THE SCHICK REACTION

AND DIPHTHERIA PROPHYLACTIC IMMUNISATION WITH TOXIN-ANTITOXIN MIXTURE.*

BY A. T. GLENNY, B.Sc., K. ALLEN, AND R. A. O'BRIEN, M.D., D.P.H.

(From the Wellcome Physiological Research Laboratories, Herne Hill, London.)

THE Schick reaction and diphtheria prophylaxis by active immunisation with toxin-antitoxin mixtures together constitute the greatest advance in our knowledge of the problems associated with diphtheria since the introduction of antitoxin. It is probable that when public opinion is sufficiently familiarised with these two methods and their significance, they will come into universal use and there will follow the same reduction in the occurrence of diphtheria that has been brought about by Jennerian vaccination for small-pox. Dr. Park of New York advocates the injection of all children between the ages of 6 months and 2 to 3 years. It is probable that the adoption of this method would lead in a very short time to the disappearance of diphtheria in the community. The methods are, therefore, of importance to those responsible for public health and to the immunologist.

We owe the intradermic skin reaction to Schick, and the active immunisation (which was foreshadowed many years ago by that prolific worker, Theobald Smith) to von Behring. Most of the work in this field has been carried out by von Behring and his colleagues in Germany, and by Park and Zingher in New York. It is a pleasure to acknowledge the generous manner in which Dr. Park and his colleagues have placed their material at our disposal.

Technique of the Reaction.

The Schick test consists in the intradermic injection of 0·2 c.cm. of a dilution of diphtheria toxin containing in 0·2 c.cm. 1/50 m.l.d. for a guinea-pig. The quantity of toxin actually used is about 0·0004 c.cm. of an ordinary aged toxin. When we use a concentrated toxin similar to one recently prepared by Dr. P. Hartley, we inject about 0·000 016 c.cm., which contains 0·000 000 02 g. of nitrogen.

Four types of reaction are shown: "negative" given by people with antitoxin in their blood; "positive" by those without antitoxin. The positive consists in a persistent red flush at the spot where the unheated toxin was injected, whereas no reaction appears where the heated toxin was given. A third group ("pseudo") give a reaction, but have antitoxin in their blood. These people respond to the injection of broth in which diphtheria bacilli have been grown, and which has subsequently been heated to 75°C. for 10 minutes. This heated toxin contains practically no "true" toxin; it produces no reaction in the skin of a guinea-pig and large quantities can be injected subcutaneously into guinea-pigs without harming them. The response given by these people is called a pseudo-reaction and is due to some unknown constituent. It is commonly stated to be due to bacillary protein, but we cannot find any authority for this statement and we propose to investigate the truth of it. The fourth group ("combined") consist of those who are sensitive to toxin and also to the "pseudo" constituent. At the site where the heated toxin was injected they show a reaction, while at the site where the unheated toxin was injected there appears a much larger reaction, which is the sum of the "true" toxin reaction and the pseudo-reaction. These people are not immune to diphtheria. (We are emphatically of opinion that in carrying out the test injection of toxin alone may lead to dangerous confusion. It is imperative that the control, heated toxin, be injected into one arm and the unheated into the other.)

The pseudo-reaction is rather puzzling. Park and Zingher in their very large experience have not found it in infants or young children whether immunised or not; it begins to appear at 5–10 years of age, and becomes much more frequent in adult life. If it is a specific response to the protein of diphtheria bacilli, then one would expect it to appear more or less in parallel with the immunity to diphtheria toxin, but it does not do so. It does not appear for certain in those who have had diphtheria and recovered, and who months later may have antitoxin in their blood. It does not occur in the babies either of those mothers who show a pseudo-reaction or of those who do not. One German observer records a series in which 10 of a group of 20 mothers showed pseudo-reaction, whereas none of the 20 babies did so. The cell sensitiveness is therefore not transmitted diaplacentally. The concentrated toxin referred to earlier unfortunately produced a pseudo-reaction, although its nitrogen content is extremely low. Ordinary toxin boiled for 30 minutes has produced a pseudo-reaction in one of us.

Preparation of Toxin for the Schick Test.

We have hitherto advised the issue of diluted toxin only for several reasons. There is an error in "filling" capillaries with toxin, as described by Zingher last year. We are of opinion that the clinician is liable to introduce an error in dilutions owing to the difficulty of getting the whole of a minute drop of toxin, which is about 1/50 of a c.cm., into the diluting fluid. Earlier in our work a batch of toxin diluted in the laboratory sometimes failed to pass our guinea-pig test. It is evident that the conditions must be very rigidly controlled, or the dilution of toxin will not be of standard strength. A serious mistake might arise if practitioners used weak toxin and, because of erroneous reactions, concluded that certain patients were immune and might be safely left exposed to infection—e.g., in a school epidemic—or, on the other hand, might be safely left without the injection of antitoxin though suffering from membranous sore throat. (Recent work makes us hope that we have succeeded in finding a stable dilution.)

We therefore make a toxin dilution each week and test it intracutaneously on normal guinea-pigs. We find the m.r.d.—i.e., the minimal dose that will produce a reaction when given alone—and also the dose that, when mixed with varying quantities of antitoxin, will give a reaction. Our current toxin dilution ready for the Schick test, when further diluted 20 times, causes a reaction in the guinea-pig's skin; 0·1 c.cm. when mixed with 0·0004 of a unit of antitoxin and injected intradermically, must also produce a definite reaction.

Stability of Diluted Toxin.—The details of tests presented show that the diluted toxin when kept at 0°C. undergoes practically no deterioration in four weeks, whereas after seven weeks a loss of about 30 per cent. in value is found. After seven days at 15°C. the loss in potency is just detectable, while after 14 days the loss is about 30 per cent. When kept at 37°C. the dilution deteriorated seriously in one day.

Reliability of the Test.

During the past few years we have tested some of the laboratory staff many times and have obtained consistent results. Park and Leete found in repetitions of tests on the same patients by the same observer a discrepancy of a few per cent. only. We were interested to observe that most if not all of these discrepant observations relate to patients who had been positive, and, later, became negative. We have experimental evidence that the very small amount of toxin present in the Schick test dose will produce an increase in the antitoxin concentration of the blood. The details of some experiments on rabbits show that five days after the injection of a Schick test dose the antitoxic content of the rabbit's blood had risen above the level at which the Schick

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reaction fails to appear in human beings. If the same effect, as shown, had been produced in human beings, the first Schick test would have given a positive result, whereas another test of five days or more later would have given a negative result.

The Schick test is roughly quantitative. A positive reaction does not indicate the total absence of immunity. The test does not divide the population into black and white, but rather into those darker or lighter than a certain shade of grey. If Schick had originally determined on 1/500 of an m.l.d. instead of 1/50, it is practically certain that numbers of people who give a positive response to the present standard Schick would give a negative reaction with the weaker dilutions, and would be considered immune to diphtheria. Roughly speaking, people with at least 1/30th of a unit of antitoxin per c.cm. of blood give a negative reaction, while those with less than 1/30th, or with no antitoxin at all, give a positive reaction. As the result of clinical experience Schick decided that it was safe to assume that people with 1/30th of a unit were immune to clinical diphtheria.

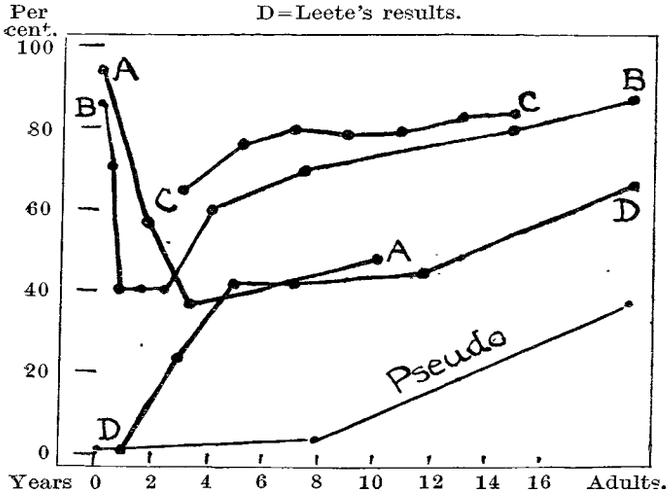
Results Obtained.

Recent observations on 18 members of the laboratory staff showed that 10 of them contained less than 0·0005 of a unit of antitoxin per c.cm. of blood —i.e., they probably contained none at all. Eight contained from one-fifth of a unit to 1 unit. Of these eight, six gave pseudo-reactions. Observations made from 1913 up to the present show that some of the laboratory staff have in their sera a greater concentration of antitoxin now than they had seven years ago. It was very interesting to find that three people, who had had abundant chance of becoming infected while working in diphtheria wards, gave positive reactions and had no antitoxin in their sera. It would appear that non-immune people may escape infection, and a suggestion arises that there may be some protective mechanism, bactericidal or otherwise, in addition to the antitoxin defence. There may be such an additional defence, but, on the other hand, we are told that in the history of infectious diseases hospitals many resident medical officers who have worked for years in diphtheria wards have assumed that they were immune, but have, later, succumbed to diphtheria. Our colleagues, presumably, did not suffer from the disease because they were fortunate enough to escape the implantation of bacilli on their mucous membrane.

The only clinical observations published in England are those made by Leete. His figures are of the same

Age Distribution of Negative Schick Reactions.

A=Schick's results. B and C=Park's results. D=Leete's results.



order as those of Park. Leete has published a series of observations in England. His results on patients over 4 years old, in the main, agree with the results of other investigators, shown on the graph. The discrepancies between Leete's observations on very young children are probably due to the smallness of the number of patients available. It may be that the differences in level between the curves obtained by Park and these other investigators are due to differences in population or to the use of a slightly different strength of toxin. We have some experimental evidence to suggest that the prescribed standard of "1/50 m.l.d. for a guinea-pig" may not be precise enough. We have not, however, completed our observations on this point.

Toxin-antitoxin Mixture.

The toxin-antitoxin mixture we have used complies with the American Government regulations. Although von Behring does not give the full details of his mixture, it is fairly certain, from the perusal of various German articles, that his mixture contains relatively more antitoxin than the American and ours. One would expect the antitoxin response of injected patients to be less than after the use of the American mixture, and so far as we can judge from the German literature, this is what is found. In 1 c.cm. of mixture, there are three L+ doses of toxin and about 3·5 units of antitoxin. A sample test of our current batch showed that three guinea-pigs injected with 1 c.cm. were all alive and well 30 days later, while of 17 guinea-pigs injected with 5 c.cm. of the mixture two died of an intercurrent disease (probably *B. Gaertner* infection) which was epidemic at the time, and 14 showed diphtheritic paralysis, most of them dying between the twentieth and twenty-ninth day. The injection of the toxin-antitoxin mixture into normal rabbits produced a satisfactory degree of immunity within 8–12 weeks. A quicker method of testing the antigenic suitability of a mixture consists in injecting it into a rabbit already possessed of a low degree of immunity. In one rabbit thus injected the concentration of antitoxin per c.cm. of blood rose from 0·004 units per c.cm. to 0·08 units four days later, and in six days to 0·5 units.

With regard to the period that elapses before an injected child becomes immune, it has been found that about 90 per cent. of children who have received three weekly injections of 1 c.cm. of a mixture give a negative response to the Schick test three months later. A negative response indicates a concentration in the blood of at least 1/30th of a unit of antitoxin. It is quite possible that a much lower content of antitoxin reached within a period of some weeks in the blood of a child who has been actively immunised recently would be sufficient to produce a considerable degree of protection against clinical diphtheria. Experimental evidence obtained by one of us (A. T. G.), and as yet unpublished, convinces us that the first minute amount of toxin conveyed into the system of the patient from the diphtheria bacilli that had gained a lodgment in the throat would produce an immediate and great increase of antitoxin in the patient's blood sufficient to neutralise further quantities of toxin produced by the attacking bacilli.

Conclusion.

Unfortunately we cannot bring forward a long series of results of the injection of toxin-antitoxin mixture. English physicians in charge of schools, institutions, and infectious disease hospitals have apparently been too busy up to the present to be able to investigate these two methods. In America the Schick reactions that have been carried out number many tens of thousands, and thousands of active immunisations have been done. The main purpose of this paper is to try to induce those who are fortunate enough to have large clinical opportunities to apply these two methods. We hope that some investigations may be carried out in London, for we believe that until much more experience has been gained in England, it would be very desirable for the laboratory investigator and the clinician to work in close coöperation. We may by working together succeed in explaining difficulties and discrepancies that are bound to be met with when new methods such as these are first used.