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THE DISTRIBUTION OF SODIUM BETWEEN THE AQUEOUS HUMOUR AND BLOOD PLASMA OF CATS

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In an earlier paper [Davson, Duke-Elder & Benham, 1936] determinations of the relative concentrations of Na, K, and Cl in the serum and aqueous humour of the cat were reported, and it was concluded that the results did not warrant the postulation of a secretory origin of the aqueous humour. Since then Benham, Duke-Elder & Hodgson [1938] have shown that there is an excess of osmotic pressure in the aqueous humour over that in the serum provided the fluids are removed from the man or the dog under local anaesthesia or with amytal or nembutal as general anaesthetics; if ether is used then the osmotic pressure difference is in the reverse direction. More recently, Hodgson [1938] has shown that in the man and the dog the mean ratio of chloride in the serum to chloride in the aqueous humour is about 0·89, compared with a theoretical value on the basis of a Donnan distribution of about 0·96.

It is clear therefore that the problem needs a critical re-examination, and in the present work an attempt has been made to supply some of the deficiencies which have since transpired in respect to the earlier work of Davson *et al.* In this earlier work the aqueous humour was divided into three portions for determinations of the separate ions; owing to the limited quantity of fluid it was not possible to make duplicate determinations, so that the errors were larger than they would have been had triplicate determinations of a single ion been compared. It was thought at the time that single determinations on all three ions would have more value, since, if deviations from the theoretical Donnan ratios occurred, the relative magnitudes and directions of the deviations of the positive and negative ions would have given a clue to the nature of the disturbing factor.

The author, however, is now of the opinion that more accurate analyses of a single ion, i.e. Na^+, will give a more decisive answer to the problem. The reasons for this are as follows:

(*a*) The Na^+ is the main cationic contributor to the osmotic pressure of the blood and aqueous humour; consequently any differences in concentration between these fluids caused by experimental technique or changes in hydration of the animal will be comparatively rapidly levelled out by osmosis of water. This is not the case with K^+, where changes in the concentration of the fluids with respect to this ion must be levelled out by a process of diffusion and may take hours for completion [Davson & Quilliam, 1939, in preparation].

(*b*) So far there is no evidence that the Na concentration of blood is influenced by anaesthesia, but D'Silva [1934] has shown that the K concentration in the plasma of the cat rises during ether anaesthesia.

(*c*) Changes in the CO_2 tension of the blood of the order of magnitude encountered in the experimental technique used in this sort of work are reflected in negligible changes in the Na concentration of the plasma, whereas they may have considerable influences on the Cl concentration; thus there is a difference of 1·5% between the plasma Cl concentrations in arterial and venous blood [Doisy & Beckmann, 1922]. Similarly a variation in the temperature at which the blood is allowed to clot will have a minimal effect on the cation concentration of the plasma.

(*d*) A more careful investigation into the Barber-Kolthoff [1928] method of Na determination reveals that it has greater potentialities as an accurate means of determining small differences of concentration than the methods generally in use for the determination of K and Cl.

In this paper the distributions of Na between the aqueous humour and blood serum of 18 cats are recorded. Blood was drawn under nembutal anaesthesia from 6 cats and by heart puncture without general anaesthesia from the remainder. Owing to the improved technique, variations of 1% in these distribution ratios from the theoretical ratio of 1·04 may now be ascribed a significance which it would have been unjustifiable to assume in the earlier work. Hence, although the present results bear out the earlier ones in that the mean Na ratio lies fairly close to the theoretical value, they do, nevertheless, leave room for a possible secretory activity on the part of the membrane separating the eye fluids from the plasma. Owing to the divergences from theory obtained in these experiments, both Na and Cl were determined in a group of 6 cats. Duplicate estimations were possible in both instances owing to the use of the $AgIO_3$

method for Cl determination [Sendroy, 1937] which requires much smaller quantities of fluid than the Volhard method). The variations in the Cl ratios did not correspond in any regular way with those of the Na.

Experimental

Removal of fluids. When nembutal was used as a general anaesthetic, it was injected intra-peritoneally, and a cannula was placed in the carotid artery; the aqueous humour from both eyes was then removed with a syringe[1] and immediately afterwards blood was run into a centrifuge tube, allowed to clot and the serum removed. When the blood was drawn without a general anaesthetic, this was done by heart puncture after a subcutaneous injection of cocaine at the site of entry. Immediately afterwards the animal was anaesthetized with ether and the eye fluids removed.

Determination of sodium. Three 0·5 ml. lots of each fluid were measured out into silica flasks with long thin necks; 1 ml. of conc. HNO_3 and two drops of conc. H_2SO_4 were added to each and the contents heated gently for 30 min. on a sand bath. The temperature of the bath was then raised so that the HNO_3 evaporated. When the contents of the flask had evaporated to apparent dryness the latter was cooled so that the H_2SO_4 and HNO_3 remaining inside condensed; the flask was then replaced on the sand bath and more organic matter was destroyed. By cooling and heating in this manner three or four times all the organic matter was removed without raising the temperature above about 350°; further the amount of acid used for ashing was the same for all samples, thereby controlling the blank due to the presence of Na in this reagent. The contents of the flask were washed out into a silica beaker with four successive lots of 2 ml. of H_2O, the solution was evaporated to dryness on a sand bath and after the addition of 1 ml. of H_2O the Na was determined by the Barber-Kolthoff [1928] gravimetric technique. The following precautions, in addition to those described by these authors, should be observed. After addition of the reagent the mixture is stirred with a thin glass rod for at least 1 min.; the precipitate adhering to the rod may be washed off into the beaker with a few drops of the reagent. As a washing medium for the precipitate, 95% EtOH saturated with freshly prepared sodium-zinc-uranyl acetate is used; it is useless to

[1] Hodgson [1938] objects to the use of suction for the removal of the aqueous humour although he advances no reason. The danger of including significant quantities of extravasates from the plasma is negligible, considering the shortness of the time required to remove the aqueous humour.

attempt to store this wash fluid as a fine precipitate settles out continuously.

Of triplicate determinations made in this way, two usually agreed to within 1 in 500, and it was rare to find one of the three deviating from the mean by more than 1%.

Determination of chloride. This was carried out exactly as recommended by Sendroy [1937], without removal of proteins from the serum, with the exception that the excess of $AgIO_3$ was removed by filtering through asbestos. The individual results rarely differed from the mean by more than 0·5%.

Determination of total solids in the serum. About 2 ml. of serum were weighed in a bottle and evaporated to dryness on a sand bath; the bottle was then transferred to an oven at 105° and the contents dried to constant weight.

Errors in the determination of Na. In work of this kind, in which fluids of very nearly equal compositions are compared, it is essential that a fair statement of the errors should be made. In Table II, the results of which the author wishes to stress most, an idea of the accuracy of the figures presented may be derived from the following: In Exps. 1, 5, 6, 9 and 11 two of the three determinations of Na in the serum and two of the three determinations in the aqueous humour gave values differing by less than 1 in 600; in Exps. 2, 4, 7, 8, 10 and 12 two of the determinations on one fluid differed by less than 1 in 600, whilst two of the determinations on the other fluid differed by less than 1 in 300. In Exp. 3 two determinations of the aqueous humour differed by 1 in 600, whilst the discrepancy between the values for the serum was less than 1 in 100.

Results

The effect of ether anaesthesia on the Na content of the serum of the cat is shown in Table I; the blood was drawn first by heart puncture, and about 15 min. later from the carotid under ether anaesthesia. In three instances the influence of ether was negligible, and in the remaining three an increase of 1–3% was observed. These last results may account for the rather large values of the ratio $[Na]_S/[Na]_{Aq} = (R_{Na})$ observed earlier.

In Table II the Na contents of serum and aqueous humour of twelve cats are shown, together with the percentage solids in the serum. In the fifth and sixth columns the values are expressed as millimol. per kg. of H_2O, assuming the aqueous humour to contain 1% of solids. In the final column the values of the ratio $[Na]_S/[Na]_{Aq}$ are given, the figures being given to the nearest 5 parts in 1000. In Exps. 1–6 the blood was with-

TABLE I. Na contents of serum, expressed in millimol. per kg., from blood drawn first by heart puncture and then from the carotid artery after ether anaesthesia

Exp. no.	Heart puncture	Ether anaesthesia	Change (%)
1	152·5	152·0	−0·3
2	154·5	154·0	−0·3
3	149·0	149·0	0·0
4	149·0	154·0	3·4
5	144·5	146·0	1·0
6	148·0	152·0	2·7

TABLE II. Sodium contents of serum and aqueous humour of twelve cats. Exps. 1–6, blood withdrawn by heart puncture; Exps. 7–12, under nembutal anaesthesia

Exp. no.	Serum millimol./ kg.	Aqueous humour millimol./ kg.	% solids in serum	Serum millimol./ kg. H_2O	Aqueous humour millimol./ kg. H_2O	R_{Na}
1	152·3	158·0	7·5	164·7	155·9	1·055
2	154·4	157·0	8·9	169·5	158·6	1·070
3	148·7	158·9	7·8	161·3	160·5	1·005
4	149·2	159·8	7·9	162·0	161·4	1·005
5	144·3	153·9	8·5	157·7	155·6	1·015
6	147·9	154·4	9·1	162·7	156·0	1·040
7	162·3	172·0	8·2	176·8	173·9	1·015
8	147·9	152·9	8·1	160·9	154·6	1·040
9	146·4	152·8	8·5	160·0	154·4	1·035
10	152·2	158·2	9·0	167·2	159·8	1·045
11	150·7	155·3	7·0	162·0	156·9	1·030
12	145·7	153·2	8·4	159·0	154·7	1·030
		Mean (Exps. 1–6)				1·030
		Mean (Exps. 7–12)				1·030
		Standard deviation (Exps. 1–18; *vide* Table III)				0·018

drawn by heart puncture, and in Exps. 7–12 under nembutal anaesthesia. It is seen that the mean R_{Na} is the same for the two groups. It is clear, however, that individual ratios may differ by as much as 3·5% from the theoretical value of 1·04 [Van Slyke, 1926], and there seems to be no correlation between the protein content (i.e. the percentage solids) and the magnitude or sign of the deviation.

In Table III are shown the results of six experiments on the distribution of both Na and Cl between the serum and aqueous humour; blood was drawn by heart puncture and allowed to clot under paraffin. In the

TABLE III. Results of simultaneous determinations of sodium and chloride in the serum and aqueous humour of six cats. Blood was withdrawn by heart puncture

Exp. no.	R_{Na}	R_{Cl}	% solids in serum	$R_{Na} \times R_{Cl}$
13	1·005	0·950	8·4	0·955
14	1·050	0·965	7·7	1·010
15	1·030	0·950	8·6	0·980
16	1·005	0·910	6·8	0·915
17	1·045	0·940	8·4	0·980
18	1·035	0·955	8·3	0·990
Mean	1·030	0·945	—	0·970

final column the products $R_{Na} \times R_{Cl}$ are shown; on the basis of the Donnan equilibrium and assuming that activities are equal to concentrations, this product should equal unity. It is clear that marked deviations from unity occur, and further that a low R_{Na} value does not always correspond to a low R_{Cl}, as would be expected were NaCl, as such, being excreted into the aqueous humour. The mean R_{Na} is equal to that for Exps. 1–12 (Table II).

Discussion

The results of the experiments described in this paper show that the distribution of Na between the serum and aqueous humour of the cat may vary from the theoretical ratio derived on the basis of a Donnan Equilibrium; the variation is irregular, however, and the means of the successive groups are equal and differ only by 1% from this theoretical ratio. The product $R_{Na} \times R_{Cl}$ is neither equal to unity in all the cases examined, nor is it the same from one individual to another. So far a "theoretical" R_{Na} has been mentioned as though this were an invariable quantity; this, however, is not so, since its value depends on the number of indiffusible anions present in the plasma, and this will depend on the protein concentration, the relative amounts of the constituent serum proteins, and the acidity of the serum. The latter may be taken as constant, since intravital variations in acidity are not large enough to influence these results. Inspection of Tables II and III shows that the percentage solids, and hence presumably the protein concentration, varies from serum to serum, so that deviations from the "theoretical" value of R_{Na} might be expected to be related to the protein content of the serum; nevertheless there is no correlation between the protein concentration and the deviation of individual values of R_{Na} from the mean.

Let us now turn to an investigation of *in vitro* ultra-filtrates of plasma. Ingraham, Lombard & Visscher [1933] have calculated that the relative concentrations of diffusible salts in a dialysate of plasma should be equal to those from an ultra-filtrate. They then determined the distribution of ions between dogs' plasma and its ultra-filtrate. The present author has calculated the results as the ratio $[Na]_P/[Na]_{Aq}$.

The seventeen determinations gave a mean value of 1·08 with a standard deviation of 0·021. Six ratios selected at random gave values of 1·05, 1·11, 1·09, 1·09, 1·02, 1·09.

Two points emerge from these results: first that the variability of the distribution of Na between plasma and its dialysate *in vitro* is slightly greater than that found for the distribution of Na between serum and aqueous humour (standard deviation, 0·018), and secondly, the mean

R_{Na} is greater than the calculated value of 1·04. Ingraham *et al.* show, however, that this high value may be due to the proteins which depress the activity of the Na in the plasma.

Some results of Greene & Power [1931] on artificial *in vivo* dialysates of plasma may next be considered. The following values of the R_{Na} have been taken at random: 1·165, 1·11, 1·14, 1·11, 1·15, 1·11. The mean of 15 determinations by these authors was 1·10. It is clear that here, also, the deviations from the mean are greater than with the aqueous humour and serum; also the ratios are very much greater than the theoretical value of 1·04. Similar values were found by Greene, Bollman, Keith & Wakefield [1931] for the distribution of Na between plasma and ascitic fluid.

In the light of these results on *in vitro* systems and artificial *in vivo* dialysates it would be very rash to conclude that deviations from a certain theoretical R_{Na} of 1·04 of the size met with in this work are proof that a secretory process is at work in determining the formation of the aqueous humour. In fact, if a direct comparison of the various systems is permissible, it would appear that the membrane separating the aqueous humour from the blood plasma in the cat is a more efficient dialysing apparatus than the collodion sac. The results shown here do, however, leave room for a possible secretory activity, but the proof that such an activity occurs with the cat must come from other sources.

Summary

The distribution of Na between the aqueous humour and blood serum of eighteen cats is reported. The mean value of the ratios of the concentrations in the two fluids was 1·03 (theoretical value 1·04). In a group of six experiments in which blood was drawn under nembutal anaesthesia, the mean ratio was not different from that obtained with another group in which the blood was obtained by heart puncture. In a further group of six cats both Na and Cl were determined in the two fluids. The products of the concentration ratios were not equal to unity nor were they equal one to another.

The results are discussed in relation to similar determinations on the distribution of Na between plasma and its *in vitro* ultra-filtrate, and its artificial *in vivo* dialysate, and it is pointed out that the variations in the value of the ratio obtained for different cats by the present author are rather less than variations in similar ratios for these artificial systems.

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