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PROTEOLYTIC ACTIVITY OF VITREOUS-HUMOUR

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It has been found that the bovine vitreous-humour did not digest *in vitro* collagen at physiological and acidic pH. A proteolytic activity against haemoglobin in acid pH was found both in bovine and human vitreous-humours. The activity of human vitreous-humour increased significantly in endophtalmitis and glaucoma. In all pathological conditions studied the pH optima were at more acidic values than in control.

Vitreous-humour of the eye contains various proteins at the total concentration of about 1.5 mg/ml. Both collagens (types II, IX and XI) and non-collagenous proteins (pre-albumins, albumins, transferrin and immunoglobulin G) were identified in this fluid [1-3].

The mechanism of enzymatic degradation of vitreous-humour proteins is not known. Under physiological conditions the collagens are digested only by specific proteases: collagenases, and collagenolytic cathepsins [4, 5].

Our aim was to study wheather vitreous-humour contains a collagenase or collagenolytic cathepsin and a protease(s) which digest non-collagenous proteins.

MATERIALS AND METHODS

Bovine vitreous-humour (v.h.) was obtained as described previously [3] and samples of human v.h. were taken from the material removed during surgical operations of patients with various eye diseases: tumours (5 cases), cataract (7), glaucoma (6) and endophtalmitis with retinal detachment (4). For obvious reasons it was not possible to obtain control material from normal human eye therefore vitreous-humour from an eye removed because of an extensive accidental injury served as a control (it was further referred to as "normal"). The investigated samples were free of pathological material like blood, pus, etc.

Type I collagen was isolated from rat tail tendons as described by Chung & Miller [6]. Types II, IX and XI collagens were kindly supplied by Dr. Shirley

Ayad from the University of Manchester (Great Britain). The collagens were dissolved (1 mg/ml) in 0.02 M acetate buffer, 0.05 M $CaCl_2$, pH 4.8, or in 0.05 M Tris/HCl buffer, 0.05 M $CaCl_2$, pH 7.4. The vitrous-humours were dialysed against proper buffers. The incubation mixtures containing 0.5 ml of each component were as follows: a, collagen solution and a buffer; b, collagen solution and vitreous-humour; c, a buffer and vitreous-humour. All samples were incubated at 37°C for 4 h and dialysed against 0.01 M phosphate buffer, pH 7.0 at room temperature for 24 h. Then the samples were supplemented with sodium dodecyl sulphate (SDS) and 2-mercaptoethanol to 1% concn. and submitted to electrophoresis on SDS-5% polyacrylamide gel [7].

Proteolytic activity was estimated by the method of Worowski & Roszkowska [8], at pH range 2.5-8.5 with denatured haemoglobin as a substrate. The 4% haemoglobin solution was diluted (1:1) with Britton--Robinson buffer of the desired pH. To 0.4 ml of the substrate 0.1 ml of vitreous-humour was added and the mixture incubated at 37°C for 4 h. The reaction was terminated by the addition of 1.25 ml of 5% trichloroacetic acid. The control sample contained the same constituents but trichloroacetic acid was added at "zero time". The samples were filtered and tyrosine content in the acid-soluble fraction was determined by the Folin-Ciocalteau method [9].

RESULTS AND DISCUSSION

It was found that bovine vitreous-humour at pH 4.8 and 7.4 did not induce cdegradation of type I, II, IX and XI collagens. The electrophoretic mobilities of collagens treated with the humour were found to be the same as those of intact substrates. A typical electrophoregram is presented in Fig. 1.

On the other hand, normal bovine vitreous-humour digested haemoglobin at acidic pH with the optimum at pH 4.0. Similar proteolytic activity was found in "normal" human vitreous-humour (Fig. 2).

Both the proteolytic activity and pH optimum of human vitreous-humour differed largely in various pathological conditions. In the cases of eye tumours and cataract this activity was almost the same as in "normal" material but in glaucoma and endophtalmitis it was, respectively, 2-4 times higher (Fig. 2).

These results allow to conclude that vitreous-humour does not contain any collagenolytic activity either at acidic or at neutral pH, therefore it can not degrade its own collagen. It seems that collagen fibrils present in vitreous--humour undergo phagocytosis by hyalocytes or other cells and are digested in lysosomes.

On the other hand, the vitreous-humour is able to digest other proteins. It seems that the injury of various cells of the diseased eye may result in release of some cathepsins into vitreous-humour, and that these enzymes may participate in further damage of eye tissues.

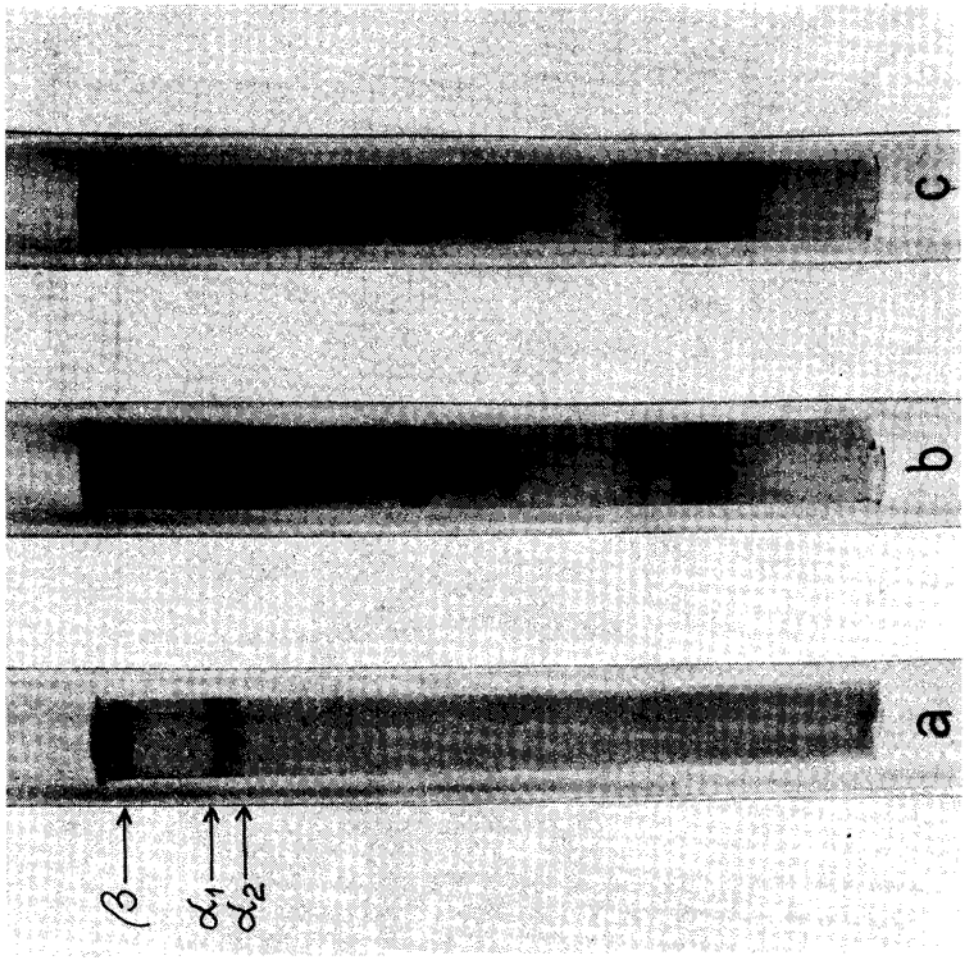

Fig. 1. The effect of incubation of type I collagen with vitreous-humour at pH 7.4. a, Type I collagen and buffer; b, type I collagen and vitreous-humour; c, vitreous-humour and buffer

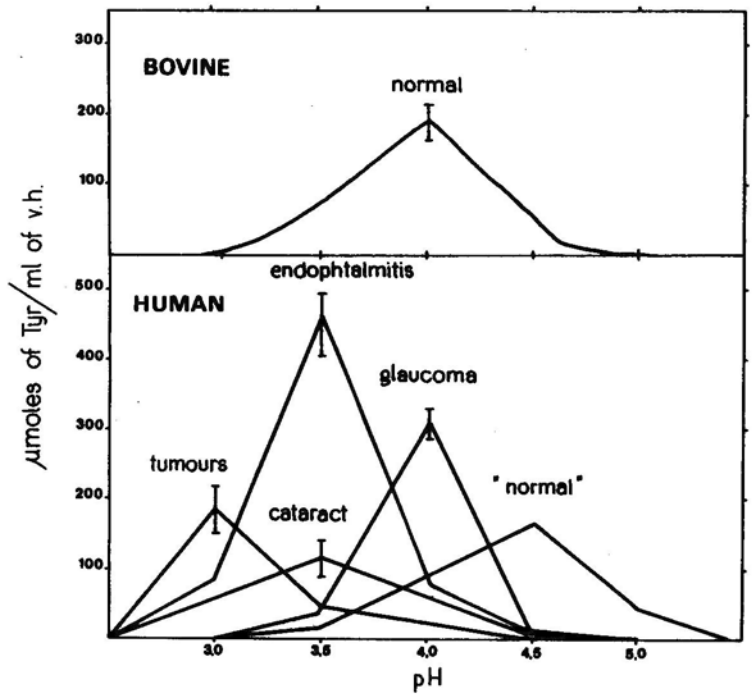

Fig. 2. Proteolytic activity of bovine and human vitreous-humours measured at different pH values with denatured haemoglobin as a substrate. Mean values ± SD are presented

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