255 (2487)

Properties of purified Dick scarlatinal toxin.

By F. M. HUNTOON.*

[*From the Biological Laboratories, H. K. Mulford Company, Glenolden, Pa.*]

The Dick scarlatinal toxin is the filtrate from the growth in broth of certain streptococci isolated from the throats of scarlet fever cases, as has been shown by the Dick's, Dochez and Sherman.

The toxic substance is produced in the largest quantities after the addition of considerable amounts of horse blood.

The best toxin in our hands has been produced by 7 day growths. It has been found possible to purify such toxins to a very considerable degree. If the material is treated with ammonium sulfate up to 60 per cent saturation, with the addition of 1 per cent acetic acid, a voluminous precipitate occurs, taking with it very little of the toxin. On the addition of more ammonium sulfate, no more precipitate occurs until around 70 per cent saturation. This increases up to about 75 per cent saturation. This precipitate carries with it the toxic substance. Such a precipitate is collected and redissolved in distilled water with the addition of sufficient NaOH to neutralize the acid present and is then dialyzed until free of salt. A product is thus produced which contains three quarters of the original toxicity as measured by the skin test, and contains so little nitrogen as to be within the realm of experimental error.

The second method of purification consists in the addition of 20 per cent sodium chloride, 1 per cent acetic acid, filtering and saving the filtrate. This filtrate is then dialyzed, during which time it about doubles in volume. This is found to have about one half the original toxicity and with the nitrogen content of 50 mg. per hundred cc. as contrasted to 850 mg. per hundred cc. in the original material.

Material purified by the ammonium sulfate method and at the same time concentrated so that a skin test was positive with

* Introduced by T. S. Githens.

1/20 of a cc. of a 1 :6000 dilution, was trypsinized by the addition of 1 :1000 Fairchilds Trypsin in 2 per cent sodium bicarbonate.

A control of the toxin with bicarbonate present, was kept under the same conditions as was the trypsin control, with no toxin present. After 24 hours these were tested at a dilution of 1 :5000 and gave no reaction with the trypsin lot; a plus reaction with the toxin control; and a minus with the trypsin control. These tests were repeated after 4 days, using a stronger dilution of the trypsin lot, with a similar result, so that it may be stated that the toxin is affected by trypsin.

Purified material, which gave a strong reaction in 1 :1000, when heated to 90° for 1 hour in a dilution of 1 :500, gave no reaction at all, even when tested on those sensitive to horse proteins. A dilution of 1 :500, mixed with convalescent scarlet fever serum in equal proportions, incubated for 30 minutes at 37° C., and injected into the skin, gave no reaction.

The toxic substance is totally insoluble in acetone, absolute alcohol, and ether, nor is it inactivated by these materials. Therefore it is not of a lipoidal nature.

CONCLUSIONS.

The toxic substance in the Dick toxin is not of the nature of a globulin, but is precipitated with the higher albumin fractions.

It is a protein substance which is destroyed or inactivated by trypsin.

It is inactivated by heat (90° for 1 hour).

Its tendency to come down in a narrow range of ammonium sulfate precipitation renders a high degree of purification possible. This, in the preparation of material for testing humans for susceptibility, makes it possible to produce solutions with a minimum tendency to produce pseudo reactions.

Lastly, the material is neutralized by convalescent serum, but is not neutralized by normal horse serum or by the serum of horses immunized against the scarlet fever streptococcus in the ordinary manner (non toxic emulsions).