9035

Purification of Botulinus Toxin.

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It was shown[1] that a purified botulinus toxin of fairly high potency (MLD = 10^{-7} gm. per mouse) may be prepared by simple acid precipitation (precipitate I) of filtered culture fluid. Samples of 10-fold toxicity, however, were obtained by dialysis of the filtered acidified mother liquors. Under these conditions a fine sediment (precipitate II) appears which contains practically all the remaining toxicity of the solution and shows a potency of about 10^{-8} gm. per mouse. It is evident from these facts that some diffusible components of the culture fluid are able to hold the toxic substance in solution at a pH of about 4. The high amino acid content of the tryptic digest broths was suspected of being responsible for this effect.

In more recent experiments a peptic digest medium was therefore used as a substrate. According to expectations the toxin could now be precipitated almost completely by the addition of hydrochloric acid without dialysis. This latter somewhat cumbersome procedure can thus be entirely eliminated. The crude acid precipitable material possesses the same high potency of 10^{-8} gm. as found previously. The yield amounts to roughly 50%; very little poison is left in the filtrate. The precipitate may be further purified by dissolution in sodium acetate buffer and reprecipitated with n/10 hydrochloric acid. The most potent product obtained in this manner showed a toxicity of 4.10^{-9} gm. = 2.10^{-7} gm. per kg. mouse. The yield of the reprecipitated product has been variable and not large. It is possible, however, to avoid great losses by taking into consideration that botulinus toxin gradually deteriorates at room temperature and that the rate of inactivation is enhanced by the presence of trivalent anions.[2] Furthermore the pH has to be adjusted carefully to the flocculation point of the protein (pH 4.4) in order to avoid loss of potency in the opalescent supernatant.

This procedure, then, may be recommended for the preparation of relatively large amounts of highly potent botulinus toxin. It makes use of the most simple equipment and reagents. The medium before as well as after growth is acidified in carboy lots. Separation

[1] Snipe, P. T., and Sommer, H., *J. Infect. Dis.*, 1928, **43**, 152.

[2] Sommer, E. W., and Sommer, H., *J. Infect. Dis.*, 1928, **43**, 496.

from the sediment is performed by syphoning at first, and later by centrifuging. It is not necessary to remove the organisms before precipitation, since the bacteria, bacterial proteins and toxin are thrown out of solution in one operation at a pH of 3.5-4.0. After redissolution in sodium acetate the organisms may be removed by centrifuging and filtering through a Seitz filter. The volume, by this time, is small enough to be conveniently handled in liter bottles and centrifuge tubes.

It is reasonable to assume that this method should not be readily applicable to the purification of the acid-sensitive diphtheria toxin, as Eaton[3] points out, although Locke and Main[4] have prepared substances of high titer by fractional acid precipitation. On the other hand there is no apparent reason why potent tetanus toxin should not be obtained in the same manner. In fact, in one experiment performed in 1928, a tetanus toxin was obtained from tryptic digest broth by the dialysis procedure, killing mice in amounts of approximately 2.5×10^{-8} gm. This compares favorably with substances (MLD $= 3.8 \times 10^{-8}$) made recently by Eaton[5] by the more complicated procedure of precipitation with metal salts.

In conclusion it must be emphasized that these highly potent protein preparations should not be considered pure substances. The following fact illustrates this point. A sample of botulinus "toxin" prepared from washed and dried organisms by dissolution in phosphate buffer showed a potency of 10^{-8} gm. of dried organisms per mouse. Since this crude bacterial product was not much less toxic than the purest substance obtained by the above procedure, the chemically pure poison must be assumed to have a potency many times greater.

Summary. A simplified purification procedure, based on acid precipitation, is described for the preparation of highly potent botulinus toxin.

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[3] Eaton, M. D., *J. Bact.*, 1936, **31**, 347.

[4] Locke, A., and Main, E. R., *J. Infect. Dis.*, 1928, **43**, 41.

[5] Eaton, M. D., PROC. SOC. EXP. BIOL. AND MED., 1936, **35**, 16.