PROTEIN STUDIES ON THE HUMAN VITREOUS BODY*

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INVESTIGATIONS of the vitreous body are beset with numerous technical difficulties. Its large water content and the instability of its structure make it difficult to obtain unchanged vitreous body in large amounts. Through the slit lamp and in fixed histological preparations (Lauber, 1936; Baurmann, 1926; Comberg, 1924; Duke-Elder, 1930), the human vitreous body appears as a structured mass.

In order to circumvent the criticism that such structures are caused artificially, new methods of investigation had to be evolved. Through the ordinary microscope fresh vitreous body demonstrates no structural elements. On the other hand, investigations with the ultramicroscope (Friedenwald and Stiehler, 1935), darkfield microscope, and phase-contrast microscope (Redslob, 1932; Matoltsy and others, 1951; Grignolo, 1952) have not led to any single conclusion, but have given rise to the present view of the vitreous body as a gel, permeated by a net of submicroscopic fibrils.

As Mörner (1893) has shown, this structural part can be split off by filtration. The chemical and physical analysis of the filter remains (Pirie and others, 1948) resulted in a collagen-type protein and a polysaccharide acid which could be identified as hyaluronic acid (Meyer and Palmer, 1936). The further structural analysis of these elements by means of the electron microscope and *x*-ray diagrams (Pirie and others, 1948; Schwarz and Schuchardt, 1950; Schwarz, 1951; Matoltsy and others, 1951) led to the discovery of three main types of fibrils:

- (i) long unbranched threads with an indication of fine axon periods;
- (ii) delicate, unbranched, entangled threads with a distinct axon period of 500–850 Å;
- (iii) broad, striated fibrils with an axon period of 500–800 Å, which demonstrated the typical relationship of collagen.

This structural residual protein consists of various components which can be separated and demonstrated by electrophoresis. To Hesselvik (1939), we owe the thorough electrophoretic analysis of the proteins of the vitreous body. Since the introduction and development of paper-electrophoresis, it has become possible to investigate the protein composition of single

*Received for publication November 13, 1952.

vitreous bodies in spite of the slight amount of protein contained. The protein components of the vitreous body, like the serum proteins, have the property of binding water and thus contributing to the colloid osmotic pressure. The strength of this binding can be measured by the rapidity with which heavy water penetrates the vitreous body of the living eye (Cagianut and Verrey, 1949). With a view to determining the influence of changes of pressure, we have now compared our results in the normal vitreous body with those obtained in glaucomatous eyes.

FIG. 1.—Paper electrophoresis (Cases 1 and 2).

FIG. 2.—Composition of vitreous body proteins according to results of electrophoresis.

Methods

Vitreous body was investigated in freshly enucleated eyes. Immediately after operation the eyeball was opened by equatorial section, and the vitreous body carefully dissected out. This was diminished to about $\frac{1}{10}$ of its original volume through a 10 per cent. Dextran solution, and the concentrated solution so obtained was examined by paper electrophoresis (Fig. 1). In some cases further samples could be ultracentrifuged. The sedimentation was accomplished in a centrifugal field of 180,000g. and the 1·3 per cent. solution of human vitreous protein was examined in physiological saline without any addition (Fig. 2). In one case the ultra-violet spectrum of the protein solution was registered (Fig. 3).

The electrophoresis apparatus of Wunderly and others (1951) was used; the filter paper was Munktell (Sweden) type strips. The pH, buffer solution, and tension corresponded to the usual values for serum. The dried filter-strips were stained with bromine-phenol blue and the resulting coloured bands colorimetrically determined with the Beckman spectro-photometer after dilution of the dye. The electrophoresis diagram was prepared in the usual way (Fig. 4).

All the vitreous humours examined showed a good correlation with regard to their protein composition. Examples of four clinical cases are shown in the Table.

FIG. 3.—Ultra-violet absorption spectra.
G, vitreous humour.
Ri, lens protein.
Re, retro-retinal fluid.

FIG. 4.—Electrophoresis diagram.

TABLE
PROTEIN COMPOSITION OF VITREOUS IN FOUR CASES

Case No.	Diagnosis	Albumin rel. per cent.	Globulin				
			α ₁	α ₂	β	γ ₁	γ ₂
1	Melanoma	61·0	7·6	5·4	10·4	7·6	8·0
2	Hydrophthalmos	77·8	3·8	5·4	7·8	5·6	4·3
3	Glaucoma	68·0	7·6	8·0	7·6	8·0	
4	Glaucoma	74·2	6·2	—	6·9	12·7	

Case Reports

Case 1, female, aged 70.—Ocular symptoms had first begun 4 weeks previously with the appearance of a shadow in front of the left eye.

Right eye normal both externally and ophthalmoscopically. Visual acuity 1.0.
Left eye showed no external irritation; anterior segment normal, lens and vitreous body clear. Tension 20 mm. Hg Schiötz, bullous detachment of retina with a tumour of the choroid below and nasally. Visual acuity 0.2.

Therapy.—Enucleation. The completely water-clear vitreous body of normal consistency was processed immediately.

Case 2, female, aged 32.—Congenital hydrophthalmos of both eyes. Recent episodes of increased pressure with pain.

Amaurosis. Both eyes staphylomatous with protrusion of the very thin sclera. Corneal diameter increased; large band-shaped corneal degenerations on both sides. Tension not constant, about 30 mm. Hg Schiötz.

Therapy.—Enucleation. The clear, somewhat fluid vitreous body was processed immediately.

Case 3, male, aged 52.—Chronic glaucoma in the left eye of several years' duration. For several days he had suffered intractable pain.

Left eye blind from chronic glaucoma. Tension 49 mm. Hg Schiötz. Early infection of anterior segment due to corneal ulcer. No response to treatment.

Therapy.—Enucleation. The clear, somewhat thready vitreous body was processed immediately.

Case 4, male, aged 68.—Occlusion of central vein of left retina 15 years before had been followed by development of a secondary glaucoma.

Left eye blind owing to secondary glaucoma. Tension 77 mm. Hg Schiötz. Painful infection of anterior segment with corneal dystrophy, resistant to treatment.

Therapy.—Enucleation. The clear, rather viscous vitreous body was processed immediately.

Results

The main portion of the human vitreous protein contains albumin, as demonstrated by electrophoresis and examination in the ultra-centrifuge. The fraction is homogenous and makes up 60–80 per cent. of the total protein. When the pressure is raised, this protein component seems to be increased, but any relationship with the duration or intensity of the increased pressure could not be demonstrated. It will be remembered that the colloid-osmotic pressure of a 1 per cent. solution of albumin amounts to 75 mm. H₂O, while that of a 1 per cent. solution of globulin amounts to only 19 mm. H₂O. (Wunderly and Wuhrmann, 1947). At the same time, the globulin sub-fractions are markedly diminished numerically, but the distribution within this non-homogenous group shows only a slight variation without specific characteristics. The most important result of this investigation is the demonstration that even under pathological conditions the proteins of the vitreous body contain no macroglobulins but only components of normal molecular size.

It would seem that the structural proteins of the human vitreous body consist mainly of a well-defined homogenous protein fraction similar to serum albumin. This interpretation is supported by the findings obtained by ultraviolet spectroscopy. The absorption of light in this spectral region depends for the most part on the content of tryptophane, tyrosine, and phenylalanine in the protein. The extinction co-efficients of these amino-acids are related as 27:8:1, although serum albumin contains only 0.1–0.2 per cent. tryptophane, while serum globulin contains 2–3 per cent. These important differences in chromophoric groups permit the characterization of a protein through the measurement of its specific absorption in the ultra-violet. The spectra of lens protein and retro-retinal fluid are illustrated for comparison (Fig. 4). The shape of the curve of the protein of the vitreous body, which shows a smaller absorption than lens protein and retro-retinal fluid and has its maximum at 280 mμ, corresponds to the large quantity of albumin contained therein.

The results of the analysis show that the protein fraction of the human vitreous body undergoes no important changes under conditions of increased pressure in the eye. The importance of the second structural component, hyaluronic acid, has not yet been ascertained.

We are indebted to the laboratories of the Hoffmann-La Roche Co., Basel, for the ultracentrifugal diagrams.

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