

It will be seen that the glycerin-buffer trypsin solutions stored in the icebox are stable over long periods. By slightly increasing the incubation time for digestion (at the most by two or three minutes), the identical end-point may be obtained for an indefinite period (three years at least) with a single standard solution of trypsin.

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PURIFICATION OF DIPHTHERIA ANTI-TOXIN

A PROTEIN has been obtained from equine anti-diphtheria plasma which is highly antitoxic and which satisfies the criteria for a pure protein. The preparation contains about 700,000 to 1,000,000 anti-toxin units per gram protein nitrogen by the Ramon flocculation test and about 700,000 anti-toxin units per gram by guinea pig protection test. This is about 20 times the activity of the original antisera and agrees approximately with that reported by Pappenheimer and Robinson¹ and by Pope² for anti-toxin prepared by a different method. The material is completely precipitated by diphtheria toxin.

Dr. Alexandre Rothen has examined a sample of the protein in the ultra-centrifuge and in the Tiselius electrophoresis cell. Both methods show the presence of only one protein at pH 7.3. A slow migration to the anode is observed at this pH ($\mu_{\infty} = 4 \times 10^{-6}$ cm²/sec. volt). The rate of sedimentation ($S^{26} = 5.77 \times 10^{-13}$) indicates a figure of about 80,000 for the molecular weight. At pH 8.0 the protein migrates also slowly to the anode and is homogeneous. At this pH the material shows some aggregation in the ultra-centrifuge. Salt precipitation shows that some denaturation occurs at pH 8.0 and becomes marked in slightly more alkaline solution.

The solubility of this sample varied slightly, however, with different quantities of solid indicating the presence of more than one protein. A second more highly purified preparation showed constant solubility, independent of the quantity of solid present. The protein therefore satisfies the criteria for a pure protein.

Several samples have been obtained in the form of fine, poorly formed needles and thin plates. The protein has been prepared from three different lots of plasma precipitated with four different lots of toxin.

The method of preparation consists in precipitating the toxin anti-toxin complex. This precipitate is dissolved by the addition of acid and the toxin destroyed by digestion with trypsin at about pH 3.7. The anti-toxin is then purified by fractionation with ammonium

sulfate at pH 7.2. The purified antibody is soluble in 0.50 saturated ammonium sulfate pH 7.4 and insoluble in 0.65 saturated ammonium sulfate.

Twenty-five to 50 per cent. of the original antibody may be recovered after the removal of the toxin. This crude antibody has a titre of from 300,000 to 500,000 units per gram protein nitrogen. It exhibits only one moving boundary in the electrophoresis cell, but there is marked spreading and the solubility test shows this crude preparation to be far from homogeneous.

The toxin and anti-toxin were furnished by the Biological Laboratories of E. R. Squibb and Sons. The animal protection tests were carried out by Dr. W. E. Bunney, at E. R. Squibb and Sons, New Brunswick, N. J.

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¹ A. M. Pappenheimer, Jr., and E. S. Robinson, *Jour. Immunol.*, 32: 291, 1937.

² C. G. Pope, *Brit. Jour. Exp. Path.*, 19: 245, 1938.