THE OSMOTIC PRESSURE OF THE AQUEOUS HUMOUR AND ITS PHYSIOLOGICAL SIGNIFICANCE.

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In two previous papers(1) it has been suggested that the vascular pressures in the eye showed nothing incompatible with the hypothesis that the intra-ocular fluids were formed by dialysation from the blood. The osmotic pressure of the aqueous humour and its value relative to that of the blood is a question of equal importance; and in the consideration of the mechanism of the formation and absorption of this fluid these two inter-related pressures must always be viewed together.

The literature of the subject shows a wide divergence of opinion. Two methods have been employed in the determination of the osmotic pressure of the aqueous humour: a physical method depending on the lowering of the freezing point, and a biological one based on the plasmolysis of red blood corpuscles.

The difficulty of the small quantity of fluid available compelled those employing the cryoscopic method to experiment with the collected aqueous humour of many animals, and to compare this with a "typical" sample of serum. The earlier observers consistently arrived at the conclusion that the aqueous was hypertonic to blood serum, the average ratio being about 11 : 10—Dreser (ox) (2), Kunst (ox) (3), Richon-Duvignaud (rabbit) (4), Botazzi and Sturgio (ox) (5), Scalinci (dog) (6). Later, van der Hoeve (ox) (7), finding variations in either direction, pronounced them isotonic, a conclusion corroborated by Osborne (ox) (8); while Deiter (rabbit) (9), using a micro-apparatus and comparing the two fluids of the same animal, found in three experiments that the aqueous humour was isotonic in one and hypotonic to the serum in two. Largely owing to technical difficulties the method has therefore given inconclusive results. Further, an exact comparison cannot be said to result from the lumping together of the aqueous humour of several animals; and the comparison is rendered still more questionable when it is remembered that in the estimation a temperature difference of 0·001° C.—a quantity difficult to measure with any approach to accuracy—registers to the quite appreciable variable of 9 mm. Hg.

Using the method of plasmolysis the earlier observers were again united in considering the aqueous humour hypertonic to serum—Hamburger (horse) (10), Kunst (3), Manca and Deganello (oxen) (11)—a conclusion supported by Manca (12), using the hæmatocrit. Later investigators, however, using the same method, failed to get the same consistent results. Römer (ox) (13) considered that the friability of the red cells varied in itself up to 50 p.c., and he arrived at the same conclusion as Nuel (14) and Rissling (15) (using various animals), that any differences found were within the limits of the experimental

errors involved in the method, and that the aqueous humour was isotonic with the blood serum "more or less." The method cannot pretend to great accuracy, and in addition to the source of error depending on the variable behaviour of the red cells, there are others depending on the treatment of the serum.

From a consideration of these results it would seem apparent that, whatever their exact relationship, the osmotic pressure of the aqueous humour and the blood are not far removed in magnitude; and in comparing values so nearly allied, and in dealing with solutions so widely different in their molecular aggregation, it would seem preferable to employ as far as possible a direct method of measurement. If the aqueous humour is a dialysate, the difference between the osmotic pressure of this fluid and the plasma can be determined by such a method, which is very much more sensitive than those hitherto employed, and will allow the comparison of the two fluids of the same animal to be made under conditions as constant and as near to the normal as experimental manipulations permit.

In the present experiments the total osmotic pressure was divided into two fractions—that due to the colloids and that due to the crystalloids. The osmotic pressure of the former was measured directly by employing a micro-osmometer capable of dealing with the small quantities of fluid available, provided with a membrane impermeable to colloids. Through this membrane the crystalloids could permeate freely, and any variation in their distribution through osmotic interchange was determined by estimating their concentration before the experiment commenced and after equilibrium had been established. These estimations were confined to the aqueous humour, since, being a comparatively simple and dilute solution, the results obtained therein are more readily interpreted than corresponding results obtained in blood. Any change in the total concentration of dissociated salts was determined by electrical conductivity measurements: the aqueous is to all intents and purposes a physiological salt solution and practically protein-free, and it may be taken that the measure of its conductivity under constant conditions provides an index of any change in its salt content. Any change in the concentration of undissociated crystalloids, which are represented largely by sugar, was determined by the chemical estimation of this substance. The difference in osmotic pressure due to the non-diffusible substances was therefore read off directly on a manometer as mm. Hg; since the membrane offered no permanent resistance to the passage of diffusible substances and they would therefore pass freely from one side to the other until osmotic equilibrium had been established, a determination

of any change in their distribution would be an index of the difference in the component of the osmotic pressure due to their influence. A summation of these two fractions was taken as providing a relative determination of the total osmotic pressure of the two fluids.

A micro-osmometer was designed as illustrated, and throughout all the experiments it was used immersed in a thermostat kept at 18° C.

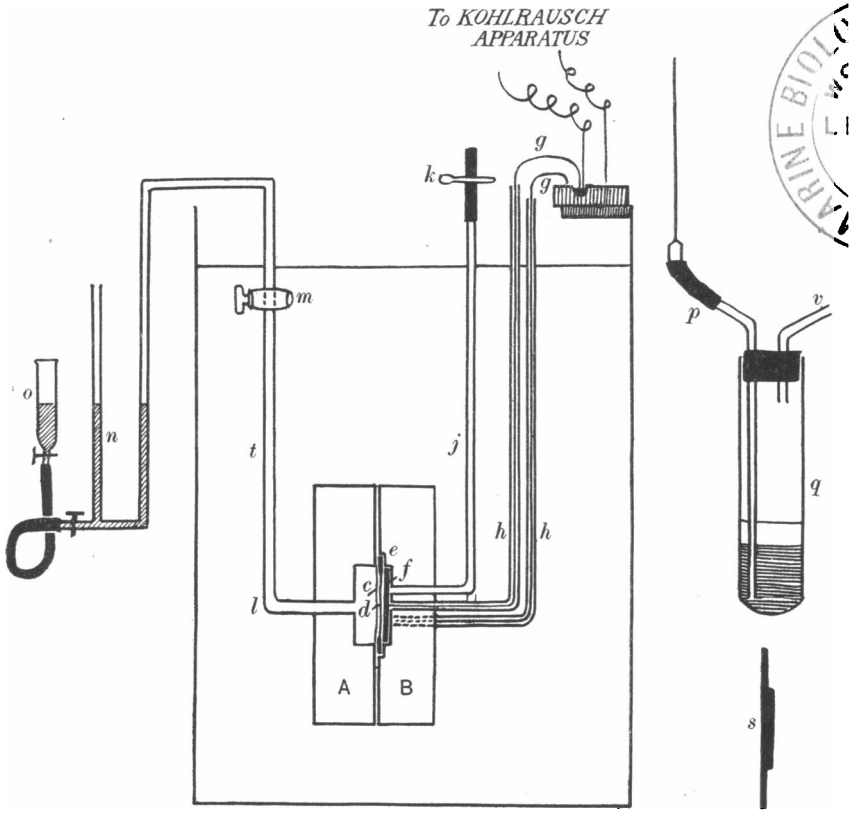

Fig. 1.

It was made of glass, two plates of which (A and B), with the opposing surfaces accurately ground, were clamped together. (In the figure the clamp is not shown in position.) In each plate a rectangular cell was cut: that in A—the "blood cell"—was made of such capacity as to contain 0·5 c.c. of blood (1 × 1 × 0·5 cm.); that in B—the "aqueous cell"—to contain 0·2 c.c. of aqueous (1 × 1 × 0·2 cm.). This was the size of cell which was found most convenient to work with, as the total quantity of aqueous obtainable from the eye of the average rabbit is from 0·25

to 0·30 c.c., and a cell of such size, while being shallow enough to allow osmotic equilibrium to establish itself fairly rapidly, at the same time permitted the spacing of two rectangular electrodes of sufficient area at a sufficient distance apart to ensure reliable conductivity measurements. Between the two a membrane of cellophane[1] was interposed (*c*), supported by a stout copper gauze (*d*), the joint being made water-tight by surrounding the gauze on both sides by a thin rubber washer peripherally, which fitted accurately into a shallow ledge (*e*) cut from the plate (B). The washer projected slightly from the sides of the aqueous cell so that the electrodes at no place came into contact with the gauze, and this latter was further insulated by coating it with bitelite, thus preventing any interference with the measurements of the conductivity, as was verified by standardisation with potassium nitrate. Two platinum electrodes (*f*) were cemented on to the parallel sides of the aqueous cell, and led off by platinum wires (*g*, *g*), cemented into holes running through the thickness of the plate (B). These were carried above the level of the surface of the water in the thermostat by glass capillaries (*h*, *h*) ground and cemented into the plate of the osmometer. Connection was then made through mercury contacts with a Kohlrausch apparatus. From each cell, fitting securely by ground joints into holes running through the thickness of the glass plates, two tubes ran upwards above the water surface: the one (*j*)—the tube from the aqueous cell—terminated in a small rubber tube, which could be opened or closed by a clamp (*k*); the other (*l*)—the tube from the blood cell—could be closed by a stop-cock (*m*), beyond which connection was made to a mercury manometer (*n*) and levelling bulb (*o*).

The majority of the experiments were done on rabbits. The aqueous humour was taken from both eyes and mixed. It was withdrawn under sterile conditions by means of a syringe dried with alcohol and ether. A special needle was made with a broad lance point, and this was introduced into the cornea near the limbus obliquely; such a needle is inserted with less disturbance than the ordinary round pointed instrument, and with the latter it is difficult to prevent the aqueous from escaping round it at the moment of its introduction. 2 p.c. cocaine was instilled into the conjunctival sac as an anæsthetic; it has been repeatedly demonstrated that this procedure does not alter the properties of the intra-

[1] See Verney (this *Journal*, 61. p. 319, 1926), who has tested the permeability of cellophane to crystalloids and its impermeability to serum proteins. I am indebted to Dr Verney for demonstrating his apparatus to me before his work was published, from which I have freely borrowed several ideas.

ocular fluids to any appreciable extent. Before the needle was introduced the cornea was dried with blotting paper to avoid contamination by tears. Blood was taken, also with aseptic precautions, from the ear, the central artery or the marginal vein being used as the case required. In order to obtain accurate comparisons it was considered necessary that the blood and the aqueous should be taken from the same animal. Plasma in preference to serum was employed: the osmotic pressure of the two are different, if only by a small amount, and it is the former which comes into equilibrium with the aqueous *in vivo*. It was also considered essential that the blood should be protected from air throughout, since the osmotic pressure varies with the carbon dioxide content; that it should be uncontaminated by anticoagulants, at any rate until the corpuscles had been separated off, since, in the majority of cases, these substances are known to alter the distribution of its constituents; and that throughout all the manipulations it should be kept sterile, since with the activity of micro-organisms a fall in the osmotic pressure of its protein constituents takes place. A sterile needle, connected with a glass tube (*p*), both of which were coated internally with sterile paraffin wax, was therefore inserted into the artery or vein after the ear of the animal had been cleaned and shaved. The tube, supported in a rubber cork, led down to the bottom of a centrifuge tube (*q*), also rendered sterile and paraffin-coated, so that it opened under a layer of liquid paraffin. By sucking a second small tube (*v*), arranged after the manner of a wash-bottle, blood was drawn into the bottom of the centrifuge tube and lay underneath the layer of paraffin without at any time having been in contact with the air. The cork with the two tubes was then withdrawn, and the blood, still under paraffin, was immediately centrifuged. The middle layer of plasma was then pipetted off, and was ready for introduction into the blood cell.

Before use the osmometer was sterilised, the rubber washers and cellophane by autoclaving, the glass cells by keeping them in a solution of perchloride of mercury, and drying them with alcohol and ether. The inside of the instrument was then coated with sterile paraffin wax, care being taken to leave the surfaces of the electrodes clear. To compare the conductivity of the aqueous humour before and after the experiment under constant conditions, it was necessary to obtain the first measurements after the fluid and the cell containing it had assumed the constant working temperature, and before it had opportunity of coming into association with the diffusible constituents of the plasma. After 0·2 c.c. of the aqueous had been put aside for the determination of its glucose

content and its refractometric value, a sterile vulcanite plug (*s*) was inserted into the open side of the aqueous cell, clamped into place and sealed with paraffin wax. The cell was then filled with aqueous from the syringe, and the tube (*j*), after its lower end had been dipped into sterile liquid paraffin, was pushed home into position so that the aqueous rose a little way up its lumen, its exposed surface being protected from the air by a layer of liquid paraffin; the tube was then sealed into position with paraffin wax. The half-cell was then immersed in the thermostat, and allowed to remain for half an hour until temperature equilibrium had been reached, when the conductivity was measured. Thereafter it was taken out of the thermostat, the clamp (*k*) shutting the outlet of the tube was closed, and, the cell being turned with its inner side uppermost, the plug (*s*) was taken off, the cellophane membrane and its supporting gauze were put into place, the washer being sealed with paraffin wax, and the blood cell (A), also paraffin-coated, was clamped into position. Into this the plasma was deposited directly from the centrifuge tube, and a small quantity of heparin added as a precaution, in addition to the paraffin, against subsequent clotting. With the stop-cock open, the tube (*l*) was then pushed home until the plasma surface rose to the mark (*t*), when it was sealed into place with paraffin wax, and the osmometer immersed again in the thermostat. The clamp (*k*) was then opened, and the top of the tube (*l*) was connected with the manometer, whose level was adjusted by the levelling bulb so as to exert a pressure upon the meniscus of plasma approximating that which experience had shown to be the final pressure reading: the stop-cock was then closed. At the end of twenty-four hours the pressure in the air confined between the meniscus and the stop-cock was measured by manipulating the levelling bulb of the manometer until the level of the plasma had reached its original mark as determined by observation through a horizontal microscope. The apparatus was then allowed to stand for two hours to verify the attainment of equilibrium: it was always found that this had been reached within the first few hours. Then, this being the case, the reading on the mercury manometer, corrected for the difference in level of a column of plasma between the meniscus and the cell and for the capillarity of the tube, gave the osmotic pressure of the non-diffusible constituents. The conductivity was then again measured, the osmometer dismantled, and the aqueous was withdrawn for the estimation of its refractive index and its sugar content.

The "sugar" was estimated by the Hagedorn-Jensen method. The refractive index was taken in the thermostat under constant temperature

conditions with a Dipping refractometer (Zeiss) fitted with an auxiliary prism to enable it to deal with one drop of fluid: the instrument reads to an accuracy corresponding to ± 3·7 units of the fifth decimal place of n_D. The difference—if any—in the refractive index before and after the experiment demonstrated the efficiency of the membrane in keeping back colloid material. The refractometric method seemed peculiarly appropriate for the purpose, since the power to refract light is a function of the size of the molecules—the property with which we are largely concerned—and is additive, being independent of their chemical nature.

Both arterial and venous blood were used; as also was the normal aqueous humour obtained on first performing a paracentesis upon the eye, and the reconstituted aqueous formed secondarily under the abnormal pressure conditions brought about by the evacuation of the anterior chamber.

The results obtained are tabulated below.

I. *Experiments on rabbits using normal aqueous humour.*

(*a*) Colloid osmotic pressure.

	No. of rabbit	Manometer reading mm. Hg	Diff. in levels mm. plasma	Capillarity of tube mm. plasma	Corrected colloid os. pres. mm. Hg	Mean pres. mm. Hg	Variation mm. Hg
With arterial blood	1	20·8	+32	−12	22·3	21·66	+0·64
	2	19·2	+30		20·5		−1·16
	3	20·5	+35		22·2		
With venous blood	4	21·5	+38	−12	23·5	22·1	+1·4
	5	18·8	+30		20·1		−1·0
	6	21·0	+35		22·7		

(*b*) Electrical conductivity of aqueous humour—$\lambda_{18° C.} \times 10^5$.

	No. of rabbit	Conductivity before exp.	Conductivity after exp.	Difference	Mean difference
With arterial blood	1	1293	1293	0	− 3·3
	2	1313	1306	−7	
	3	1342	1339	−3	
With venous blood	4	1363	1385	+22	+17·4
	5	1300	1328	+28	
	6	1353	1355	+ 2	

(*c*) "Sugar" content of aqueous humour: grm. p.c. (*d*) Refractive index.

	No. of rabbit	Before exp.	After exp.	Difference	Mean difference	Before exp.	After exp.
With arterial blood	1	0·141	0·141	0	+0·012	1·335168	1·335168
	2	0·138	0·168	+0·030		1·335244	1·335244
	3	0·154	0·159	+0·005		1·335206	1·335206
With venous blood	4	0·170	0·102	−0·068	−0·053	1·335130	1·335130
	5	0·155	0·104	−0·051		1·335206	1·335206
	6	0·164	0·122	−0·042		1·335168	1·335168

As supplementing these figures, a typical estimation of the chloride content of blood and aqueous humour in the normal rabbit is of interest. The estimations were done by Ruszynák's modification of Koranyi's method(16), and the results are expressed as NaCl. This method gives values expressed volumetrically as grm. per 100 c.c. plasma or aqueous; but for the purpose of the present enquiry, in comparing two solutions of so dissimilar molecular aggregation, these results should be corrected to allow for the mass occupied by the molecules of solute, which, owing largely to the presence of large-moleculed proteins in the blood, is very different in the two cases. Thus 100 c.c. of rabbit's plasma was found to contain 8·6832 grm. of solids at 100° C.; and the density of the plasma was found to be 1·020. 100 c.c. of the plasma therefore contains 93·6168 grm. water, and since this represents the available solvent, a correction factor of 100/93·6168, or 1·075, gives the true concentration in watery solution. The aqueous on the other hand is practically protein-free: it contains 1·0899 grm. solids per 100 c.c. at 100° C., and its density is 1·007. The corresponding correction factor to be applied is therefore 1·01.

	NaCl		
Arterial plasma	0·603 grm. per 100 c.c. plasma	× 1·075 =	0·6482 grm. per 100 grm. water
Venous plasma	0·578 grm. per 100 c.c. plasma	× 1·075 =	0·6213 grm. per 100 grm. water
Aqueous	0·668 grm. per 100 c.c. aqueous	× 1·01 =	0·6753 grm. per 100 grm. water

Further the "sugar" content of the plasma of rabbit No. 1 was found to be:

	"Sugar"		
Arterial plasma	0·136 grm. per 100 c.c. plasma	× 1·075 =	0·1461 grm. per 100 grm. water
Venous plasma	0·120 grm. per 100 c.c. plasma	× 1·075 =	0·1290 grm. per 100 grm. water
Aqueous	0·141 grm. per 100 c.c. aqueous	× 1·01 =	0·1425 grm. per 100 grm. water

From these results it is seen that, owing to the excess of non-diffusible substances in it, the plasma exerts an osmotic pressure of about 20 mm. of mercury greater than the aqueous in the rabbit. The measurements of the conductivity show that when arterial blood was being used there was little or no diffusion of the dissociated ions. The small differences found were almost within the experimental error, but the fact that they all occurred in the same sense seems to indicate that there was a tendency to migration from the aqueous to the plasma. When venous plasma was used, however, there was a definite shift of ions from the plasma into the intra-ocular fluid. It is therefore to be concluded that the total concentration of dissociated salts in the aqueous is practically the same as, but probably a little more than, that required to maintain osmotic equilibrium with the arterial blood, and considerably less than that required in the case of venous blood. These findings confirm, in

the main, those obtained by van der Hoeve(7), who, dialysing aqueous against serum found its conductivity to be the mean between a dialysate of arterial and venous serum. Similarly with the undissociated constituents as represented by the sugar: this is present in the aqueous in such a proportion as to be practically in osmotic equilibrium with the arterial plasma. When venous blood was used, the diminished concentration of sugar in the aqueous cell indicated a diffusion into the blood, that is, the initial osmotic concentration of this substance in the aqueous was greater than that in the venous plasma. The refractive index throughout showed no detectable change. Concurrent estimations of the chloride (as the preponderating dissociated constituent) and of the glucose showed that there was more chloride in the arterial than in the venous plasma, and more in the aqueous than either; and that there was more sugar in the arterial than in the venous plasma, while the quantity in the aqueous, when expressed in terms of concentration in watery solution, lay between the two, although nearer that in the arterial plasma than the venous.

II. *Experiments on rabbits using the aqueous humour formed secondarily after paracentesis of the anterior chamber.*

This re-formed aqueous humour has usually been regarded as having a completely different origin from the normal aqueous. It has long been known to contain more protein than the normal fluid, and has been variously called "albuminous" aqueous, "secondary" aqueous, or "reconstituted" aqueous. The normal aqueous contains all the colloidal constituents of plasma in minute amount (protein, fats, immune bodies, etc.), and the re-formed fluid differs from the normal in its constitution only quantitatively by containing relatively more of these difficultly diffusible substances. Further, a fluid of the same chemical composition is formed not only after paracentesis, but under all conditions wherein capillary dilatation occurs, as on radiation of the eye or the application of heat, irritant sub-conjunctival injections, mechanical and chemical irritation of the cornea, the production of venous congestion by subluxation of the eyeball or constriction of the neck, or on the lowering of intra-ocular pressure brought about by applying pressure to the eye and suddenly releasing it. Moreover, its formation is prevented under these circumstances by any agency which prevents the capillaries dilating, as stimulation of the sympathetic, the retro-ocular injection of adrenaline, or ligature of the carotid. It would seem therefore that none of these names meets the requirements of the case. In as much as it appears to differ from the normal aqueous only in resembling the plasma more closely, I propose to call it the "plasmoid" aqueous.

(*a*) Colloid osmotic pressure.

	No. of rabbit	Quantity of aqueous removed on 1st paracentesis c.c.	Manometer reading mm. Hg	Difference in levels mm. plasma	Capillarity of tube mm. plasma	Corrected os. pres. difference mm. Hg
Arterial blood	7	0·1	17·5	+32	−12	19·0
	8	0·25	14·5	+35		16·2
	9	0·32	13·2	+35		14·9
Venous blood	10	0·20	15·5	+30	−12	16·8

(*b*) Electrical conductivity of aqueous humour: $\lambda_{18°\,C.} \times 10^5$.

	No. of rabbit	Conductivity normal aqueous	Conductivity plasmoid aqueous		Difference in cond. normal – plasmoid aqueous
			Before exp.	After exp.	
Arterial blood	7	1335	1281	1278	54
	8	1272	1174	1170	98
	9	1365	1217	1217	148
Venous blood	10	1313	1236	1262	77

(*c*) Refractive Index.

	No. of rabbit	Normal aqueous	Plasmoid aqueous		Difference plasmoid – normal aqueous	Approx. protein p.c. in plasmoid aqueous
			Before exp.	After exp.		
Arterial blood	7	1·335244	1·337088	1·337088	0·001844	1·0
	8	1·335168	1·339036	1·339036	0·003868	2·0
	9	1·335244	1·339834	1·339834	0·004590	2·5
Venous blood	10	1·335130	1·338428	1·338428	0·003298	1·8

Protein content of normal aqueous of rabbit—0·04 p.c.

(*d*) "Sugar" content of aqueous humour.

	No. of rabbit	Normal aqueous	Plasmoid aqueous	
			Before	After
Arterial blood	7	0·143	0·148	0·148
	8	0·175	0·175	0·179
	9	0·165	0·172	0·177
Venous blood	10	0·150	0·155	0·111

(*e*) Chloride (as NaCl).

Normal aqueous	0·686 grm. p.c.
Plasmoid aqueous	0·536 grm. p.c.

III. *Experiments with different species of animals, using normal aqueous humour and venous plasma.*

Animal	Corrected osmotic pressure mm. Hg	Approximate protein p.c. in blood
Rabbit	20·27	5–6
Cat	31·33	7–8
Dog	29·80	7–8
(Man—Deiter [9], using Krogh's modification of Sørensen's apparatus	31·7	7–8)

It is seen that on removing progressively larger quantities of the aqueous humour from the eye, the re-formed fluid, as judged from its refractive index, contains a progressively larger quantity of colloid material. The approximate percentages of protein corresponding with the refractive indices found are given in the table: these were calculated by utilising a comparatively large quantity of horse aqueous humour and subtracting the refractive index of a protein-free preparation of this

fluid as obtained by the technique elaborated by Brailsford Robertson(17) from its normal refractive index, and correlating this value with gravimetric protein estimations carried out on the same fluid. From the results obtained it is seen that the difference in osmotic pressure between the plasmoid aqueous and the plasma due to the non-diffusible constituents is progressively smaller in the same animal as the colloid content of the former increases. Further, it is seen that in the normal aqueous of different species of animals this difference varies with the protein content of the blood. At the same time the measurements of the electrical conductivity and the estimations of the sugar suggest that, in the concentration of their diffusible substances, the plasmoid aqueous bears the same relation to the plasma as the normal aqueous does, that is, it is practically in osmotic equilibrium with the diffusible constituents of arterial blood, being—if anything—in slightly higher concentration than these, while the dissociated salts are in less concentration, and the glucose in greater concentration than that required to maintain equilibrium with venous blood.

Discussion.

The physiological interest in the comparison of the osmotic pressures of the intra-ocular fluids and the blood lies in the influence which the result must have on any theory of the origin of the aqueous humour. If this latter is a secretion elaborated by an expenditure of energy by the cells of the ciliary epithelium, it remains open for it to have an osmotic pressure greater than, equal to, or less than the plasma. If, however, it is a dialysate from the plasma, when it is equilibrated with the latter, its refraction and its conductivity should remain unchanged. The earlier writers, who held that the aqueous was a transudate and who at the same time found that its osmotic pressure was higher than that of the serum, maintained an impossible position which they apparently failed to recognise. And those who, advocating the same origin and looking upon the intra-ocular fluids as in rapid circulation through the eye, regarded the osmotic pressure of the two as being equal, overlooked the fact that the concentration of negative ions in the aqueous, as shown by the chloride content, is higher than that in the blood.

It is hoped in a future publication to deal with the question of the circulation of the aqueous; but as far as considerations of osmotic pressure go, at the end of each of the experiments detailed above the fluids on either side of the membrane (aqueous and plasma) were in Donnan's equilibrium. During the course of the experiments, when arterial blood

was used, neither the undissociated constituents (as judged by the sugar estimations), nor the ionised constituents (as judged by the conductivity measurements) had altered appreciably in concentration—if anything, they had slightly decreased. Before the experiments commenced, therefore, that is, as these fluids occurred *in vivo*, it follows that their molecular concentration bore the same relationship. When venous blood was used, the smaller concentration of sugar in the aqueous cell at the end of the experiment betrayed a diffusion of this substance into the plasma. The chemical analysis carried out concurrently showed that the sugar content of venous plasma is less than that of arterial plasma: the sugar concentration in the aqueous is seen to be greater than that in the former, and slightly less than that in the latter. At the same time the increased electrical conductivity of the aqueous indicated a diffusion of ionised salts in the opposite direction. As a result of the ionic interchange dependent on the addition of carbon dioxide to the plasma there is an increase of osmotic pressure in the change from the arterial to the venous condition, due to the increase of the bicarbonate content undergoing a greater change in molarity than the concomitant decrease of the chloride content. The concentration of the ionised constituents of the aqueous would therefore appear to be slightly greater than that in the arterial, and considerably less than that in the venous blood. It seems to be the case that capillary blood is more nearly related to the arterial than to the venous blood, a relationship suggested by the findings of Verzár and Keller(18) on the oxygen saturation of "finger blood," and of Foster(19), who found that the sugar content of the latter was practically identical with that of arterial and widely different from that of venous blood: moreover, in the present case, any remissness in the somewhat exacting technique of keeping the plasma absolutely excluded from the air throughout the complicated manipulations would tend to reduce the arterial plasma to some extent. The deduction that appears to follow is that the molecular concentration of the aqueous is equal to that of a dialysate of capillary blood. Such a suggestion would account, moreover, for the (practical) identity of the concentration of undissociated diffusible substances (sugar) in the two fluids, and the preponderance of cations (Cl') in the aqueous. The osmotic pressure of the aqueous is therefore less than that of the blood, the difference between them being determined in part by the difference in the distribution of their ions, and in part by the difference in the concentration of their colloid constituents.

When the eye is punctured and the normal aqueous is drawn off, the anterior chamber rapidly refills with a fluid containing more colloid than normally. The experimental results detailed above show that

the excess of colloid varies with the amount of fluid originally withdrawn, until on complete evacuation the re-formed fluid closely resembles the plasma. It is said that this plasmoid aqueous is of completely different origin from the normal aqueous; that the latter is a secretion, and that the former is a transudate determined by the altered pressure conditions following the removal of the supporting pressure of the intra-ocular fluids. We find, however, that in all stages of withdrawal, the fluid retains the same molecular concentration relative to the blood as the normal aqueous, that is, osmotically, the two always remain in Donnan's equilibrium. Further, the undissociated diffusible substances (sugar) retain practically the same concentration; the slight tendency to increase of sugar in the plasmoid aqueous is probably within the error introduced by an increase in plasma sugar accompanying the excitement of the experimental manipulation, although, alternatively, it might be interpreted as indicating the introduction into the aqueous of "bound" sugar along with the increase of plasma proteins. But the conductivity was found to decrease with the addition of protein to an extent greater than the mere addition of colloid molecules warranted. The correction formula to allow for this inverse relation is (Bugarzky and Tangl(20)):

$$\lambda_c = \lambda \frac{100}{100 - 2{\cdot}5p},$$

where λ_c and λ are the corrected and determined conductivities, and p the protein p.c. Thus in rabbit No. 7, where the measured conductivity of the plasmoid aqueous was found to be 1281×10^{-5} and the protein content 1·0 p.c., the normal aqueous should have had a conductivity of 1307; whereas it was 1335. Corresponding with this, chemical estimation showed a decrease in the concentration of anions (Cl′)—a relationship explicable in terms of a system in Donnan's equilibrium. It seems reasonable, therefore, to suggest that the two—the normal and the plasmoid aqueous—may be formed by one and the same process; that on the withdrawal of the supporting pressure of the intra-ocular fluids the capillaries dilate, the amount of dilatation depending on the extent to which the eye was evacuated, the permeability of their walls becomes increased, and the dialysate then formed becomes progressively richer in colloids, while, at the same time, the ionised constituents redistribute themselves to conform with the altered conditions.

If the aqueous and the plasma are in Donnan's equilibrium, not only must their osmotic pressures bear a definite relationship, but a difference in electrical potential must exist between them whose value is given by the formula of Nernst:

$$E = \frac{RT}{F} l_n \frac{x}{y},$$

where x and y represent the concentration of cations on the two sides of the membrane. Recently, Lehmann and Meesmann(21), using a capillary electrometer and 1/10 n. KCl electrodes, one of which was introduced through a cannula into the jugular and the other through a needle into the aqueous, found in cats and dogs that a difference of from 6 to 10 millivolts existed between the two, the aqueous being positive and the blood negative. Moreover, the potential difference between them decreased on equalising their protein contents, either by increasing the protein in the aqueous by performing a paracentesis or by injecting plasma into the eye, or after dilution of the blood proteins (in frogs) by intravenous perfusion with Ringer's solution.

Considering these results in conjunction with those obtained as the values of the vascular pressures in the eye(1), it would appear that the physical forces involved—the difference in the hydrostatic pressure of the aqueous (the intra-ocular pressure) and that in the blood vessels, the difference in the osmotic pressure and in the electrical potential of the two fluids—show nothing inconsistent with the hypothesis that the intra-ocular fluids are formed by dialysation from the capillary plasma, and are in equilibrium with it.

Conclusions.

(1) Evidence is brought forward which suggests that the aqueous humour is a dialysate of capillary blood, in that, when this fluid is equilibrated with arterial blood, its refraction, conductivity and sugar content remain practically unaltered, any change which does occur being in the direction of equilibrium with venous blood.

(2) The osmotic pressure of the aqueous is the same as that of a dialysate. It is therefore less than that of the blood, the amount depending on the difference in the colloid content of the two fluids. This difference is only a small percentage (about 0·3 p.c. in the rabbit) of the total osmotic pressure (about 6000 mm. Hg).

(3) The osmotic pressure of the plasmoid aqueous bears the same relation to the osmotic pressure of the blood as does the normal aqueous. Its molecular concentration is also that of a dialysate, and, as far as the present investigation goes, there is no necessity to ascribe to it a mode of formation differing fundamentally from that of the normal aqueous.

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