THE ROUTINE PREPARATION OF SCARLET-FEVER TOXIN OF HIGH VALUE.

R. A. Q. O'MEARA.

From the Wellcome Physiological Research Laboratories, Beckenham, Kent.

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A number of attempts have been made in recent years to improve the quality of scarlet fever toxin, of which the most noteworthy is that of Hooker et al. (1933), who by a special technique obtained toxin with a value of 500,000 skin test doses per c.c.

For some time past, however, the routine toxins prepared in this laboratory have been of considerably greater potency than those described by the authors quoted above, and as the routine preparation of toxin of high value is of considerable practical importance, it is thought that an account of the method which we employ may be of interest.

Theoretical Considerations.

The first essential for good toxin production is a strain of high toxigenicity. The organism which we employ is *Str. scarlatinae* (Strain Dochez N.Y. 5), which has proved its worth in these and other laboratories.

The second essential is a medium which provides adequate growth and suitable nitrogenous constituents for the elaboration of toxin. Streptococci utilize carbohydrate as their chief source of energy, and are among those organisms which do not cause reversal of reaction. In other words, they rely for their growth mainly upon fermentable carbohydrate, from which they produce relatively large amounts of acid, principally lactic (Langwill, 1924). Unlike such organisms as *C. diphtheriae*, they do not oxidize the lactic acid which they produce. It therefore accumulates and, as it is a fairly strong acid, causes a rapid fall of pH in an inadequately buffered medium, and so tends to limit growth. It has been found by experience that there is sufficient carbohydrate in horse muscle digest to supply the energy requirements of the organism, so that this type of medium forms a satisfactory basis on which to work. To enrich the nitrogenous constituents of the medium the addition of peptone has been successful, and for the neutralization of the lactic acid formed during growth we employ sodium bicarbonate.

Preparation of the Medium.

The basic medium is a "straight line" 6-hour digest of horse muscle similar to that used by Pope and Smith (1932) for the preparation of diphtheria toxin of high value, but hydrochloric acid is used instead of acetic for acidification prior to boiling. The fermentable carbohydrate is not removed by the addition of yeast when digestion is complete. Instead 1 p.c. of peptone is added, the reaction is adjusted to pH 8·2, and phosphates are precipitated by
adding calcium chloride. Sodium bicarbonate 0·2 p.c. is then added, and the medium is sterilized by filtration through a battery of Berkefeld N candles into 4-litre bottles, which are filled nearly to capacity. A batch of medium prepared in this way may be up to 100 litres in volume, and keeps well in a cool dark place for a period of several weeks.

**Toxin Production.**

For toxin production a batch of medium which has been prepared in accordance with the above directions is warmed by placing it in the incubator at 37° for 24 hours, and is then inoculated liberally from a vigorous eight-hour culture of streptococci in the same medium. Growth is permitted to continue for 18 hours from the time of inoculation, and the toxin is then harvested by passing first through pulp filters, and later through Berkefeld N candles. The organism gives, in this medium, a constant opacity of six to seven thousand million per c.c., and the reaction falls from an initial pH of 8·2 to an average figure of 6·8.

**Constituents of the Toxin.**

The chief constituent by which a scarlet fever toxin is judged is the “erythrogenic toxin”. It is probable, however, that for the production of scarlet fever sera of greatest therapeutic value, toxins should be employed for immunization in which the other known constituents of haemolytic streptococcus filtrates, such as the streptolysin investigated by Todd (1932), and the flocculating antigen described by Dyer (1925), receive due consideration. While greatest weight is, therefore, to be given to the erythrogenic toxin, brief consideration of the other components of scarlet fever toxin prepared by the above technique may not be out of place.

**Erythrogenic Toxin.**

Scarlet fever toxin prepared by our routine method contains on the average a million skin test doses per c.c. In the table are set out the results of an illustrative comparison between our toxins and an American Standard Toxin S.T. 2, containing 35,000 skin test doses per c.c. in parallel with a laboratory standard toxin X 48, containing 30,000 skin test doses per c.c. Two individuals of different degrees of sensitiveness to the toxin were selected, and doses of 0·1 c.c. of S.T. 2, 1/700 dilution (i. e. 5 S.T.D.) and 1/3500 dilution (i. e. 1 S.T.D.) were injected intradermally. Similar doses of the laboratory standard X 48 1/500 dilution and 1/3000 dilution were injected, and for comparison two toxins, X 641 and X 644, prepared in accordance with the technique outlined in this paper, 0·1 c.c. of 1/100,000 and 1/500,000 dilutions. The reactions were read at 17 hours and 24 hours. The 24-hour readings were in the same order as the 17-hour readings, but by then most of the reactions had begun to fade.

The reactions obtained with 1/100,000 dilutions of X 641 and X 644 were much stronger than any of the others in subject B.

Reference to the table will show that in both individuals the reactions given by 0·1 c.c. of the 1/500,000 dilutions of the routine toxins are of the same order as those given by 1 S.T.D. of the standard toxins. The reactions given by the 1/100,000 dilutions are greater than those given by 1 S.T.D., and
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Skin Tests of Various Toxins on Two Individuals "A" and "B".

Dose intradermally 0.1 c.c.

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f. = faint flush.  b. = bright flush.  r. = raised reaction.  Numerals = diameters of reactions in mm.

approach those given by 5 S.T.D. of the standard toxins. The routine toxins therefore contain between one and five million skin test doses per c.c., a potency much in excess of that usually considered satisfactory for a scarlet-fever toxin.

**Combining Power.**

The combining power of the erythrogenetic toxin may be determined in terms of antitoxin by inoculating toxin-antitoxin mixtures intradermally into rabbits. I am indebted to my colleague, Dr. Buttle, for estimating the combining power of these toxins by means of a test, the details of which will shortly be published. He has found that the combining power of the new toxins X 641 and X 644 is 5 to 10 times that of the American Standard Toxin S.T. 2, and of the laboratory standard X 48, prepared by the older methods.

**Rabbit-killing Power.**

The routine toxins prepared as above have a consistently high lethal value for rabbits when injected intravenously according to the manner of Parish and Okell (1927). A dose of 5 c.c. of these toxins injected intravenously into rabbits over 4 lb. in weight has been found to kill the majority in 48 hours or less.

**Hæmolysin.**

The usual scarlet fever toxins contain little or no demonstrable hæmolysin of the type described by Todd (1932). In toxins freshly prepared by our routine method this hæmolysin is well represented, and after reduction with sodium hydrosulphite they may be shown to contain, when titrated with sheep cells, up to 50 M.H.D. per c.c., a value little inferior to that obtained by other methods. They have a high combining power value of hæmolysin when titrated with scarlet-fever antitoxin, or antistreptolysin.

**Flocculating Substance.**

The flocculating antigen produced by streptococci in culture is also represented in these toxins. Their value in this respect is not as high as can be
obtained, when desired, by other methods of culture, but it may be considered adequate for purposes of immunization.

SUMMARY.

A method is described for the routine production of scarlet-fever toxin of very high erythrogenic value. The toxin so prepared contains in addition to an abundance of the erythrogenic factor an adequate amount of the other recognized toxic products of the scarlatinal streptococcus.

Our thanks are due to Dr. McCoy and Surgeon-Commander Veldee for the American Standard Toxin S.T. 2, used for purposes of comparison. We are furthermore indebted to several colleagues in England and America who have very kindly tested our toxin on human beings and animals. Some of the results show that our toxin is of the remarkably high value we have indicated, but in some cases significantly lower values have been returned. These lower titrations are probably due to deterioration caused by the phenol used as preservative in the toxin. This point is under further observation. The deleterious effect of phenol on certain other toxins is well recognised.

REFERENCES.

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TYPE-SPECIFIC AND GROUP-SPECIFIC SERA AGAINST STREPTOCOCCI.

H. LOEWENTHAL.

*From the Hale Clinical Laboratories, London Hospital.*

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The problem of producing antibacterial sera of therapeutic value against haemolytic streptococci is contemplated to-day with some pessimism. Apart from the difficulties encountered in the production of sera with adequate protective properties, it has come to be suspected that since strains differ so greatly in serological respects, only strictly homologous sera could confer protection. Such a situation would make practical serum therapy almost impossible.

Since our expectations of the value of scarlatinal antitoxin in the treatment of septicæmia have not been entirely realized, no effort should be spared to