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Brief communication

ISOELECTRIC CHARACTERIZATION OF CLOSTRIDIUM BOTULINUM TYPE C TOXIN

The physico-chemical laws controlling natural pH gradients that have been developed by stationary electrolysis have been described in earlier papers by *Svensson* (2, 3). As demonstrated by *Svensson* (4) the resolving power attained by this method is better than 0.2 pH unit. In a later paper (5) the same author described the use of synthetic carrier ampholytes, and it was shown that a still better resolving power could be produced.

The present work aims at making a preliminary characterization of Cl. botulinum type C toxin*) by means of isoelectric focussing. The toxin contained 10^6 mouse LD50 per ml. As carrier ampholytes both synthetic ampholytes and "peptide ampholytes" were used. Basically, the synthetic ampholytes were produced according to the process of electrolysis described in reference (5). The "peptide ampholytes" were produced by papain digestion of a concentrated haemoglobin solution in dialysis tubes. The dialysate was vacuum evaporated to a concentration that was suitable for pre-electrolysis in a multimembrane**) apparatus.

Following electrolysis some 40 fractions were collected from the electrolyzer. The ampholytes from the various compartments of this apparatus were subjected to pH measurements, the solutions then being evaporated at 20°C. For making pH scales to cover the pH interval 2.1 to 9.2 some of the evaporated ampholyte fractions were mixed in adequate proportions. Of the ampholyte mixture 3 g were then dissolved in 20 ml of redistilled water. The solution was boiled for 2 min. since the protein solution had been prepared by means of papain digestion. It was then cooled to 4°C, centrifugated at 20,000 × g (average) for 30 min. and was finally passed through a Sephadex G-50 column in aqueous phase. The first fraction was discarded and the main part of the included fraction was used for preparing 65 ml heavy (with 50 % saccha-

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**) Nylon fabric.

rose) and 65 ml light ampholyte solution. The amount of toxin employed in each electrolysis was 0.05 ml crude material. It was applied by means of a special pipette either in a light solution in the upper part of the column or in a gradient shelf in the lower part. The density gradient curve showed a linear course. On completion of the isoelectric focussing the contents of the column were divided into 2.5 ml fractions. From each fraction 0.5 ml was withdrawn to be used for toxicity tests on mice weighing 16—18 g.

When performing toxicity tests in connection with application of the synthetic ampholytes, which constitute a mixture of low-molecular aliphatic polyaminepolycarboxylic acids, one has to consider the activity within the pH interval 8.0 and up that is caused by the ampholytes in this basic interval.

When making pH scales in the pH interval 2.1—9.2 toxic activity is noticed at pH 3.2 and pH 5.5. These results can be reproduced with reversed electrodes and with either type of ampholyte.

As concerns the interval around pH 5.5 these results have a close resemblance to the results obtained earlier with Cl. botulinum type A toxin; in these investigations, however, the isoelectric point has been determined by the conventional method (1). The result obtained at pH 3.2 is somewhat surprising from the point of toxicology and protein chemistry. In addition, the acid fraction is the more toxic of the two. This fraction also contains butyric acid, yet this fact does not explain the marked toxicity at this pH as the total amount of the test material is only 0.05 ml crude toxin.

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