A CONTRIBUTION TO THE PHYSIOLOGY OF THE AQUEOUS HUMOUR.

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THE aqueous humour presents the singular phenomenon of a body fluid surrounded by living tissue and yet containing so little protein that it may be regarded as practically protein-free. As is well known in physiological theory a cellular tissue if brought into contact with a saline solution containing no colloid, which could balance the protein of the cells, will imbibe fluid from the saline by virtue of the osmotic pressure of the protein. This will take place even if the saline has a higher osmotic pressure than the cell contents, though there may then be an initial reverse action. Connective tissue apart from its enclosed cells will adsorb more fluid from a saline than from the same saline *plus* protein. According to theory then the cells of the iris, at least the superficial cells, must be in a state of œdema unless they are separated from the aqueous humour by a water-proof membrane.

Another problem is that presented by the alleged higher osmotic pressure of the aqueous humour. No other fluid in the body except the urine retains for any length of time a hyperosmotic concentration. In the case of the urinary passages there is present a pluricellular mucous membrane of a special type. A hyperosmotic fluid could not be produced in the ciliary body or elsewhere by filtration only; its formation would imply the performance of work and would constitute a true secretion. But of course an iso-osmotic solution can be made hyperosmotic by the loss of water through evaporation or expression from a semi-permeable membrane.

A general study of this theory led me to test some of the text-book statements concerning the aqueous humour. The fluid was obtained from ox eyes brought rapidly from the slaughter house to the laboratory. The cornea of each eye was wiped dry and opened with a sharp scalpel, the eyeball being held so that the cornea looked downwards. Generally the aqueous humour of 18 ox eyes was pooled, though in the quantitative

sugar estimations the fluid in some cases was aspirated by a hypodermic needle from the anterior chamber.

Protein content. The protein in fresh ox serum was estimated by the Scherer coagulation method after proper dilution. Another sample of the same serum was diluted with Ringer's fluid until it possessed a protein concentration approximately equal to that of the aqueous. For nephelometric estimations I found that adding an equal volume of 1 p.c. trichloracetic acid gave a suitable precipitate. Using this method with the Kober nephelometer-colorimeter[1] I found in two estimations that the protein content was 0·08 p.c.

Total solids and ash. 13·1011 grams of aqueous humour were placed in a tared platinum vessel and exposed for two hours to a temperature of 105° C. I did not, however, test whether constant weight had been attained. The residue weighed 0·1567 gm. = 1·26 p.c. total solids by weight. This residue was ashed over a low flame with the lid on the platinum vessel. The ash residue weighed 0·1083 gm. = 0·82 p.c. mineral matter by weight. This, it will be seen, is what one would expect if the humour were a simple filtrate from the blood.

Sugar content. Two cm.^{3} of aqueous humour were aspirated direct from the anterior chamber or were taken from the pooled fluid. The reducing sugar was estimated by the Benedict method in which a standard glucose solution and not picramic acid was employed. Three estimations gave me 0·07 p.c., 0·09 p.c., and 0·12 p.c. sugar estimated as glucose. I gave this quantitative estimation to some advanced students as an exercise and their results were 0·06 p.c., 0·09 p.c., 0·14 p.c. and 0·14 p.c. Again, it will be observed, the sugar content is of the order expected in a blood filtrate.

Refractive index. With a Zeiss-Abbe refractometer ox serum at 7·1° C. gave the reading 1·3451. Aqueous humour at the same temperature gave 1·336. I took a quantity of ox serum in a test tube, immersed this for ten minutes in a water bath at 100° C. and determined the refractive index of the protein-free fluid that eventually separated from the coagulum. The reading at 7·1° C. was 1·336, *i.e.* exactly the same as the aqueous.

Freezing point. In determining the osmotic pressure by this method I employed the same Beckmann thermometer that I have used for the

[1] It is a pity that this otherwise fine instrument should possess an error in construction. As pointed out to me by my colleague Professor Laby the containing vessels into which the plungers dip should be square in section and not circular as this latter shape gives lack of uniformity in each field.

last thirteen years. I have always adjusted the mercury so that the meniscus at the freezing point should be close to the centre of the scale. The freezing mixture was separated from the fluid to be investigated by a layer of air as well as two glass walls. Inoculation with an ice crystal took place when the supercooling was about 0·75° C. Stirring was carried out by a clock-work mechanism. A too low temperature in the freezing mixture was avoided. With this technique I have taken from time to time the depression of freezing point of a number of ox blood specimens, some twenty-five to thirty in all, and invariably the first two figures of the decimal have been the same, namely 0·59° C. The experiment was carried out with the pooled aqueous humour from 18 ox eyes and the depression obtained was 0·597° C.

Discussion.

The results of these experiments confirm the view that the chief solids in the aqueous humour are metallic salts, protein being present only in traces. But the statement that the aqueous has a higher osmotic pressure than the blood is challenged. According to these results the formation of the aqueous is most easily explained by physical filtration.

As pointed out in the introduction there are certain problems presented by tissues in contact with a saline solution containing no protein or other colloid. Can it be that the endothelium of Descemet's membrane is actually water-proof, in which case the nutrition of the cornea would take place from the periphery? There are certain clinical and experimental facts which bear this out. "Absorption of fluid (by the cornea) from the aqueous occurs when the endothelium is injured by pathological processes, *e.g.* interstitial keratitis... Softening and ectasia of the cornea may ensue during life from prolonged impairment of the endothelium[1]."

Again, as is well known, loss of continuity of the anterior capsule of the lens will lead, in young subjects at least, to swelling and protrusion of the lens substance through the wound and ultimate breakdown of lens tissue. Does this capsule function in the same way as the endothelium of Descemet's membrane? Most interesting, too, are the problems concerned with the iris. We have here, what is not present in cornea or lens, a fairly rich blood supply. I do not desire to enter into the debateable question of intra-ocular pressure but one important point seems to me to have been overlooked. If in the capillaries and venules of the iris the pressure over and above the intraocular is greater than the osmotic pressure of the proteins of the blood then, according to all theory, there

[1] *The Pathology of the Eye*, by J. Herbert Parsons, p. 997.

must be filtration outwards from the iridic vessels. But if the pressure over and above the intraocular is less than the osmotic pressure of the proteins of the blood then the aqueous humour will be absorbed. The argument is not affected even if the osmotic pressure of the humour is slightly higher than that of the blood. If Parsons'[1] estimates are correct, *viz.* that the intraocular pressure is 20 to 30 mm. Hg whilst the total capillary pressure is 40 to 50 mm. Hg then, as the proteins of the blood exert, according to Starling, an osmotic pressure of 30 mm. Hg, there must be a movement of aqueous humour into the iris capillaries with a head of pressure equal to 10 mm. Hg. As no one has measured the pressure in the iridic vessels this estimate must remain a surmise; but experiments with pigments have placed beyond doubt[2] that the iris, at least on its anterior aspect, does absorb fluid and so we may reason backwards that there the capillary pressure over and above the intraocular is less than 30 mm. Hg; also that no water-proof membrane envelopes the anterior surface of the iridic curtain—it is stated that there is no endothelium at this region.

If this reasoning is correct the iris should always be reckoned as a participant in the absorption of aqueous humour by virtue of the proteins of its blood. Diminished absorption would then be caused by pathological thickening of the vessel walls, high capillary pressure over and above the intraocular, or reduced iridic surface as occurs in mydriasis. Clinical evidence, I take it, is in favour of this view. Of course, such absorption could not explain the leakage that occurs in an excised eye under pressure; here one must fall back on the lymphatic path.

Results

(1) The osmotic pressure of ox aqueous humour was found to be equal to that of blood. Other quantitative estimations support the view that the humour is a blood filtrate.

(2) The osmotic pressure of the blood proteins in the iridic vessels will cause absorption of aqueous humour if the excess pressure in these vessels is less than 30 mm. Hg.

[1] *The Pathology of the Eye*, by Herbert Parsons, p. 1047.
[2] J. Herbert Parsons, *loc. cit.* p. 991.