CXII. THE IONIC EQUILIBRIUM BETWEEN THE AQUEOUS HUMOUR AND BLOOD PLASMA OF CATS.

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THE origin of the aqueous humour and the nature of the equilibrium existing between it and the blood plasma are problems of considerable significance in relation to the cause of glaucoma. The view that it is the secretion of "ciliary glands" has been gradually abandoned in recent years in favour of the thesis that it is a dialysate from the plasma in the capillaries similar to the cerebrospinal and glomerular fluids. This theory derives its main support from analyses of the blood and aqueous humour of the horse by Duke-Elder [1927] who found an excess of cations in the plasma over those in the aqueous humour and the reverse for anions in accordance with expectation on the basis of the Donnan equilibrium; diffusible non-electrolytes were approximately equally distributed. However, recent work has cast doubt on the validity of the dialysis theory. Thus Walker [1933] found that (a) the inorganic P concentrations of the aqueous humours of all species examined averaged 50% of those in the corresponding plasmas, (b) in rabbits, dog and man the urea concentration was 68% of the plasma value, (c) the uric acid concentration in fowls and man was 66% of the plasma value and (d) reducing substances were generally present in lower concentration than in the plasma. Adler [1933] showed that in cats the concentration of urea in the aqueous humour was always less than in the plasma. Both Walker and Adler made their determinations on the blood and aqueous humour of the same animal, whereas Duke-Elder carried out a complete analysis of a pooled specimen of aqueous humour derived from the eyes of some thousand horses and compared the results with those of a similar analysis of a single sample of blood obtained from the M.R.C. farm. The significance of Duke-Elder's results depends therefore on the limits of variability from individual to individual of the concentrations of the substances determined in the blood and aqueous humour. In the light of this consideration and the claims of Walker and Adler that the capillary membrane separating the aqueous humour from the blood plasma shows a selectivity incompatible with the assumption that it is an inert membrane, we have carried out careful and accurate analyses of Na^+ , K^+ and Cl^- in the aqueous humours and bloods of cats. The values for any given aqueous humour were compared with those of its corresponding plasma. The results obtained demonstrate that so far as these ions are concerned there is a Donnan equilibrium between the aqueous humour and the blood plasma.

EXPERIMENTAL.

Cats were anaesthetised with Et_2O and bled from the carotid; during the bleeding the aqueous humour was removed from both eyes with a syringe. After clotting, the serum was removed and 5 ml. were measured into a centrifuge-tube. Proteins were removed with trichloroacetic acid, the washings evaporated to dryness in a silica flask and the residue was ashed with conc. HNO_3 [Kutz,

1931]. The ashed residue was made up to 10 ml. with distilled water and used for the Na^+ and K^+ determinations. For determinations of Na^+ and K^+ in the aqueous humour, 0.5 ml. samples were measured into silica flasks and ashed directly with HNO_3 . The ashed residues were washed out into silica beakers, evaporated to dryness and taken up with 1 ml. of water. Na^+ was determined by the method of Barber and Kolthoff [1928], K^+ by Kramer's method [1920] and Cl^- directly on the serum and aqueous humour by a modification of the method of Van Slyke and Sendroy [1923]. The modification consisted in filtering off the AgCl precipitate through an asbestos filter before titration with KCNS . All methods gave errors of rather less than 1 %.

RESULTS.

In Table I are shown the results of experiments on six individual cats (Exps. 1-6) and on the pooled aqueous humours and plasmas of four cats (Exp. 7). In this latter case the individual samples of plasma and aqueous humour

Table I.

Exp. no.	Na			K			Cl		
	Aqueous humour	Serum	R_{Na}	Aqueous humour	Serum	R_{K}	Aqueous humour	Serum	R_{Cl}
1	155	161	1.04	—	—	—	130	124	0.96
2	153	157	1.03	6.25	6.50	1.04	128	123	0.96
3	147	161	1.09	—	—	—	122	117	0.96
4	162	170	1.05	6.20	6.05	0.97	135	135	1.00
5	—	—	—	6.30	6.80	1.08	129	122	0.95
6	151	161	1.07	6.15	6.75	1.10	127	123	0.97
7	150	160	1.05	6.00	6.50	1.08	129	123	0.95

Concentrations are expressed in millimols. per kg. of water.

were pooled in proportion. This rendered possible the use of 5 ml. samples of aqueous humour for ashing just as with plasma so that triplicate determinations of all three ions could be made; although trichloroacetic acid was unnecessary in the case of the aqueous humour, it was added so as to make the conditions identical. In the table the R values indicate the ratios of the concentrations of the ions, expressed as millimols. per kg. of H_2O , in the serum and aqueous humour; thus R_{Na} equals $[\text{Na}]_{\text{s}}/[\text{Na}]_{\text{aq}}$. Blanks in the table indicate that insufficient material was obtained for all three analyses.

It is evident that in all cases R_{Na} is greater than unity as would be expected on the basis of the Donnan equilibrium; in all cases but one R_{Cl} is less than unity; the values of R_{K} were more variable than the others but similar in trend to those of R_{Na} . The values for Exp. 7 represent the pooled serum and aqueous humour of four cats with a considerably smaller margin of error in the actual determinations.

DISCUSSION.

Van Slyke [1926] has shown that human plasma in equilibrium with its dialysate should give values for R_{Na} and R_{K} of 1.04 and for R_{Cl} 0.96; the experimental values obtained show a fairly satisfactory agreement with expectation. Thus Exp. 7, which is the most significant of all, gave values for R_{Na} and R_{K} of 1.05 and 1.08 respectively and for R_{Cl} 0.95. However, the variability of the values of R as seen in Exps. 1-6, especially of R_{K} , cannot be attributed to experimental errors in the determination of the ions. It must be emphasised that the Donnan ratios should be expressed in terms of activities and there seems no reason to suppose that the activity coefficients will be the same in

both aqueous humour and serum; furthermore the procedure for obtaining the fluids, anaesthesia, insertion of a hypodermic needle through the tough cornea *etc.* must have temporary influences on the local concentrations of these ions; for example D'Silva [1934] has shown that adrenaline, which is liberated during the anaesthetising process, causes an increased blood K^+ . Thus it appears possible that the variations are primarily artificial, so that the assumption that the membrane separating the aqueous humour and the blood plasma is normally capable of selective accumulation of ions is unwarrantable on the basis of the experimental results described in this paper. On the other hand, the results of Walker and Adler show for non-electrolytes and inorganic P that the membrane is capable of maintaining concentration gradients. It seems improbable that the gradients exist merely in virtue of the low permeability coefficients of the solutes concerned, so that changes in the blood concentration are reflected only after some time in corresponding changes in the aqueous humour concentration, since the concentration gradients observed in a large number of experiments were always in the same direction, the serum concentration being in all cases higher than that of the aqueous humour.

As a main conclusion then it may be taken that the aqueous humour is a dialysate from the blood plasma, showing the ionic distribution characteristic of such a system; superimposed on this simple system is the phenomenon of selective absorption which manifests itself in concentration gradients of certain molecules, but most probably not of Na^+ , K^+ and Cl^- .

The relation of these findings to the problem of glaucoma may be briefly indicated. So long as it was maintained that the aqueous humour was a secretory product of "ciliary glands", the normal intra-ocular pressure could be described as the secretion pressure of the glands and its pathological increase in glaucoma could be explained as the result of hypersecretion. By regarding the aqueous humour as a dialysate from blood plasma, the intra-ocular pressure has to be considered as similar to any other tissue pressure, being made up of the hydrostatic capillary pressure partly balanced by the difference in osmotic pressure between the aqueous humour and plasma. To account for the raised intra-ocular pressure in glaucoma now, no such simple explanation is available; there is no change in the protein content of the blood, so that a simple change in the colloid osmotic pressure cannot be said to be the cause of glaucoma, as it is claimed to be the cause of renal oedemas. Similarly there is no evidence that glaucoma is due to raised blood pressure. Thus the necessity for a well founded theory of the conditions which determine the formation of the aqueous humour is apparent.

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