A Soluble Protein derived from Elastin

During an investigation of the early stages of hydrolysis of various mucoids and connective-tissue proteins, it was observed that, on partial hydrolysis with acetic acid or oxalic acid at 100°, aspartic acid was in each case the first amino-acid to appear in the free condition, followed at a somewhat later stage by glutamic acid[1]. Since elastin has an unusually low content of the dicarboxylic amino-acids[2], it was thought that, with this protein, hydrolytic cleavage at some proportion of the aspartic or glutamic acid residues might yield soluble degradation products of high molecular weight.

Experiment showed that on extracting collagen-free elastin (prepared from ligamentum nuchæ of cattle) with 0·25 *M* oxalic acid at 100° C. for periods of 1 hr., the whole of the material passed into solution after five or six successive extractions. Removal of the oxalic acid from the mixed extracts by dialysis through 'Cellophane' resulted in the loss of about 5 per cent of the nitrogen as small peptides. Paper chromatograms carried out with the diffusible fraction showed aspartic acid as the only free amino-acid; but since pyrrolidone carboxylic acid (in which form glutamic acid might be found) gives no reaction with ninhydrin, the presence of this compound in low concentration could not be excluded.

The non-diffusible protein was soluble in water at temperatures below 25° C., giving a pale yellow mobile solution. On raising the temperature to 25–30° C. in the presence of dilute buffer (*p*H 4–6), a precipitate consisting of liquid droplets separated. The droplets showed no birefringence under crossed nicols and immediately redissolved on reducing the temperature. On centrifuging at 37° C. the droplets coalesced to form a lower layer of viscous liquid; refractive index measurements showed the two liquid phases to consist of aqueous protein solutions of different concentration.

When dialysed against buffer mixtures of ionic strength 0·20, the protein showed a single symmetrical peak in the electrophoresis apparatus at all *p*H values over the range *p*H 2–9. If the ionic strength was reduced to 0·02, there was partial resolution of two components over the range *p*H 3–5; but under more alkaline conditions no separation was observed at this ionic strength. At ionic strength 0·20 the value for the isoelectric point from the electrophoretic mobility measurements was 3·9, while that from membrane potential determinations was 4·0. On reducing the ionic strength to 0·02, there was a considerable change in the position of the isoelectric point; determinations in the dilute buffer gave 4·9 from electrophoresis measurements and 4·8 from membrane potentials.

The osmotic pressures of different fractions were determined for solutions buffered with sodium phosphate with a *p*H value of 7·4 and an ionic strength of 0·1. The initial solutions gave molecular weights of 60,000–65,000. However, when the initial solution was dialysed against buffer using a highly permeable collodion membrane, the non-diffusible fraction (which represented 60 per cent of the whole) gave a mean molecular weight of 84,000. This indicated that the primary extraction product was not homogeneous. Subsequent fractionation resulted in the separation of a major component (representing about 75 per cent of the protein) which showed the characteristic property of reversible heat precipitation, and a minor component which gave no precipitate at any temperature up to 100° when dissolved in buffer of low ionic strength. This latter component gave a mean molecular weight of 6,000 from osmotic pressure measurements.

In a number of experiments carried out with the ultracentrifuge by Dr. R. A. Kekwick, the primary extraction product and the protein derived from it by dialysis with a highly permeable membrane both gave a single peak at 270,000*g*, the rate of sedimentation being approximately the same in both cases. No indication of the presence of the minor component could be observed in the photographs.

The shape of curves obtained by titrating the protein in the presence of sodium chloride suggests a high content of α-carboxyl and α-amino groups not found in native elastin. This indicates that primary bonds have been broken during the hydrolytic treatment, and the evidence as a whole suggests that the mild hydrolysis has resulted in the fragmentation of the fibrous protein at certain reactive sites, thus liberating a large part of the material as molecules of rather uniform character. Full details of this work will be published elsewhere.

We wish to thank Dr. R. A. Kekwick for his part in examining the protein with the ultracentrifuge, and Mr. J. R. Bendall for a titration study. We have also particularly benefited from discussion with Dr. E. C. Bate-Smith.

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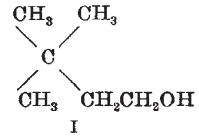
[2] Neuman, R. E., *Arch. Biochem.*, **24**, 289 (1949).

Pharmacological Activity of the Carbon Analogue of Acetylcholine

It has been shown by Adams and Whittaker[1–4] that both forms of mammalian cholinesterase hydrolyse aliphatic esters, those of 3 : 3-dimethylbutanol (I), the carbon analogue of choline (II), being particularly rapidly hydrolysed. Thus human[2] and horse[5] erythrocyte cholinesterase hydrolyse 3 : 3-dimethylbutyl acetate at 1·8 and 1·6 times the rate of acetyl-β-methylcholine respectively. It is therefore of interest to know something of the pharmacological properties of this compound.



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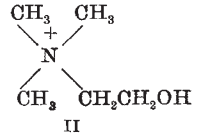

II

Two preparations have been used, namely, the isolated eserinized frog rectus abdominis muscle and the isolated guinea pig ileum. The carbon analogue of acetylcholine, in relatively high concentration, causes a contraction of the frog rectus (see p. 606), but its activity is only about 1/12,000 that of acetylcholine, that is, considerably less than that of choline, which was tested on the same preparation. In the guinea pig ileum, full contractions were never observed. Usually, sufficiently large doses