against diphtheria.

When a person becomes infected and develops the disease, toxin is produced by the organism and in course of time becomes fixed to the tissues, as a result of which clinical symptoms appear. In the prevention of any disease caused by the formation of toxin by an infecting organism, it appears dangerous to rely upon rapid antitoxin-production by the host, following stimulation from toxin produced in infection. This seems especially dangerous in the case of diphtheria which has a very short incubation period usually stated as being
two to four days. The incubation period is the time elapsing between the date of infection and the date of appearance of symptoms, but fixation of toxin to the tissues may occur some time before symptoms appear. It appears to us that circulating antitoxin is essential to neutralize and so prevent fixation of the toxin produced in the initial stages of infection. The production of antitoxin as a result of stimulation by toxin formed by the infecting organism is unlikely to be initiated before the fourth or fifth day following this toxin production, and in persons with poor potential immunity it is more probable that seven to ten days elapse before any antitoxin is produced. By this time sufficient toxin may have been produced by the organism and fixed to the tissues, to have caused the development of severe symptoms.

Strong evidence in support of this reasoning is to be found in the report of Hartley et al. (1950) who found that considerable titres of antitoxin were produced in some persons after admission to hospital as cases of clinical diphtheria.

We are of the opinion that good antitoxic titres at the time of infection are of much greater importance than good potential immunity, because the latter alone is unlikely to afford complete protection though it may ensure that illness should be relatively mild. In any event good antitoxic titres, unless produced as a result of very recent immunization, seldom exist without well-developed potential immunity.

The present and future aims of diphtheria immunization must be the maintenance of sufficient antitoxin to ensure complete protection into adult life. It is very significant that in epidemics in Norway and Denmark there was a heavy incidence of cases among the adult population, suggesting that antitoxic values had lapsed in the absence of recent infection and the adult population had become relatively unprotected. It is possible that this would also occur in Great Britain in the event of an epidemic in the near future.

Now that the carrier rate has become greatly reduced, it appears that we can no longer rely upon “natural boosting” for the maintenance of antitoxic values, and better methods of artificial immunization should be instituted. We have shown (1947) that continued antitoxin-production can occur for many months or years after the last injection of an antigen has been given. It now remains to find the way to bring about this state in human prophylaxis.

We are convinced that immunization must be started in early infancy, and that possibly in the present state of knowledge a relatively crude prophylactic such as A.P.T., containing products of metabolism other than specific toxoid, should be used for the first injection. Doubt has occasionally been expressed as to whether modern highly purified antitoxin is as efficient therapeutically as the old unconcentrated serum, suggesting that other specific and useful antibodies might have been removed in the concentration process. If there is any truth in such assertions it would seem desirable to include products of metabolism, other than specific toxoid, in prophylactics. On the other hand, P.T.A.P. (made from purified toxoid) appears to be a more potent primary stimulus than A.P.T., but this prophylactic is still in the experimental stages.
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It seems very probable that the ideal course of immunization should include the use of more than one type of prophylactic. For the preliminary course one or two injections of a precipitated or adsorbed antigen might be used, while later doses could consist of purified toxoid. This would be expected to diminish the chances of reactions after boosting doses, although as shown by Pappenheimer and Lawrence (1948) some persons develop allergic reactions after injections of highly purified toxoid.

There are many gaps in our knowledge at the present time, and these can only be filled by a series of long-term investigations. The Schick test cannot be used in research work; the information given by the result is very limited and the possible boosting effect caused by the injection of the test toxin may lead to misleading conclusions at a later stage. In all investigations blood samples should be examined at definite chosen times after injections have been given and the immunization details carefully checked. As pointed out by Dudley, such work can only be done by the collaboration of an "anti-diphtheria worker" with an experienced immunologist.

We are fortunate in collaborating with hospital pathologists, but much can also be done by the joint work of enthusiastic field workers in close touch with experienced research workers. The former should have at their disposal human subjects willing to assist, possibly for some years, in the investigation by submitting to a few injections and to periodic bleedings. The immunologist must have facilities for antitoxin titrations on a large scale and considerable knowledge of immunization procedure gained from animal experience. It is essential for laboratory workers to be able to deal with their side of the work from a quantitative standpoint and at least one of the team should be able to submit their combined results to statistical analysis.

After the establishment of a satisfactory procedure, only periodic checks would be necessary to ensure that the methods in use were still sound, although general conditions of natural stimulation might have changed in the course of time. If such methods had been adopted in the early days of immunization, the effect of the reduction in natural stimulation from infection would have been fully realized some time ago.

REFERENCES

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