from the original point of attack of DFP on the protein. Further work is under way to elucidate the sequence of the amino acids in the isolated peptide, the localisation of the DIP group and the chemical composition and properties of the peptide resulting from the alkaline treatment.

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Received December 22nd, 1954

* Cotazym Organon Oss (Holland).

Purification of cholinesterase from ox red cells

In a previous communication[1] a method was reported for the preparation of soluble cholinesterase from ox red cells presenting a 250–400 fold purification. These preparations still suffered from various disadvantages; they were still of limited purity and had a tendency to lose solubility particularly after freeze drying.

A new method will be described which yields preparations approx. 10 times as pure as that of the previous ones which can be satisfactorily freeze dried without loss of solubility.

Analytical methods. As described previously[1].

Procedure. Stroma was prepared as before[1]. Instead of being freeze dried it was now added to one l of phosphate buffer 0.02 *M* pH 8.0 containing 20 g NaCl and 2 ml cotazym (a pancreatic extract purchased from N.V. Organon, Oss; 3 tablets are dissolved in 22 ml of distilled water and centrifuged to remove insoluble material). This mixture is adjusted to pH 8.0 with NH_4OH 25% and after vigorous shaking incubated for 16 hours at 37° C. After incubation the pH is readjusted to 8.0 and the mixture centrifuged in the Servall vacuum centrifuge SS2 (approx. 14,000 rpm). The supernatant is saturated to 0.7 with ammonium sulphate and allowed to stand at 5° C for 1 hour. After centrifugation in the Servall centrifuge the precipitate is dissolved in distilled water. This solution is dialysed overnight against three different lots of distilled water at pH 7.0. The dialysate is centrifuged and the supernatant adjusted to pH 6.2. Alcohol is added to a concentration of 9% *v/v* at —2° C. The precipitate is discarded by centrifugation at 2,000 rpm at —2° C. The alcohol concentration of the supernatant is increased to 32.8% *v/v* at —5° C. The precipitate is dissolved in 100 ml phosphate buffer pH 7 (0.01 *M*).

To samples of one ml of the solution calcium phosphate gel[2] is added until the supernatant on centrifugation is optimal as regards enzyme concentration and purity. Usually two volumes of the gel have to be added in two stages. The pH should be kept at 7.0. The bulk is then treated with calcium phosphate gel according to the results obtained on the sample. The gel is separated by centrifugation and the supernatant saturated to 0.8 with ammonium sulphate at pH 6.5. After standing overnight at 5° C the precipitate is spun down and dissolved in 0.01 *M* phosphate buffer (pH 7.0), dialysed and freeze dried.

Preparations with an average activity of 100,000–350,000 units per mg N were obtained.

To our knowledge the mammalian cholinesterase prepared in this way is considerably purer than any reported in the literature or commercially available.

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Received December 22nd, 1954