water vehicles and has proved to be very economical, easy to perform, reliable and consistent in results, and is adaptable to the testing of materials with organic matter as well as those without.

In the examination of materials selective for gram negative organisms, as acriflavine, the colon bacilli can be very properly substituted as a test organism, since its resistance is equally high and constant.

At present the tests are performed at room temperature, since staphylococci and diphtheroids are not as variable to slight changes of temperature as the less constant typhoid bacilli. But if a constant temperature is required, it probably can be secured very easily by making a simple water bath, heated by electric lamps and regulated by an easily made mercury contact system.

OBSERVATIONS ON DIPHTHERIA TOXOID AS AN IMMUNIZING AGENT

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BOTH diphtheria toxoid and diphtheria toxin-antitoxin derive their immunizing potency from altered diphtheria toxin. The freshly prepared toxin has such an instant action on the human tissues that only minute amounts can be used, and immunization with it is therefore a long drawn out process.

Diphtheria toxoid was used for animal immunization in the first experimental work in producing antitoxin and it has continued to be used to some degree ever since.

The utilization of a toxin-antitoxin mixture by von Behring led to the use by us and others of toxin-antitoxin rather than toxoid. During the past three years Glenny and Hopkins[1] in London and Ramon[2] in Paris have been suggesting the substitution of toxoid for toxin-antitoxin. Independently of them we[3] had begun to test the value of toxoid preparations.

Diphtheria toxin can be changed to toxoid by various means, such as storage at moderate temperatures, heating to 50 to 54° C. for a few hours, or exposure to chemicals.

The accepted method at the present time is to add 0.2 to 0.3 per cent of formalin to the toxin, the amount depending on the protein content and the duration of storage in the incubator.

The advantages claimed for the toxoid are that it is simpler to prepare; is more stable; is somewhat more potent, and it contains no horse serum globulin. In our opinion toxoid is no easier to prepare than toxin-antitoxin. Experience and the following out carefully of every detail of a proper method are necessary in both cases. To the inexperienced a toxoid is easier to prepare because it is a single substance instead of two substances and the difficulty of making a proper mixture is avoided.

The question of stability is more debatable. The longer a toxin is stored, and to a certain extent the greater the percentage of change from toxin to toxoid, the less rapid will be any further change.

The toxin used for toxin-antitoxin is really a mixture of toxin and toxoid. If the toxin-antitoxin mixture is made so that when mixed it has just the right toxicity, it will drop off in potency faster than a toxoid preparation, but if it is made with some excess of toxicity and stored for several months before it be-

comes suitable for use it will then remain as stable or nearly as stable as the toxoid preparations.

The objection that toxin-antitoxin has a minute amount of horse serum globulin, and as shown by Hooker suffices to cause a moderate serum sensitization, has some weight. It can be obviated by following the suggestion of Hooker of using goat antitoxin. Banzhaf has already prepared such a preparation, and this has been used in a number of children by Schroder and Zingher. Re-tests have not yet been made. We have never seen any dangerous reactions following the use of serum in those who previously had toxin-antitoxin. We feel certain that there is no objection to giving previously injected children an intramuscular injection of serum. We need more experience before being as sure as to the effect of this slight degree of sensitization on the reaction to an intravenous dose. There is a tendency to attempt to use a toxoid or a toxin-antitoxin preparation that has no toxic effect on guinea pigs. This is very effective in animals, but has a decided drawback. Thus the toxoid sent us by Dr. O'Brien from the Burroughs Wellcome laboratory can be given in 5 c.c. doses to guinea pigs, and has remarkable immunizing potency in this dose. In man, however, one-half a c.c. is advisable because the pseudo reaction to the bacillus proteins makes too severe a local reaction. It practically is the same as the old 3 or 5 L plus preparations. The comparative reactions from 1 c.c. and from ½ c.c. of the non-toxic toxoid and 1 c.c. of the 1/10 L plus toxin-antitoxin are shown in Table II. Glenny and Hopkins and Ramon believe that the amount of absorption of antitoxin by a preparation is a pretty exact index of its immunizing power.

This may be true as between different preparations made in the same dilutions from the same materials, and as far as possible in the same manner, but it is far from true if used to test preparations made in different ways and of different degrees of toxicity. The larger the amount of the original toxin in the human dose, the greater will be its antitoxic neutralizing value.

Table I shows the relative absorption of antitoxin by a human dose and the relative potency of different toxoid preparations, as judged by the immunizing re-

TABLE I

Preparation	Human Dose	Units of Antitoxin bound by the test dose	Toxicity in 250 grown guinea pigs	Human Doses used as test injections in guinea pigs	After one Dose and one Schick test Percentage of 15 guinea pigs giving a negative Schick reaction at end of four weeks
B. Wellcome Toxoid 1923	0.5 c.c.	3.5	5 c.c. no effect	2.0	70
Same 8 mos. later	0.5 c.c.	3.5	5 c.c. no effect	2.0	60
B. Wellcome Toxoid 1924 preparation	0.5 c.c.	3.5	5 c.c. no effect	1.0	35
Banzhaf 0.25% formalin Toxoid 437	0.5 c.c.	2.5	5 c.c. sl. paralysis	2.0	76
Toxoid 350	0.5 c.c.	2.5	5 c.c. died 18 days	2.0	70
Banzhaf 0.1% formalin Toxoid 437Z	0.1 c.c.	0.37	0.2 c.c. sl. paralysis	1.0	15
Toxoid 377Z	0.1 c.c.	0.20	0.2 c.c. sl. paralysis	1.0	50
Banzhaf heated 54°C. 5 hrs. Toxoid			5 c.c. no effect	1.0	24
Toxin-antitoxin as comparison T.A. 110 0.1L plus			1 c.c. paralysis 21 das.	0.5	85
T.A. 69 0.1L plus			5 c.c. paralysis 27 das.	1.0	65
T.A. 86 0.1L plus			1 c.c. paralysis and recovery	0.5	10
T. A. Solution of precipitate from mixing Toxin and Antitoxin					
Precipitate of toxin 377 Banzhaf			1 c.c. kills 30 days	0.2	80
Precipitate of toxin 517 Banzhaf			1 c.c. slight paralysis	1.0	100

TABLE II

COMPARISON BETWEEN THE REACTIONS DUE TO THE SUBCUTANEOUS INJECTIONS OF THE NONTOXIC TOXOID (BURROUGHS WELLCOME & CO.) AND THE STANDARD 1/10 L PLUS TOXIN-ANTITOXIN IN CHILDREN (SCHRODER).

29 children (Schick + 10 Schick — 19) receiving 1 c.c. of toxoid. Subcutaneous reaction + 16; ± 9; ∓ 3; — 1.

23 children (Schick + 9 Schick — 14) receiving 0.5 c.c. of toxoid. Subcutaneous reaction + 5; ± 12; ∓ 3; — 3.

29 children (Schick + 5 Schick — 24) receiving 1 c.c. standard 1/10 L plus toxin-antitoxin. Reaction + 6; ± 6; ∓ 4; — 13.

Average diameter of the 1 c.c. toxoid reaction 5 cm., 1/2 c.c. toxoid 1.7 cm., 1 c.c. T-A 1.7 cm. One c.c. of undiluted toxoid gives a larger percentage of pseudo-reactions and more intense reactions than the 1/10 L plus T. A. The 1/2 c.c. dose of toxoid gives a greater number of marked pseudo-reactions but of no greater intensity than the 1/10 L plus T.A.

sults in guinea pigs of a single injection followed by a stimulating Schick test at the end of the third week and a final Schick test at the end of the fourth week. A completely negative Schick test is considered as indicating immunity.

The results of these tests indicate that the best toxoid preparations we have at present are nearly, but not quite, as effective as the best toxin-antitoxin preparations. If only the non-specific proteins could be removed, larger doses of toxoid could be used, and even better results obtained.

RESULTS OF IMMUNIZATION IN MAN

One c.c. of original toxoid 377 diluted 1 in 14 to 1 in 20, given to 659 children originally Schick positive, when re-tested by Zingher four to six months afterwards gave 448 negative reactions, or 68 per cent effective results. Schroder gave three injections of the same preparation to 187 adults with 87 per cent apparent success. As control tests were not used by Schroder with the first injections this should be reduced to about 80 per cent.

With toxoid 437, Zingher got better results—1,525 children giving a positive reaction received three injections of 1 c.c. of a 1 to 20 dilution of the original toxoid. At the re-test two and one-half to three months later, 1,342, or 88 per cent, gave a negative reaction.

Comparing the results we find that toxoid 437, containing a larger amount of antitoxin neutralizing substance, gave the better results in man, but the force of this observation is lessened by the fact that in the test on guinea pigs the reverse result was obtained.

The local and constitutional effects of the diluted slightly toxic toxoid as noted by Zingher and Schroder were very similar to those following the standard 1/10 L plus preparation of toxin-antitoxin of the same toxicity.

The reaction following the use of nontoxic toxoid was wholly due to the proteins, as there was no active toxin present. In Table II a comparison between the Burroughs and Wellcome toxoid and toxin-antitoxin is given.

SUMMARY

At the present time toxoid preparations have been developed which are nearly, if not quite, equal to the best toxin-antitoxin preparations. The toxoid preparations which have no toxicity in guinea pigs cannot be properly used in human beings in doses larger than ½ c.c. because of the marked pseudo-reactions. Toxoid that is slightly toxic can be diluted at least ten times and so reduce the amount of proteins. It is a question whether the slightly toxic diluted toxoid preparations are as stable as the nontoxic ones. Intensive experimental work should be done in both toxoid and toxin-antitoxin preparations so as to be able to constantly have thoroughly potent and stable preparations. After one or two years we should be able to decide which has the greater advantages.

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