# XI. THE BIOCHEMISTRY OF THE AQUEOUS HUMOUR.

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THE view at present most generally accepted regarding the origin and nature of the aqueous humour of the eye is that it is secreted by the cells of the ciliary epithelium, that it circulates through the eye, and that it finds an exit therefrom largely by way of the canal of Schlemm. Alternatively it is held by some that this fluid is formed from the blood by a process of simple transudation through the capillary walls and circulates in a similar manner. The evidence whereon the secretory theory is based is largely anatomical, resting on the histological structure of the ciliary epithelium and its resemblance to a gland—a form of evidence at best inconclusive and susceptible of varying interpretations. Some animals possess no ciliary body at all; others, possessing a ciliary body, show no specific glandular formation; and finally, in those animals (*e.g.* man) wherein the anatomical evidence is most complete, the resemblance to true glands is questioned by many authorities. Moreover, even in these latter it can be shown that the formation of the intra-ocular fluids, though largely associated with the ciliary body, is by no means confined to it. The "circulation" of the aqueous humour has been determined and measured only by unsound physiological experiments wherein the normal pressure conditions have been upset by opening the eye, or by making injections into the eye, or by employing other procedures which can be interpreted as calculated to initiate artificially an abnormal circulation.

In a series of experiments on the intra-ocular pressure it was indicated [1926, 1] that the pressure equilibrium of the eye might be explained more consistently on the hypothesis that it was of a hydrostatic rather than of a hydrodynamic nature. These suggested the probability of a third hypothesis which has more recently been put forward, that the aqueous humour is formed by dialysis from the blood, and that, apart from a minimal circulation determined by metabolic interchange and muscular activity, it is in equilibrium with it. A further series of experiments on the vascular pressures of the eye [1926, 3] demonstrated that the physical forces involved in the production of the aqueous humour did not necessitate the intervention of any secretory energy; the difference between the intra-ocular pressure and the hydrostatic pressure in the ocular arteries and veins, and the difference in osmotic pressure between the aqueous humour and the capillary plasma were found to be compatible with a dialysis hypothesis. It was shown also that the pressure equilibrium forbade a hydrostatic outflow of aqueous humour into the veins under normal pressure conditions [1926, 2], and that, in addition, the differences in osmotic pressure [1927] and electrical potential between the plasma and the aqueous humour were of an order such as to satisfy the requirements of a system in thermodynamical equilibrium.

In complicated physiological experiments the number of imperfectly controllable variables frequently leads to equivocal results and rarely to a pragmatic conclusion; whilst the attempt to eliminate the variables tends to introduce conditions so abnormal as to defeat its own ends. It was therefore felt that the study of the aqueous humour from the biochemical point of view would not be without interest. The main difficulty which presented itself was the small quantity of intra-ocular fluid which is available from any one animal, and the minute concentration of some of the constituents. In order to get a general comparison between the aqueous humour and the blood, large quantities of horse aqueous humour were obtained, and the pooled fluid was analysed concurrently with a typical sample of horse serum. By this procedure a qualitative comparison could be made, and though it cannot lay claim to quantitative accuracy, a relatively close approximation can be obtained. The principal constituents of each class of substance were then selected and micro-estimations made of these, comparing each with the corresponding concentration in the arterial and venous plasma of the same animal. Finally, changes in the chemical constitution of the aqueous humour were studied under experimentally produced variations in the blood and in the eye.

## I. The general chemistry of the aqueous humour.

A considerable amount of work has been done on the general chemistry of the aqueous humour—in the horse notably by Mestrezat and Magitot [1921]—but in no case has a systematic analysis been directly compared with the blood.

*Collection of material.* The aqueous humour was taken from the eyes of horses immediately after their being slaughtered. In this animal each eye contains 1 to 2 cc. of fluid; only the first cc. was withdrawn, since on complete evacuation the last drawn fluid becomes contaminated with plasma exuded from the capillaries. The cornea was cleaned and dried, a nick made through three-quarters of its substance with a sharp knife, and the needle of a 1 cc. syringe inserted through it, care being taken to avoid the iris and to cause as little pressure disturbance as possible. The serum was a typical sample taken from the farm of the Medical Research Council.

The chemical analysis is given in the accompanying table.

*Methods of analysis.* The total protein was estimated in the aqueous humour by acidifying 100 cc. and coagulating by heat; the precipitate was washed with water and alcohol, dried at 100° and weighed. 10 cc. of serum

Quantities in g. per 100 cc.

| | Aqueous humour | Serum |
|---|---|---|
| Water ... ... ... ... | 99·6921 | 93·3238 |
| Solids (dried at 100°) ... ... | 1·0869 | 9·5362 |
| Total protein ... ... ... | 0·0201 | 7·3692 |
| Albumin ... ... ... ... | 0·0078 | 2·9557 |
| Globulin ... ... ... ... | 0·0123 | 4·4135 |
| Non-protein N ... ... ... | — | 0·0239 |
| Total N ... ... ... ... | 0·0268 | — |
| Urea ... ... ... ... | 0·028 | 0·027 |
| Amino-acids ... ... ... | 0·029 | 0·035 |
| Creatinine ... ... ... | 0·002 | 0·002 |
| "Fats" ... ... ... ... | Trace (0·004) | 0·13 |
| Cholesterol ... ... ... | ? Nil | — |
| "Sugar" ... ... ... ... | 0·0983 | 0·0910 |
| Sodium ... ... ... ... | 0·2787 | 0·3351 |
| Potassium ... ... ... | 0·0189 | 0·0201 |
| Calcium ... ... ... ... | 0·0062 | 0·0101 |
| Magnesium ... ... ... | 0·0026 | 0·0028 |
| Chlorine ... ... ... ... | 0·4371 | 0·3664 |
| Inorg. P ($P_2O_5$) ... ... ... | 0·0033 | 0·0030 |
| Inorg. S ($SO_4$) ... ... ... | 0·0061 | 0·0058 |

diluted to 100 cc. with distilled water were treated similarly. The albumin was determined by precipitating the globulins by adding an equal volume of saturated ammonium sulphate solution to 100 cc. which had been allowed to concentrate in a desiccator, allowing this to stand for 24 hours, and filtering; the filtrate was acidified with $N/10$ sulphuric acid and filtered; the precipitate was redissolved in the original volume of distilled water, neutralised with dilute caustic soda, and re-precipitated with half-saturated ammonium sulphate, the albumin being precipitated again from the filtrate with acid. This process of fractionation was repeated five times. The final filtrate was acidified and boiled, the precipitate washed with water, alcohol, and ether, dried at 100° and weighed. 10 cc. of diluted serum were treated similarly. The globulin was taken as the difference between the total protein and the albumin.

The total nitrogen of the aqueous humour was estimated by a micro-Kjeldahl determination; the non-protein nitrogen of the serum by the same method on a protein-free filtrate (Folin and Wu). Urea was estimated by the urease method: the amino-acids by Van Slyke's method: and the creatinine colorimetrically against a standard creatinine picrate solution.

By the term "fats" is meant the figures obtained by weighing an ether extract of dried residue. The cholesterol was estimated after extraction by precipitation with digitonin.

"Sugar" is taken as reducing substance estimated as glucose by the Schaffer-Hartmann method.

The total mineral ash was obtained by calcination. The material thus left was dissolved in HCl; barium chloride and baryta were added, and the phosphates and sulphates removed; barium salts were removed by ammonia and ammonium carbonate; the filtrate was evaporated to dryness and the ammonium salts removed by heat; the residue was dissolved, treated again with ammonia and ammonium carbonate, filtered, acidified with HCl and evaporated to dryness. This residue was redissolved; the potassium was estimated by precipitation with $H_2PtCl_6$, and the amount of sodium calculated by difference. The calcium was precipitated as oxalate, and estimated as CaO, the magnesium as ammonium magnesium phosphate and estimated as magnesium pyrophosphate. The chlorides were determined by Ruszynák's modification [1921] of Koranyi's method; the inorganic phosphates gravimetrically as pyrophosphate; and the inorganic sulphates by conversion to benzidine sulphate and titration with sodium hydroxide.

The results of the chemical analysis show that the constituents of the aqueous humour, when compared with those of serum, may be divided into three groups of substances depending on the physical state of their molecules in solution.

1. *Colloidal substances. Partition coefficient serum/aqueous humour* $> 1$.

All the colloidal substances are found in the aqueous humour in much smaller concentration than in the serum. Proteins are present in the former in very small quantities; but it is to be noted that although this is the case the different fractions (as determined by precipitation by ammonium sulphate) are found in approximately the same proportion as they occur in the blood. Thus the albumin/globulin ratio in the former is 38·8/61·2, in the latter 39·9/60·1. Further it was shown by dialysing the globulin fraction against distilled water that in the aqueous humour as in the serum it was composed partly of euglobulin and partly of pseudoglobulin, although, unfortunately, the minuteness of the quantities involved precluded any reliable quantitative estimation. Moreover, it can be shown that they are specifically identical[1]. Similarly the "fats," also indiffusible substances, are found in correspondingly minute quantities in the aqueous humour.

Since all the colloidal constituents of the serum appear only in traces in the aqueous humour the two solutions are of very dissimilar molecular aggregation; in comparing the distribution of the diffusible constituents it is therefore necessary to apply a correction factor to allow for the difference in solid displacement due to the unequal mass of solute. Thus 100 cc. of the horse aqueous humour contain 1·0869 g. of solids and 99·6921 g. of water, whilst the same quantity of serum contains 9·5362 g. of solids (largely protein) and 93·3238 g. of water. A correction factor therefore of 100/99·6921 or 1·003 applied to the aqueous humour and of 100/93·3238 or 1·07 applied to the serum gives comparative results expressed as concentrations dissolved in an equal quantity (100 g.) of water.

2. *Diffusible non-dissociated substances. Partition coefficient* $= 1$.

The total N of the aqueous humour was found to be 0·0268 g. %. Deducting 0·0032 g. % to allow for 0·02 % protein, the non-protein nitrogen becomes 0·0236 g. %—a close approximation to the non-protein nitrogen

[1] Unpublished researches, at present being extended, with Dr Percival Hartley.

content of the serum (0·0239 g. %). The amino-acid content at any one time is probably too variable a quantity to permit reliable deductions to be made from it when the method of collecting materials is borne in mind. The creatinine appeared to be present in both in equal quantities. The urea and the "sugar" also appeared in practically the same concentrations.

| | Aqueous humour | | Serum | |
|---|---|---|---|---|
| | G. per 100 cc. solution | G. per 100 g. water | G. per 100 cc. solution | G. per 100 g. water |
| Urea | 0·028 | 0·028 | 0·027 | 0·0289 |
| "Sugar" | 0·0983 | 0·0986 | 0·0910 | 0·0974 |

The diffusible non-dissociated substances are therefore partitioned between the two fluids in approximately equal amounts.

### 3. *Dissociated diffusible substances.*

The dissociated substances are seen to be unequally distributed, even when corrected for solid displacement. In each case the cations have a partition coefficient greater than one, and the anions a partition coefficient less than one.

| | Aqueous humour | | | Serum | | |
|---|---|---|---|---|---|---|
| | Per 100 cc. solution | | Per 100 g. water | Per 100 cc. solution | | Per 100 g. water |
| | g. | Milli-mols per litre | g. | g. | Milli-mols per litre | g. |
| *Cations:* | | | | | | |
| Sodium | 0·2787 | 121·2 | 0·2795 | 0·3351 | 145·6 | 0·3585 |
| Potassium | 0·0189 | 4·8 | 0·0190 | 0·0201 | 5·1 | 0·0215 |
| Calcium | 0·0062 | 1·5 | 0·0063 | 0·0101 | 2·5 | 0·0108 |
| Magnesium | 0·0026 | 1·1 | 0·0026 | 0·0928 | 1·2 | 0·0030 |
| *Anions:* | | | | | | |
| Cl′ | 0·4371 | 123·1 | 0·4384 | 0·3664 | 103·2 | 0·3920 |
| $PO_4'''$ | 0·0044 | 1·38 | 0·0044 | 0·0040 | 1·26 | 0·0043 |
| $SO_4''$ | 0·0061 | 1·8 | 0·0062 | 0·0058 | 1·7 | 0·0062 |

It is seen that in the serum there is a considerable excess of basic radicles, which is shown especially in the case of sodium and calcium. This is accounted for by the fact that in blood part of these are associated with protein as protein salts, and as such are rendered indiffusible. Rona and György [1913] found that 15 to 28 % of the total quantity of sodium was indiffusible for this reason, and Rona and Takahashi [1913] that 30 to 40 % of the total calcium was similarly in association with protein. Conversely, the acidic radicles show a predominance in the aqueous humour, the chlorides showing a higher relative concentration than the phosphates and sulphates. This again suggests comparison with the work of previous observers, since all the chlorides of serum have been found to dialyse [Ascher and Rosenfeld, 1907; Creveld, 1921], while the phosphates have been held back to some extent in loose combination with the protein. The increase in the concentration of anions as a whole and the diminution of cations strongly suggest that the distribution of the ionic activities between the aqueous humour and the blood is subject to the thermodynamical laws relating to two fluids in the membrane equilibrium described by Donnan. Thus the relative concentrations expressed as normal chlorine and sodium are:

$$Cl_{aq} : Cl_{serum} = 123 : 103$$
$$Na_{aq} : Na_{serum} = 121 : 145.$$

The theoretical relation characteristic of such an equilibrium

$$[Na^+]_{aq} \times [Cl^-]_{aq} = [Na^+]_{serum} \times [Cl^-]_{serum}$$

therefore becomes $$121 \times 123 = 145 \times 103$$
or $$148{\cdot}83 = 149{\cdot}35.$$

The distribution of all the constituents of the aqueous humour thus appears to be determined by physical laws and provides no evidence of the expenditure of any secretory energy in its elaboration, whilst the concentration of its ionised substances seems to preclude a simple transudation, but rather suggests that it is formed by dialysis from the blood through a membrane which is almost impermeable to colloidal micelles and is in equilibrium with it.

## II. The "sugar" and salt of the aqueous humour.

In order to compare the aqueous humour with the blood under more exact conditions than were possible in the method of collection of material detailed above, two of the most abundant and easily manipulated constituents were chosen—"sugar" as being representative of the non-dissociated substances, and chlorides as being representative of the ionised constituents—and their relative concentrations compared. The two fluids—aqueous humour and plasma—were in each case derived from the same animal and were retained in a condition approximating the normal as closely as possible.

Rabbits were used. The aqueous humour was withdrawn under sterile conditions by means of a syringe dried with alcohol and ether. The needle had a broad lance point which was introduced into the cornea obliquely near the limbus; such a needle is inserted with less disturbance than the ordinary round pointed instrument, and with the latter it was found difficult to prevent aqueous humour escaping round it at the moment of introduction. Cocaine (2 %) was instilled into the conjunctival sac as an anaesthetic; it has been repeatedly demonstrated that this procedure does not appreciably alter the properties of the intra-ocular fluids. Before the needle was introduced the cornea was dried with blotting paper to obviate any contamination with lacrymal secretion which is of widely different composition. Blood was taken also with aseptic precautions from the ear, the central artery or the marginal vein being used as the case required. Plasma in preference to serum was employed, since it is the former which comes into equilibrium with the aqueous humour *in vivo*; the use of anti-coagulants was dispensed with, since these substances are known to alter the distribution of its constituents to such an extent as to make estimations carried out *in vitro* useless for comparative purposes with the actual state of the blood *in vivo*. Blood was therefore sucked directly through a paraffined needle and tube into a paraffined centrifuge tube under a layer of paraffin, and, after centrifuging, the middle layer of plasma was pipetted off without its ever having been in contact with air. The centrifuging was done rapidly, and in this way both arterial and venous plasma were obtained in a state as near to that in which they occur naturally as experimental manipulations permitted.

The results are expressed in g. per 100 cc. solution, and g. per 100 g. water. 100 cc. rabbit's plasma were found to contain 8·6832 g. solids (dried at 100°), and the density was 1·023. Correspondingly, 100 cc. aqueous humour contained 1·0899 g. solids (dried at 100°), and its density was 1·007. The factors 1·07 and 1·003 respectively therefore give the concentration of solution in 100 g. water.

The "sugar" (*i.e.* reducing substance) was estimated by the Hagedorn-Jensen method.

| | No. of rabbit | G. per 100 cc. solution | G. per 100 g. water |
|---|---|---|---|
| Aqueous humour | 1 | 0·141 | 0·141 |
| | 2 | 0·138 | 0·139 |
| | 3 | 0·175 | 0·175 |
| Arterial plasma | 1 | 0·136 | 0·145 |
| | 2 | 0·139 | 0·148 |
| | 3 | 0·170 | 0·182 |
| Venous plasma | 1 | 0·120 | 0·128 |
| | 2 | 0·111 | 0·118 |
| | 3 | 0·143 | 0·153 |
| Average aqueous humour | | 0·151 | 0·151 |
| " arterial plasma | | 0·148 | 0·158 |
| " venous plasma | | 0·125 | 0·133 |

The sugar concentration of the aqueous humour lies between that of arterial and venous plasma, a relation which suggests that it comes into equilibrium with capillary plasma. The concentration appears to be more closely related to the arterial than to the venous plasma; this finding may be compared with that of Foster [1923], who showed that the sugar content of "finger blood," and therefore presumably of capillary blood, was very nearly identical with that of arterial and widely different from that of venous blood. The results obtained may be compared with those of previous observers on the sugar content of aqueous humour. Osborne [1919] found the concentration in both to be the same; Ask [1914] found the sugar in the aqueous humour to be 0·01 to 0·02 % greater than in the blood, Deiter [1925] 0·004 % greater; whilst Haan and Creveld [1921, 1, 2] obtained a value for the aqueous humour 0·045 % less than that for the blood. These last authors accept the hypothesis of the dialysis of the aqueous humour, and, taking the sugar content of the capillary plasma as being the mean of the arterial and venous plasma and finding the concentration in the aqueous humour less than this, they conclude therefrom that glucose is partially retained in combination with plasma proteins. Until more exact knowledge of the sugar content of capillary blood is available it would seem dangerous to draw any such conclusions from the above figures. The results obtained in the present investigation suggest, however, that the sugar content of the aqueous humour lies between that of the arterial and venous plasma and support the hypothesis of the dialysis of the aqueous humour.

*Salt.* The chloride was estimated by Ruszynák's modification of Koranyi's method, and is expressed as NaCl.

| | No. of rabbit | G. per 100 cc. solution | G. per 100 g. water |
|---|---|---|---|
| Aqueous humour | 1 | 0·668 | 0·670 |
| | 2 | 0·597 | 0·599 |
| | 3 | 0·641 | 0·643 |
| Arterial plasma | 1 | 0·603 | 0·645 |
| | 2 | 0·543 | 0·581 |
| | 3 | 0·590 | 0·631 |
| Venous plasma | 1 | 0·578 | 0·618 |
| | 2 | 0·501 | 0·536 |
| | 3 | 0·573 | 0·613 |
| Average aqueous humour | | 0·635 | 0·637 |
| ,, arterial plasma | | 0·579 | 0·619 |
| ,, venous plasma | | 0·551 | 0·589 |

The chloride content of the aqueous humour is higher than that of the plasma even when expressed as g. in an equal weight of water. The results obtained thus agree with those of Ascher [1922] and Creveld [1921]—and again suggest that the aqueous humour is a dialysate from the blood.

## III. The chemical composition of abnormal aqueous humour.

If the intra-ocular fluids are a dialysate of the plasma there are two possible methods of changing their composition: by altering the permeability of the dialysing membrane (*i.e.* the capillary wall), and by altering the composition of the blood.

### 1. *Alteration of the permeability of the capillary walls.*

In a state of dilatation the permeability of the capillary walls is increased, and under these circumstances a larger proportion of colloidal molecules is able to penetrate through their walls. This occurs in the eye as elsewhere, and, along with the increased protein content of the aqueous humour, definite changes in the distribution of ionised substances occur.

(*a*) *Dilatation of the capillaries by paracentesis.* When the eye is punctured and the intra-ocular fluids are withdrawn the capillaries, deprived of the supporting pressure of the aqueous humour, undergo immediate dilatation. The fluid re-formed under these conditions I have elsewhere [1927] called "plasmoid aqueous." The aqueous humour was withdrawn from the eyes of rabbits, using the same technique as was employed previously, and the re-formed fluid was similarly withdrawn 20 minutes after the first paracentesis.

The chemical constitution of the normal and plasmoid aqueous humours was compared in respect of their colloid, sugar, and chloride contents.

*Colloid content.* The colloids, which are largely composed of protein, were estimated refractometrically. A dipping refractometer (Zeiss) was used provided with an auxiliary prism to enable it to deal with one drop of fluid and the temperature was kept constant by a thermostat; the error of the instrument is $\pm$ 3·7 units of the fifth decimal place of $n_D$.

The increase of refractivity found in the "plasmoid aqueous" shows an increase in its content of colloids (see table below)—a fact which has long been known. Considering the total colloid as proteins, the approximate corresponding percentages of this substance are also given. The figures do not pretend to any great accuracy. They were calculated by the technique suggested by Robertson [1915] applied to a large quantity (100 cc.) of horse aqueous humour. The refractive index of a protein-free preparation of this fluid was subtracted from that of the normal horse aqueous humour, and the differential result thus obtained was correlated with a gravimetric estimation of the total protein in the original fluid. That this refractive index was due to increase of protein, both albumin and globulin, was seen by special tests for these substances. The Noguchi globulin test gave a granular flocculent precipitate whilst Pandy's reaction gave a typical blue-white cloud; both these reactions are absent from the normal aqueous humour. Similarly the Nonne-Apelt test gave a positive reaction for globulin, and on filtering and acidifying the filtrate the presence of increased albumin was verified.

| | Refractive index |
|---|---|
| Normal horse aqueous humour ... ... ... | 1·335130 |
| Protein-free horse aqueous humour ... ... | 1·335091 |
| Refractive index of protein ... ... ... | 0·000039 |
| Protein ... ... ... ... ... ... | 0·024 % |

The aqueous humour of the rabbit was found to have on the average a higher refractive index than that of the horse.

| | Refractive index |
|---|---|
| Normal rabbit aqueous humour ... ... ... | 1·335168 |
| Protein-free rabbit aqueous humour ... ... | 1·335091 |
| Refractive index of protein ... ... ... | 0·000077 |
| Protein ... ... ... ... ... ... | 0·04 % (approx.) |

| No. of rabbit | Aqueous removed cc. | $n_D$ Normal aqueous | $n_D$ "Plasmoid aqueous" | Difference (plasmoid − normal) | Approx. % protein |
|---|---|---|---|---|---|
| 1 | 0·1 | 1·335244 | 1·337088 | 0·001844 | 1·0 |
| 2 | 0·2 | 1·335130 | 1·338428 | 0·003298 | 1·8 |
| 3 | 0·25 | 1·335168 | 1·339036 | 0·003868 | 2·0 |
| 4 | 0·32 | 1·335244 | 1·339834 | 0·004590 | 2·5 |

Concurrent sugar and salt estimations showed the following results. The sugar was estimated by the Hagedorn-Jensen method and is expressed as g. per 100 cc.

| No. of rabbit | Normal aqueous | "Plasmoid aqueous" | Difference |
|---|---|---|---|
| 1 | 0·143 | 0·148 | 0·005 |
| 2 | 0·155 | 0·173 | 0·018 |
| 3 | 0·173 | 0·175 | 0·002 |
| 4 | 0·165 | 0·172 | 0·007 |

The increase of sugar in the "plasmoid aqueous" is probably largely to be explained by an increase in the blood sugar occurring during the experiment due to emotional excitement on the part of the animals. An effort was made to accustom the rabbits to experimental procedures before performing any of the above experiments upon them; but without success. Thus in one experiment the blood sugar at the start was 0·154 %, and at the end had risen to 0·177 %.

The salt was estimated by the Ruszynák method and is expressed as g. NaCl per 100 cc.

| No. of rabbit | Normal aqueous | "Plasmoid aqueous" | Difference |
|---|---|---|---|
| 1 | 0·641 | 0·600 | 0·041 |
| 2 | 0·597 | 0·561 | 0·036 |
| 3 | 0·680 | 0·536 | 0·144 |
| 4 | 0·500 | 0·421 | 0·079 |

There is therefore a constant decrease in the quantity of chloride with increasing concentration of protein.

(*b*) *Dilatation of the capillaries by radiant energy.*

(i) *Ultra-violet rays.* Rabbit—10 minutes' irradiation at 2 feet from a quartz mercury vapour lamp, water-cooled. The left eye protected.

| Rabbit ... ... | Left eye normal | Right eye irradiated |
|---|---|---|
| Refractive index | 1·335168 | 1·340898 (approx. 3 % protein) |
| "Sugar" | 0·154 | 0·172 |
| Chloride | 0·693 | 0·520 |

(ii) *Infra-red rays.* Rabbit—10 minutes' irradiation at 2 feet from a carbon arc, ultra-violet filtered off through 1 cm. thickness of glass. The left eye protected.

| Rabbit ... ... | Left eye normal | Right eye irradiated |
|---|---|---|
| Refractive index | 1·335247 | 1·341990 (approx. 3·5 % protein) |
| "Sugar" | 0·125 | 0·131 |
| Chloride | 0·661 | 0·501 |

It is probable that both of these agencies act in the same way, the radiant energy being absorbed by the pigment of the iris, and there converted into heat which causes capillary dilatation.

(*c*) To show that the changed chemical constitution was due to the dilatation of the capillary walls, a paracentesis was done after 1 cc. of 1 : 1000 adrenaline with cocaine had been injected behind the eyeball. This counteracts to a large extent the dilatation caused by the paracentesis. Wessely [1908] first showed that the aqueous humour formed secondarily under these conditions contained less protein than the usual "plasmoid aqueous": the present analysis demonstrates that throughout it is to a less extent of a plasmoid nature, but resembles more closely the normal fluid.

| Rabbit ... ... | Left eye—normal | Right eye—paracentesis after adrenaline |
|---|---|---|
| Refractive index | 1·335130 | 1·335244 |
| "Sugar" | 0·152 | 0·175 |
| Chloride | 0·638 | 0·630 |

It is suggested that whilst the normal aqueous humour has the chemical constitution which one would expect were it in membrane equilibrium with the capillary plasma, the abnormal humour formed under experimentally varied conditions changes its composition in a corresponding manner. On increasing the permeability of the separating membrane and allowing more colloid to enter the eye, the increase is proportional to the amount of capillary dilatation produced. With the tendency to equalisation of the amount of colloid on either side of the membrane the excess of cations largely disappears, while, allowing for the increased glucose content of the blood which accompanies the excitement of the experimental manipulations, the non-dissociated constituents ("sugar") remain practically constant. Moreover, on preventing capillary dilatation the aqueous humour formed secondarily after puncture of the eye is not far removed from the normal. It would appear, therefore, that the view is unjustified which ascribes to the normal humour and the "plasmoid aqueous" fundamentally different origins—that the former is a secretion and the latter a transudation; it would seem rather that the intra-ocular fluids are formed under both conditions by the same process of dialysation through a membrane of varying permeability.

2. *By varying the chemical constitution of the blood.*

A series of experiments was done on cats wherein varying quantities up to 50 cc. of a 10 % solution of gum arabic were injected intravenously: no change in the refractive index of the aqueous humour was ever detected. Where colloidal substances are susceptible to delicate biological tests, however, their presence can be detected in proportions corresponding to the traces of protein normally present, as is seen in the case of the various substances associated with immunity [Poleff, 1914, and others]. Difficultly diffusible drugs are also found in traces—*e.g.* the organic compounds of arsenic [Neame and Webster, 1923]. All these substances are found in the "plasmoid aqueous" in quantity.

Diffusible substances find their way freely into the aqueous humour. Thus in experiments carried out on the cat [1926, 1] when 10 cc. of a 30 % glucose solution were injected intravenously, the sugar content of the right eye before the experiment was found to be 0·146 %, whilst that in the left 15 minutes after the injection was 0·204 %. Similarly with sodium chloride; the concentration of this substance was found to rise from 0·641 to 0·698 % after the intravenous injection of 10 cc. of a 30 % solution. This corresponds with the clinical findings of Ask [1914] and Deiter [1925], who found that the sugar content of the aqueous humour rose parallel with the blood in diabetics, and of Gala [1924], who found that in cases of chloride retention the aqueous humour varied similarly, and the experimental findings of Löhlein [1910] and Haan and Creveld [1921, 2], who found that such substances as fluorescein, potassium ferrocyanide, and potassium iodide were partitioned between the aqueous humour and the blood in the ratio of their diffusion constants.

SUMMARY.

From the biochemical point of view neither the normal nor the abnormal aqueous humour shows evidence of the existence of any special secretory mechanism in its elaboration but appears to be rather a dialysate of the capillary blood.

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