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CHANGES IN IONIC DISTRIBUTION FOLLOWING DIALYSIS OF AQUEOUS HUMOUR AGAINST PLASMA

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There have been frequent attempts to determine, by studies of ionic distribution, whether the aqueous humour and blood plasma are in thermodynamic equilibrium. It has been customary to adopt, as a criterion of equilibrium, the theoretical distribution of monovalent ions calculated by Van Slyke (1926) from the base-binding power of the plasma proteins. This distribution corresponds to a ratio: *concentration of sodium in plasma/concentration of sodium in aqueous humour* (R_{Na}) equal to 1·04, and to a similar ratio for the chloride ion, R_{Cl}, equal to 0·96. Studies on the cat certainly suggest a close approximation to thermodynamic equilibrium between the two fluids. However, a study of the chloride distribution in the dog (Hodgson, 1938; Davson & Weld, 1941) revealed a considerable discrepancy from Van Slyke's theoretical ratio, a mean value for R_{Cl} of 0·92–0·93 being obtained; the distribution of sodium, on the other hand, was almost completely theoretical ($R_{\mathrm{Na}} = 1{\cdot}04$). There thus seems to be an 'excess' of chloride in the aqueous humour of the dog and, to a less extent, in that of the cat. Studies on dialysates of plasma, obtained with collodion sacs (Ingraham, Lombard & Visscher, 1933; Greene & Power, 1931), suggest, however, that Van Slyke's theoretical ratios of 1·04 and 0·96 are wrong in that they fail to take into account the possibility that the activity coefficients of the ions are not the same for the two fluids; the studies indicate that R_{Na} should be 1·08 or even higher, whilst R_{Cl} should be 0·98–0·99. If this is true, the ratios of 1·03 and 0·945 for sodium and chloride in the cat, for example, are not to be taken as evidence of approximate thermodynamic equilibrium between the two fluids but rather of the existence of an excess of sodium and chloride in the aqueous humour. If aqueous humour and blood plasma are not in thermodynamic equilibrium in respect to sodium and chloride, dialysis of the one fluid against the other should result in the passage of these ions across the separating membrane. If, moreover, our interpretation of the values of R_{Na} and R_{Cl} is correct, the passage should be from the aqueous humour into the blood plasma. In the experiments to be described, such movements have been consistently found.

METHODS

General. Dogs and cats were anaesthetized with nembutal; aqueous humour was removed from both eyes with a needle and syringe; blood was obtained by heart-puncture, heparinized, and equilibrated with alveolar air at body temperature; the plasma was separated by centrifugation in a corked tube. For studies on chloride or 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 & Evans (1920), tied to the end of a piece of capillary tubing (Fig. 1*a*), which was then sealed with sealing-wax. The sac was washed with a few drops of aqueous humour and then immersed in sufficient of this fluid to cover it. A collar of rubber tubing round the capillary tubing 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 hr. 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 in the dialysis system was taking place, consequently the apparatus illustrated in Fig. 1*b* was devised. It consisted of an outer glass container, *A*, into which fitted a ground stopper, *B*. The base of the stopper formed

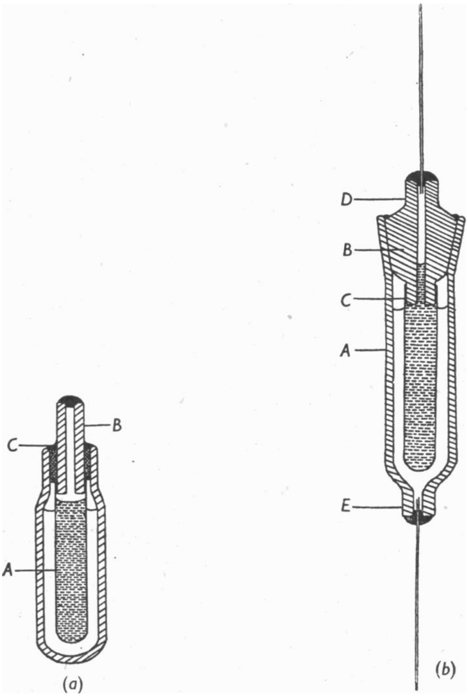

Fig. 1. (*a*) Simple dialysis system. The collodion sac, *A*, is tied to a piece of capillary tubing, *B*, and submerged in aqueous humour. *C* is a rubber collar. (*b*) Completely sealed system. For description see text.

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 means of sealing wax, a length of glass tubing drawn out to capillary thinness as shown in the diagram. 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 made 8 mm. in diameter and the inside of the container about 11 mm.; if the sac was 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 or 2 ml. of solution inside the sac, but it is not of course necessary, though it is 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 which was made to rotate 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 2–3 hr. 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 dialyses; 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 ashed as described earlier (Davson, 1939). The coefficient of variation of means of triplicate determinations was estimated to be 0·23 % (thirty-four sets of determinations); since the effects described in this paper involve changes of as much as 1–2 % there is no doubt of their statistical significance. Chloride was determined on 0·2 ml. samples by the Sendroy technique (1937); the coefficient of variation of means of triplicates was 0·6 % (twenty-nine sets of determinations).

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

Conductivity. The conductivity cell was in the form of a pipette of capacity 0·3 ml. with a flattened bulb into which were sealed two platinum wire electrodes, blackened in the usual manner. Connexion 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 and aqueous humour samples agreed to one part in a thousand.

pH. The measurements were made with a glass-electrode against a calomel half-cell on a direct-reading valve millivoltmeter. Alternate measurements were made on dialysed and undialysed fluids which were transferred as rapidly as possible to the measuring cell so as to avoid serious losses of CO_2.

RESULTS

Chloride. The results with chloride on dogs are quite definite (Table 1); as a result of a decrease in A, the concentration of chloride in the aqueous humour, and of a rise in P, the corresponding concentration in the plasma, 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; moreover, Van Slyke's calculated figure of 0·96 for the ratio differs significantly from the experimentally determined ratio after dialysis, 0·98. In cats the effects are not so large, as we should expect since the average value of R_{Cl} is, on the basis of earlier work, normally 0·945; nevertheless, there is a definite change in the ratio following dialysis when this is low in the original fluids. 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 from Table 1 that the decrease in concentration in the aqueous humour does not always equal the increase in plasma concentration; this results from several causes. Thus the volumes of the two fluids were not always equal; evaporation occurred in some experiments, and this tended to obscure the loss of chloride from the aqueous humour; however, even when these effects were excluded by the use of the new type of sac (Exps. 17–24), the rise in concentration in the plasma was on occasions larger than the fall in the aqueous humour.

TABLE 1. The change in the Gibbs-Donnan ratio, R_{Cl}, following dialysis of plasma against aqueous humour

Exp.	A_1	P_1	R_1	A_2	P_2	R_2	ΔR
1	138·4	121·2	**0·875**	130·3	126·0	**0·965**	+0·09
2	145·4	130·0	**0·895**	140·1	134·6	**0·960**	+0·065
3	142·2	127·1	**0·895**	133·1	133·2	**1·000**	+0·105
4	134·5	123·9	**0·920**	129·0	129·9	**1·007**	+0·087
5	127·7	119·2	**0·935**	125·0	122·0	**0·975**	+0·04
6	131·7	123·0	**0·935**	132·1	128·5	**0·975**	+0·04
7	130·2	123·8	**0·950**	131·3	130·7	**0·995**	+0·045
17	128·0	118·2	**0·923**	126·9	123·0	**0·970**	+0·047
18	123·2	114·4	**0·930**	122·1	118·5	**0·972**	+0·042
19	129·9	115·9	**0·893**	121·6	122·4	**1·005**	+0·112
20	127·5	109·9	**0·865**	122·5	115·2	**0·940**	+0·075
Mean			**0·911**			**0·979**	
21	131·6	122·7	**0·932**	127·5	126·0	**0·990**	+0·058
22	127·5	123·9	**0·973**	128·9	124·6	**0·968**	−0·005
23	130·6	119·1	**0·910**	129·5	123·4	**0·953**	+0·043
24	132·3	127·7	**0·965**	132·0	128·4	**0·973**	+0·008
Mean			**0·945**			**0·971**	

Exps. 21–24 refer to cats, the remainder to dogs. A and P are the concentrations of chloride, in millimoles per kg. of water, in aqueous humour and plasma respectively. The suffixes (1) and (2) refer to the figures obtained before and after dialysis respectively.

Sodium. The results with this ion are shown in Table 2; 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, 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 Exps. 8 and 9 the sac had been maintained in distilled water before use, a circumstance which produced a diluting effect on the system and thus obscured the rise in plasma concentrations; in all succeeding experiments the sac was equilibrated with aqueous NaCl, the concentration being approximately that found in the aqueous humour. There was, however, evidence of evaporation, an effect which tended to obscure the fall in concentration of the aqueous humour (Exps. 10–12); this is well brought out by Exp. 12, in which sufficient fluids were obtained for two successive dialyses. 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 hr., whereupon the value of R_{Na} did not differ significantly from that obtained after the first dialysis, although as a result of evaporation the concentrations of sodium in aqueous humour and plasma both rose. In the later experiments (nos. 13–16) 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 difficult to account for these discrepancies; it is possible that the collodion membrane itself is not in equilibrium with the fluids surrounding it; it may tend to swell, 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 to investigate the point further. The significant fact is that all ten

TABLE 2. The change in the Gibbs-Donnan ratio, R_{Na}, following dialysis of plasma against aqueous humour

Exp.	A_1	P_1	R_1	A_2	P_2	R_2	ΔR
8	150·4	154·6	**1·027**	146·6	155·6	**1·062**	+0·035
9	150·3	155·0	**1·032**	147·4	154·7	**1·050**	+0·018
10	149·8	157·2	**1·050**	149·1	160·5	**1·077**	+0·027
11	148·5	155·8	**1·049**	149·1	158·7	**1·064**	+0·015
14	149·8	154·0	**1·028**	148·5	154·1	**1·038**	+0·01
16	147·4	152·1	**1·032**	145·7	152·8	**1·049**	+0·017
Mean			**1·036**			**1·057**	
12	155·8	160·2	**1·028**	156·6	166·9	**1·066**	+0·038
	155·8	160·2	**1·028**	158·5	168·2	**1·061**	+0·033
13	152·3	160·8	**1·056**	150·3	161·2	**1·073**	+0·016
15	152·1	158·7	**1·043**	149·3	159·3	**1·067**	+0·024
Mean			**1·042**			**1·068**	

Exps. 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 suffixes (1) and (2) refer to the figures obtained before and after dialysis respectively.

dialyses, representing the fluid from twenty-seven 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 these would be too small for detection (cf. for example Exp. 12).

Conductivity. The changes in conductivity are shown in Table 3. In all but one case 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 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 ratio of the mean deviations was found to be 1·5.

TABLE 3. Changes in electrical resistance of plasma (Δr_{Pl}) and aqueous humour (Δr_{Aq}) as a result of dialysis

Exp.	Δr_{Aq}	Δr_{Pl}	$-\frac{\Delta r_{Pl}}{\Delta r_{Aq}}$
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	−12·0
34	6·5	−4·3	1·5

Exps. 25–33 on cats; Exp. 34 on a dog. In Exps. 30 and 32, whole blood was placed in the sac instead of plasma.

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% in the base-binding power of the plasma—a change that could be achieved only by an increase of pH in the region of one unit (Van Slyke, Hastings, Hiller & Sendroy, 1928). 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 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 caused the aqueous humour to become alkaline; dialysis with the better buffered plasma tended to restore the pH to the physiological range.

The re-formed aqueous humour. Earlier work (Davson & Weld, 1941) has shown that the rapidly re-formed aqueous humour exhibits a value of R_{Cl} of about 0·96, i.e. it approaches that for a dialysate. 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 4. It will be observed that R_{Na} has closely approached unity, i.e. it has shifted farther away from the correct figure for a dialysate of plasma instead of nearer to this figure as was found with the chloride ion. The protein concentration in the re-formed fluid was of the order of 3% so that we may expect a lower value of R_{Na} at equilibrium, i.e. 1·03–1·035 instead of 1·06–1·07; the observed value, however, is much less than this.

TABLE 4. The Gibbs-Donnan ratio in the re-formed aqueous humour

Exp.	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
11	1·049	1·064	1·024	2·5
12	1·028	1·064	1·011	3

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.

DISCUSSION

The results show that the aqueous humour is not in thermodynamic equilibrium with blood plasma in so far as the distribution of sodium and chloride between the two fluids is concerned. In view of the presence of a metabolizing unit (the lens) within the eye, of the fact that arterial plasma has been arbitrarily chosen as the reference fluid, and of the circulation of the aqueous humour through the eye, this is perhaps not surprising. Nevertheless, the quantitative aspects of the results point strongly to the existence of a secretory mechanism for the maintenance of a high concentration of sodium and chloride in the aqueous humour. Thus the high concentration of sodium in the aqueous humour might possibly be accounted for on the basis of a difference of pH between plasma and aqueous humour resulting from the metabolism of the lens; the difference in pH required to produce the observed value of R_{Na} would be too small to detect with any certainty. Any explanation of the excess of sodium on the basis of a difference of pH between aqueous humour and plasma would require, however, that the chloride concentration in the aqueous humour should be below that necessary for equilibrium. The results have shown, however, that the excess of chloride in the dog's aqueous humour actually exceeds that of sodium. Again, 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. Finally, although the fact that the aqueous humour is continually drained off and replaced might account for small deviations from diffusion equilibrium, it cannot account for the high concentration of sodium and chloride in the aqueous humour: the osmotic forces brought into play are too great to be accounted for by the available filtration pressure.

We have seen that the rapidly re-formed aqueous humour shows a value of R_{Na} of about 1·01. The fluid formed under these conditions generally contains some 3% of protein. A simple exudate of plasma with this protein content should show a value of R_{Na} of about 1·030–1·035 and the observed value of 1·01 could be due to a differential leakage of albumin, to a filtration too rapid to permit of the attainment of a Donnan equilibrium, or to a secretory process. There is at present no evidence, derived from other observations on the re-formed fluid, to indicate which of these explanations is the most likely.

In the dog, the deviation of R_{Cl} from equilibrium was considerably greater than that of R_{Na}, the excess of chloride in the aqueous humour amounting to about 7%, or some 9 m.mol./l.; since the discrepancy of sodium amounted to only about 3 m.mol./l., the results suggest that some other cation is present in excess; studies on the distribution of potassium (Davson, Duke-Elder & Benham, 1936), calcium and magnesium (Stary & 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 difference in physiology between the cat and dog.

To conclude, the present work demonstrates beyond all reasonable doubt that secretory activity is concerned in the formation of the aqueous humour; whether the fluid is to be regarded purely as a product elaborated by the epithelial cells of the ciliary body or as a filtrate from plasma with its concentration modified by contact with these epithelial cells, cannot yet be decided.

SUMMARY

The concentrations of sodium and chloride were determined in the aqueous humour and plasma of cats and dogs before and after dialysis against each other by means of a collodion sac. As a result of this dialysis the distribution of these two ions changed in the sense that both sodium and chloride diffused from aqueous humour to plasma. A study of the changes in electrical conductivity of the fluids following dialysis confirmed these findings. It is concluded that the two fluids are not in thermodynamic equilibrium and that the deviations are of such magnitude and sign as to warrant the assumption of secretory activity.

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