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COMMUNICATIONS

STUDIES ON THE INTRA-OCULAR FLUIDS

Part 4 — The Dialysation of Aqueous Humour against Plasma*

BY

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THERE have been many attempts over the last quarter of a century to determine whether the aqueous humour and the blood plasma were in thermo-dynamical equilibrium. One of the most direct ways of determining whether or not this is so is to dialyse the one fluid against the other across a separating membrane; if there is a migration of substances across the membrane, it is obvious that such an equilibrium does not exist. In previous work of one of us (Duke-Elder, 1927) using pooled aqueous humour of

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several animals, it was found that such a transfer was not of great dimensions; but the present experiments, carried out in much more efficiently controlled conditions, demonstrate that a transfer from the aqueous humour into the blood-plasma is consistently found.

METHODS

General.—Dogs and cats were anaesthetised with nembutal; aqueous humour was removed from both eyes with a gauge 1 needle and syringe; blood was obtained by heart-puncture, heparinised, and equilibrated with alveolar air at body temperature; the plasma was separated by centrifugation in a corked tube. For studies on the chloride content and on conductivity, the fluids from a single animal were sufficient for an experiment; for studies on the distribution of sodium, the fluids from three animals were pooled in strict proportion.

Dialysis.—In Experiments Nos. 1-12, the plasma was placed in a collodion sac, prepared as described by Dale and Evans (1920), tied to the end of a piece of capillary tubing (Fig. 1), which was then sealed with sealing-wax. The sac was washed with a few drops of aqueous humour and then immersed in sufficient of

FIG. 1.

FIG. 2.

Dialysation apparatus

this fluid to cover it. A collar of rubber tubing round the capillary permitted the sac to stand in the fluid without touching the sides or bottom of the container. Evaporation was reduced (but apparently not always completely prevented) by covering the collar with paraffin-wax. The system was allowed to stand for about 16 hours at room temperature together with samples of the original fluids in stoppered tubes. During the course of the work it became evident that, in some of the experiments, evaporation of the fluids was taking place in the dialysing system; consequently the apparatus illustrated in Fig. 2 was devised. It consisted of an outer glass container, A, into which fitted a ground stopper, B. The base

of the stopper formed a slightly tapered projection, C, which was pushed into the top of the collodion sac, the junction being sealed with cellulose acetate paint. The stopper and the bottom of the container were pierced by holes, into each of which was sealed by sealing wax a length of glass tubing drawn out to capillary thinness. The stopper with its attached collodion membrane was placed in the container and fixed in position by wax or paint spread on the outside junction. An outer projection on the stopper, D, was pushed into a hole in a rubber bung; into the other end of this hole was pushed a glass tube joined to a length of rubber tubing so that by sucking or blowing on to this tubing the collodion sac could be collapsed or blown out. To fill the chamber, the sac was blown out, the bottom capillary placed under the required solution which was then drawn in by suction until the sac was completely collapsed. The chamber was then removed from the bung, inverted, and another similar projection, E, on the bottom of the container pushed into the hole. The sac was then filled with the other solution, normally plasma, by sucking it in through the capillary in the stopper. Some suction was maintained on the tube until the tip of this capillary was sealed with wax. The container was then removed from the bung and the other capillary likewise sealed.

By this means equal volumes of fluid were obtained inside and outside the sac. The sac was 8 mm. in diameter and the inside of the container about 11 mm. so that if the sac were cut so that it almost touched the bottom of the container, the fluid levels inside and outside were about the same. For the experiments described here two chambers were constructed to take 1 ml. or 2 ml. of solution inside the sac, but it is not of course necessary, although desirable, that the chamber should be completely filled either inside the sac or outside.

Stirring was achieved by fixing the chamber on a large pulley rotating at about 1 rev./sec.; at this speed the air bubbles in the compartments moved through the whole column of liquid and back during each cycle. Preliminary studies showed that equilibrium was established in about 30 min. but generally two to three hours were allowed.

To empty the sac the tips of the capillaries were broken off and the filling procedure followed in reverse. Even with the operations required in the repeated washing of the sac before filling, it should remain serviceable for at least half a dozen dialysations; it must, of course, be kept wet when not in use.

Chemical.—Sodium was determined by the Barber-Kolthoff (1928) technique on triplicate 0.5 ml. samples of the fluids, as described in an earlier paper (Davson, 1939); the mean standard error of the thirty-four triplicate determinations was one part in 730; since the effects described in this paper involve changes of as much as one to two per cent. there is no doubt of their statistical significance. Chloride was determined on 0.2 ml. samples by the Sendroy technique (1937), the mean standard error of triplicate determination being one part in 300.

Dry weight.—About 1 ml. of plasma was evaporated to dryness on a sand-bath and heated for about 24 hours in an oven at 105°.

Conductivity.—The conductivity cell was in the form of a pipette of 0.3 ml. capacity with a flattened bulb into which were sealed two platinum wire electrodes, blackened in the usual manner. Connection to the bridge was made by two copper wires, soldered on to the electrodes and carried round the stem of the pipette. The junctions and leads were protected with a coating of wax and lacquer. The bulb was filled by suction through a rubber tube which was then clipped off and the tip of the pipette was sealed with a length of blocked cycle-valve tubing. The cell was immersed in melting ice contained in a Dewar flask. Before filling with a sample the cell could either be washed with distilled water and dried with alcohol and ether, or merely washed out with a fluid of conductivity close to that expected in the sample. The resistances were measured on a bridge constructed of normal radio components and calibrated with a decade resistance box. Duplicate readings on plasma or aqueous humour samples agreed to one part in a thousand.

pH.—The measurements were made with a glass-electrode reading against a calomel $\frac{1}{2}$ cell on a direct reading valve millivoltmeter. Alternate readings were taken of dialysed and undialysed fluids which were transferred as directly as possible to the measuring cell so as to avoid significant CO_2 loss.

RESULTS

Chloride.—The results with chloride on dogs are quite definite (Table I); as a result of a decrease in the original concentration of chloride in the aqueous humour (A_1), and of a rise in the corresponding concentration in the plasma (P_2), the ratio, R_{Cl} , changes from a mean value of 0.91 to one of 0.98. The two fluids are quite clearly not in equilibrium with respect to chloride. In cats the effects are not so large; nevertheless there is a definite

TABLE I
THE MOVEMENT OF CHLORIDES

The change in the Gibbs-Donnan ratio, R_{Cl} , following dialysis of plasma against aqueous humour. Expts. 21 to 24 refer to cats, the remainder to dogs. Expts. 1 to 16 were done with the earlier, 17 to 24 with the improved type of apparatus. A and P are the concentrations of chloride, in millimoles per kg. of water, in aqueous humour and plasma respectively. The suffices (1) and (2) refer to the figures obtained before and after dialysis respectively.

Expt.	A_1	P_1	R_1	A_2	P_2	R_2	$R_2 - R_1$
Dog 1	138.4	121.2	0.875	130.3	126.0	0.965	+ .09
2	145.4	130.0	0.895	140.1	134.6	0.96	+ .065
3	142.2	127.1	0.895	133.1	133.2	1.000	+ .105
4	134.5	123.9	0.92	129.0	129.9	1.007	+ .087
5	127.7	119.2	0.935	125.0	122.0	0.975	+ .04
6	131.7	123.0	0.935	132.1	128.5	0.975	+ .04
7	130.2	123.8	0.95	131.3	130.7	0.995	+ .045
17	128.0	118.2	0.923	126.9	123.0	0.97	+ .047
18	123.2	114.4	0.93	122.1	118.5	0.972	+ .042
19	129.9	115.9	0.893	121.6	122.4	1.005	+ .112
20	127.5	109.9	0.865	122.5	115.2	0.94	+ .075
Mean			0.911			0.979	
Cat 21	131.6	122.7	0.932	127.5	126.0	0.99	+ .058
22	127.5	123.9	0.973	128.9	124.6	0.968	— .005
23	130.6	119.1	0.91	129.5	123.4	0.953	+ .043
24	132.3	127.7	0.965	132.0	128.4	0.973	+ .008
Mean			0.945			0.971	

change in the ratio following dialysis when this is low in the original fluids, but it would appear that in some animals the fluids are in equilibrium in respect to chloride whilst in others they are not. It will be noted that the decrease in concentration in the aqueous humour does not always equal the increase in plasma concentration, for the rise in concentration in the plasma was on occasions larger than the fall in the aqueous humour.

Sodium.—Some results with this ion are shown in Table II; they are equally consistent but not so striking in magnitude. The average value of R_{Na} changes from 1.036 to 1.057 in the dog,

TABLE II

THE MOVEMENT OF SODIUM

The change in the Gibbs-Donnan ratio, R_{Na} , following dialysis of plasma against aqueous humour. Expts. 12, 13 and 15 refer to cats, the remainder to dogs. Pooled specimens from three animals used in each experiment. A and P are the concentrations of sodium, in millimoles per kg. of water, in aqueous humour and plasma respectively. The suffices (1) and (2) refer to the figures obtained before and after dialysis respectively.

Expt.	A ₁	P ₁	R ₁	A ₂	P ₂	R ₂	R ₂ — R ₁
Dog 8	150.4	154.6	1.027	146.6	155.6	1.062	+ .035
9	150.3	155.0	1.032	147.4	154.7	1.050	+ .018
10	149.8	157.2	1.050	149.1	160.5	1.077	+ .027
11	148.5	155.8	1.049	149.1	158.7	1.064	+ .015
14	149.8	154.0	1.028	148.5	154.1	1.038	+ .01
16	147.4	152.1	1.032	145.7	152.8	1.049	+ .017
Mean			1.036			1.057	
Cat 12	155.8	160.2	1.028	156.6	166.9	1.066	+ .038
	155.8	160.2	1.028	158.5	168.2	1.061	+ .033
13	152.3	160.8	1.056	150.3	161.2	1.073	+ .016
15	152.1	158.7	1.043	149.3	159.3	1.067	+ .024
Mean			1.042			1.068	

and from 1.042 to 1.068 in the cat. Once again we find that the change in aqueous humour concentration is not always balanced by that in the plasma.

In Experiments 8 and 9 the sac had been maintained in distilled water before use; this produced a diluting effect on the system which obscured the rise in plasma concentration; in all succeeding experiments the sac was equilibrated with saline; there was, however, evidence of evaporation, an effect which tended to obscure the fall in concentration of the aqueous humour (Experiments 10-12). This is well brought out by Experiment 12; in this experiment sufficient fluids were obtained for two successive dialyses, and as a result of the first dialysis the value of R_{Na} rose from 1.028 to 1.066. New lots of the same fluids were then placed inside and outside the sac and the system left for another 16 hours; it will be seen that the value of R_{Na} did not differ significantly from that obtained after the first dialysis, but that as a result of evaporation the concentrations of sodium in aqueous humour and plasma both rose. In the later experiments (Nos. 13 to 16) evaporation was excluded by the use of the new type of chamber; R_{Na} invariably increased as a result of dialysis as before, but even under these conditions the loss in concentration in the aqueous humour was not entirely accounted for by a rise in the plasma concentration. It is not easy to explain

these discrepancies. It is possible that the collodion membrane itself is not in equilibrium with the fluids surrounding it; it may tend to turgescence, to adsorb or desorb ions, the particular effect observed depending on its previous history. In view of the large number of animals required and the extreme laboriousness of the chemical technique, it was not thought worthwhile investigating the point further; all ten dialyses, representing the fluid from 27 animals, concurred in showing that the ratio R_{Na} increased as a result of dialysis; dilution or concentration of the system by a few per cent. should produce slight errors in the ratio, but too small to be detected (cf. for example Expt. 12).

Conductivity.—The changes in conductivity are shown in Table III. In all cases there was an increase in the resistance of the aqueous humour and a decrease in that of the plasma, *i.e.*, the changes were such as could be produced by a movement of sodium

TABLE III
CONDUCTIVITY EXPERIMENTS

Changes in electrical resistance of plasma (ΔR_{Pl}) and aqueous humour (ΔR_{Aq}) as a result of dialysis. Expts. 25 to 33 on cats; Expt. 34 on a dog. In Expts. 30 and 32, whole blood was placed in the sac instead of plasma.

Expt.	ΔR_{Aq}	ΔR_{Pl}	$\frac{\Delta R_{Pl}}{\Delta R_{Aq}}$
Cat 25	1.7	-3.6	2.1
26	2.5	-8.7	3.5
27	0.6	-0.7	1.2
28	1.8	-2.1	1.2
29	1.0	-1.8	1.8
30	1.2	-2.6	1.45
31	7.3	-10.6	1.45
32	0.5	-1.7	2.4
33	0.1	-1.2	2.0
Dog 34	6.5	-4.3	1.5
Mean	2.2	-3.7	1.7

and chloride from the aqueous humour into the plasma. It may be computed, from the characteristics of the dialysis system, that the ratio: *Decrease in Resistance of Plasma/Increase in Resistance of Aqueous Humour* should be in the region of 1.4; the mean ratio is 1.7.

pH.—The increase in the value of R_{Na} might conceivably be due to an alkalinity of the dialysis system resulting from the loss of CO_2 ; the observed changes, however, would require an increase of about 50 per cent. in the base-binding power of the plasma, a change that could be achieved only by a change of pH in the region of one unit. It was not feasible to make pH measurements on the fluids used for analysis; separate dialysis

experiments were therefore carried out with both types of sac to determine whether any significant change in the pH of the two fluids took place as a result of the dialysis. It was found, in general, that the pH of the plasma was unaffected by dialysis whilst the aqueous humour tended to become more acid; presumably this is because the losses of CO_2 in the manipulation cause the aqueous humour to become alkaline; dialysis with the better buffered plasma tends to restore the pH to the physiological range.

The re-formed aqueous humour.—Earlier work (Davson and Weld, 1941) has shown that the aqueous humour re-formed after paracentesis is in close approximation to equilibrium with plasma

TABLE IV

THE SODIUM DISTRIBUTION IN PLASMOID AQUEOUS HUMOUR

The Gibbs-Donnan ratio, R_{Na} , in the re-formed aqueous humour. R_1 and R_2 are the ratios obtained with the normal aqueous humour before and after dialysis respectively; R_3 is the ratio obtained with normal plasma and re-formed aqueous humour.

Expt.	R_1	R_2	R_3	Protein in Re-formed Aqueous Humour
9	1.032	1.050	1.012	—
10	1.050	1.077	1.010	3 per cent.
11	1.049	1.064	1.024	2.5 per cent.
12	1.028	1.064	1.011	3 per cent

in respect to chloride: in the present work the fluid, re-formed within about half an hour of withdrawal of the normal aqueous humour, was analysed for sodium; the results are shown in Table IV. It will be observed that R_{Na} has become quite close to unity.

DISCUSSION

The results of these experiments show that the aqueous humour is not in thermo-dynamic equilibrium with blood plasma in so far as the distribution of sodium and chloride between the two fluids is concerned, for these substances are both in excess in the aqueous humour and migrate from this fluid to the plasma on dialysation. It might be suggested that the high concentration of sodium in the aqueous humour could be accounted for on the basis of a difference of pH between plasma and aqueous humour resulting, for example, from the formation of lactic acid in the metabolism of the lens; but such an explanation, even if it were acceptable, could not account for the fact that the excess of

chloride in the dog's aqueous humour actually exceeds that of sodium. The fact that arterial blood-plasma was arbitrarily chosen as the reference fluid may give rise to question, but the concentrations of sodium in arterial and venous plasma are not appreciably different, so that it is unlikely that the concentration in the capillary plasma—the fluid with which the comparison should be made—differs measurably from that in the arterial plasma used. We are therefore forced to the conclusion, already arrived at in our earlier chemical work, that energy is expended in transferring sodium and chloride from the blood to the aqueous, that is, that a process of secretion exists.

In the dog, the deviation of the concentration of chloride from equilibrium was considerably greater than that of sodium, the excess of chloride in the aqueous humour amounting to about seven per cent. or some 9 millimoles per litre; since the discrepancy of sodium amounts to only about 3 millimoles per litre, the results suggest that some other cation is present in excess; studies on the distribution of potassium (Davson, Duke-Elder and Benham, 1936), calcium and magnesium (Stry and Winternitz, 1932) have not revealed excesses of these ions in the aqueous humour of a magnitude sufficient to account for the excess of chloride. Presumably an organic cation is present. In the cat the deviation of the chloride ratio from the equilibrium value was considerably smaller, and on occasions it was found that the chloride ion was actually at equilibrium; in this respect, therefore, there seems to be a significant quantitative difference in physiology between the cat and dog.

The plasmoid aqueous humour formed after paracentesis is an entirely different case. It is seen that the ratio for the concentration of sodium (R_{Na}) as seen in Table IV approximates unity and this is most simply interpreted as indicating that during the rapid process of filtration associated with the re-filling of the anterior chamber, the diffusible ions filter through the blood-aqueous barrier in amounts proportional to their concentrations in the plasma. There is thus no attempt initially to form a Donnan equilibrium: only at a later stage would the resultant potential impose a different ionic distribution. This, of course, is in accord with the view that in the conditions of stress resulting from the collapse of compensatory pressure on the outer side of the capillary walls, the normal blood-aqueous barrier is disrupted and direct transference takes place through widely opened spaces between the endothelial cells so that a direct transudation of substances—and sometimes of whole blood—takes place.

SUMMARY

Direct dialysation of the aqueous humour against the blood plasma shows by chemical analyses and conductivity measurements that there is a transference of sodium and chloride from the former to the latter. The two fluids are therefore not in

equilibrium and in respect to sodium and chloride secretory energy must be postulated in the formation of the intra-ocular fluid.

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AN INVESTIGATION INTO THE MODE OF HEREDITY OF CONGENITAL AND JUVENILE CATARACTS*

BY

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It has been generally accepted since the investigations of Nettleship (1905) and Bateson (1909), that congenital and juvenile cataracts are inherited as dominant characteristics. It is reasonable that this should be so, for the literature provides many examples of cases in which such cataracts have been traced through several generations, and it leaves no doubt about the existence of dominant types.

There are, however, many reported cases of congenital and juvenile cataracts appearing sporadically, or in small numbers within the same family, and in which they have not been traced to succeeding generations. Such cataracts are frequently recorded as "familial"; they are often of the congenital or juvenile variety, and the possibility of their hereditary nature has received little consideration.

Franceschetti (1930) makes the following statement in *Kurzes Handbuch der Ophthalmologie*: "Das häufige isolierte Auftreten bei Geschwistern liesse auch an recessive Vererbung denken. Da aber Konsanguinität selten ist, kommt unregelmässige Dominanz in Frage."

Waardenburg (1932) says: "Auch die angeborene Katarakt wird im allgemeinen in dominanter Weise vererbt. Es liegen aber

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