COMMUNICATIONS

CHYMOTRYPSIN AND ZONULYSIS*

PRELIMINARY COMMUNICATION

BY

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INTRODUCTION

α -CHYMOTRYPSIN is now widely used to facilitate the operation of cataract extraction. Although some preliminary experiments have been reported by Barraquer, the biochemical action of this enzyme on the zonule has not been studied hitherto. It is of great importance to know whether chymotrypsin attacks only the zonule or whether it might also affect other tissues of the eye, particularly the vitreous body, the anterior surface of which would be exposed to chymotrypsin during the enzyme treatment of the zonule.

The proteolytic enzyme, chymotrypsin, has three actions. It splits peptide bonds inside the protein molecule, attacking these bonds at the carboxyl end of aromatic amino acids (endopeptidase activity). By its exopeptidase action it is also able to liberate amino acids which have free amino groups from the end of the protein molecules. It can also hydrolyse certain esters non-specifically (esterase activity).

Very little is known of the chemical constitution of the zonule. Histological methods have demonstrated the presence of a mucopolysaccharide, but the nature of the protein component has not so far been elucidated, in spite of numerous studies by Bairati (1946) who showed that the protein of the zonular fibres differs from other known structural proteins such as collagen and elastin.

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PRESENT INVESTIGATIONS

The following preliminary investigation of the biochemical action of α -chymotrypsin shows that, whilst the enzyme affects the zonular fibres, it also exerts its proteolytic action on other tissues, such as the vitreous body, which may be undesirable.

MATERIALS AND METHODS

α -Chymotrypsin.—This was purchased from the Armour Laboratories, Eastbourne, as a freeze-dried, crystalline product containing 5000 Armour Units per 6 mg.

Estimation of Chymotrypsin Activity.—A modification of the method described by Kunitz (1947) was used; this measures the absorption at 280 $m\mu$ of the aromatic amino acids liberated from proteins by the action of the chymotrypsin.

Lens homogenate, aqueous humour, or vitreous humour was incubated for varying times in a bath at 37° C. together with 1.0 ml. 0.1 M borate buffer, pH 8.0; 0.2 ml. 0.05 M calcium chloride; 0.2 ml. chymotrypsin solution in 0.9 per cent. sodium chloride (50 Armour Units/ml.); and distilled water to a final volume of 2 ml.

The reaction was stopped by precipitating the proteins with 0.4 ml. 5 per cent. $ZnSO_4 \cdot 7H_2O$ and 0.4 ml. 0.3 N $Ba(OH)_2$; the concentrations of these two solutions had previously been adjusted, so that 0.4 ml. $ZnSO_4$ was exactly neutralized by an equal volume of $Ba(OH)_2$ when phenolphthalein was used as indicator.

The precipitated proteins were removed by centrifuging and the optical density of the supernatant solution was determined at 280 $m\mu$ in a Unicam Spectrophotometer.

Parallel samples, one of which was deproteinized at once without incubation and the other containing no chymotrypsin, were used as controls.

RESULTS AND DISCUSSION

Preliminary experiments, using both ox and rabbit eyes, have shown that α -chymotrypsin does not have any visible effect on the appearance of the zonular fibres. Some fibres were carefully excised and observed under a dissecting microscope. There was no change in their appearance even after 40 minutes treatment with a solution of chymotrypsin (50 units/ml.).

It has, however, been possible to demonstrate that the enzyme weakens the zonular fibres. After removal of the cornea and iris, an ox eye was pinned to a cork board and a fine needle was impaled through the lens at its equator. An upward pull on the lens was exerted by a cotton thread connected to both ends of the needle and passing vertically over a pulley to a small pan containing weights. This exerted a slight stretch on the zonular fibres. When a 16-g. weight was used, the position of the lens remained

unchanged for over 3 hours. The zonule was kept moist during this period with 0.9 per cent. NaCl. When the zonule was treated with a solution of chymotrypsin, however, the fibres broke within 20 minutes and released the lens.

Effect of Chymotrypsin on Various Tissues of the Eye.—The proteolytic action of this enzyme was studied on ocular tissues such as the vitreous and aqueous humours and lens homogenate. The zonule was not used in these experiments since it was not possible to dissect it out completely free from the vitreous body.

The ability of chymotrypsin to liberate aromatic amino acids from protein molecules (exopeptidase activity) was used for the estimation of its proteolytic activity. The optical density at 280 m μ was determined before and after incubating various ocular tissues with chymotrypsin. All samples were deproteinized as described under Methods, since the proteins will also absorb at 280 m μ due to their aromatic amino acid content. The results are expressed in terms of an increase in optical density at 280 m μ , and no attempt was made to convert this into actual amounts of amino acid liberated, since equimolar amounts of different amino acids do not have the same optical density at this wavelength.

The Table shows the values obtained after 10 minutes incubation of ocular tissues in the presence of chymotrypsin, as well as that of casein, a protein standard.

TABLE
PROTEOLYTIC ACTION OF α -CHYMOTRYPSIN ON SOME OCULAR TISSUES

Ocular Tissue					Increase D ₂₈₀ /mg. Protein/10 min.
Lens Homogenate	Rabbit	..	..	..	0.02
	Ox	..	..	..	0.06
Vitreous Humour	Ox	..	..	..	0.24
Aqueous Humour	Rabbit	..	..	..	0.16
	Ox	..	..	..	0.25
Casein	..	..	..	..	0.13

It can be seen that a fairly rapid breakdown of proteins occurs in all the

tissues which have been investigated, and that this reaction proceeds linearly with time (Figure).

FIGURE. — Proteolytic activity of chymotrypsin.

It appears from these results that the effect of α -chymotrypsin is not restricted specifically to the zonule as has been suggested by some authors, and it is clear that the enzyme is capable of attacking other structures such as the vitreous body. It is, however, possible that the injection of the chymotrypsin locally over the relatively large surface of the zonule and its rapid removal by washing out, restricts its action mainly to this structure.

SUMMARY

The proteolytic action of α -chymotrypsin on various ocular tissues has been investigated. It has been demonstrated that aromatic amino acids are liberated from the proteins in the aqueous and vitreous humours as well as lens homogenates after incubation with this enzyme.

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