CLII. DISTRIBUTION OF UREA IN BLOOD AND AQUEOUS HUMOUR

By GRAHAM HARVEY BENHAM

*From the Department of Physiology, Biochemistry and Pharmacology,
University College, London*

(Received 25 May 1937)

EVIDENCE was put forward [Davson *et al.* 1936; Benham *et al.* 1937] to show: (a) that the distributions of Na^+ , K^+ and Cl^- in the blood and aqueous humour of cats are such that, so far as these ions are concerned, a Gibbs-Donnan equilibrium exists between the two fluids, and (b) that the total osmolar concentrations of the two fluids show values consistent with the hypothesis that the aqueous humour is a dialysate of the capillary blood, in dynamic equilibrium with it across the capillary walls. Previous evidence advanced by Duke-Elder [1927] showed that the theory of the origin of the aqueous humour as a secretion from the ciliary glands was not upheld when the facts of chemical, hydrostatic, osmotic and electric equilibria were taken into consideration.

According to the theory of dialysis, however, diffusible non-electrolytes such as urea and glucose should be distributed equally on the two sides of the membrane, if it is assumed that the membrane is a simple non-selective one.

In view of the discrepant results obtained by Walker [1933], in which he showed the ratio $\frac{\text{urea aqueous}}{\text{urea blood}}$ as varying between 0.49 and 0.95, it was thought desirable to perform a micro-estimation of urea in the aqueous humour and blood.

Method

The conductimetric method of Ranganathan & Sastri [1936], modified in a few details, was used. This method depends on the fact that the increase in electrical conductivity of the urea-urease system is a true measure of the liberation of electrolyte by the hydrolysis of urea. The changes in conductance were found to be considerable and observable even when using 0.05 mg. urea. The method is to be recommended for use with small quantities, owing to its simplicity and rapidity.

Apparatus

The apparatus for measurement of conductivity consisted of a valve-oscillator, as a source of alternating current, and a telephone receiver for detecting the null-point. The bridge components were a constant-value ratio-box, a 6-dial non-inductive resistance box and a specially constructed cell of the Ostwald type. The capacitance of the cell was balanced by means of a system of condensers, which could be plugged in at will in parallel with the resistance-box. All experiments were done at $25 \pm 0.02^\circ$.

Procedure

1 ml. of *M*/20 phosphate buffer pH 7 and variable quantities of a known urea solution were placed in the cell and made up with distilled water to 3.5 ml. The equilibrium value for the resistance of this solution was obtained after 30 min. (R_1). 0.5 ml. of a freshly prepared urease solution, the diluting effect of which was determined in blank experiments, was then added, and the contents

were well mixed without violent shaking. Readings were taken every minute for the first 5 min. and subsequently at intervals until a constant value was again obtained (R_2). Table I gives a typical set of readings. Values are represented as specific conductance L , obtained by dividing the cell-constant K by R , the resistance. The cell-constant in these experiments was 0.8208.

Table I

Urea added mg.	R_1 ohms	R_{11} (corrected for diluting effect of urease) ohms	R_2 (on addition of urease) ohms	Time min.
0.393	311.0	344.0	361.0	1
			360.0	2
			335.0	3
			322.0	4
			310.0	5
			306.5	10
			306.0	20
			306.0	40
			306.0	45

$$L_{11} \left(\frac{K}{R_{11}} \right) = \frac{0.8208}{344.0} = 0.002379,$$

$$L_2 \left(\frac{K}{R_2} \right) = \frac{0.8208}{306.0} = 0.002685,$$

whence the change in specific conductance $L_2 - L_{11}$

$$= 0.000306.$$

$$= 3.06 \times 10^{-4}.$$

The temporary increase in resistance on addition of urease is due to the initial cooling effect of the latter.

Table II shows the changes in specific conductance ($L_2 - L_{11}$), corresponding to quantities of urea from 0.197 to 1.965 mg.

Table II

mg. urea	$(L_2 - L_{11}) \times 10^4$	mg. urea	$(L_2 - L_{11}) \times 10^4$
0.197	1.40	1.179	8.91
0.393	3.06	1.376	10.09
0.589	4.66	1.572	11.18
0.786	6.22	1.768	12.30
0.982	7.55	1.965	13.60

Fig. 1. Result of plotting $(L_2 - L_{11}) \times 10^4$ against mg. urea.

The result of plotting mg. urea against $L_2 - L_{11}$ gives a graph which is very nearly a straight line, as shown in Fig. 1. Since the total change in conductance is a quantitative measure of the electrolyte released during the reaction, the graph can be used to determine small quantities of urea.

Application

Samples of aqueous humour and blood were removed from cats, dogs and rabbits. It is advisable not to use whole blood for urea estimations, for Anderson & Tompsett [1936] have shown that, unless special precautions are taken, the results are apt to be high. Accordingly serum was used and, in one set of experiments, heparinized plasma (kindly supplied by Dr C. F. Code).

The procedure was the same as for pure urea solutions, except that 0.4 or 0.5 ml. of water was replaced by serum or aqueous humour; in every case the quantity in the cell was made up to 3.5 ml. without the urease, and 4 ml. with the urease. It was found that, at such a dilution, the changes in conductivity in the presence of serum proteins did in fact correspond to the changes obtained in protein-free solutions. In all cases known quantities of urea were added, and the amount recovered in excess of that added was assumed to be that originally present in the sample. For example, the following figures show the recovery obtained in this way in the case of a sample of serum:

	Amount added mg. urea	Amount recovered mg. urea	Amount in sample
(I)	0.589	0.735	0.146
(II)	0.589	0.740	0.151
(III)	0.786	0.929	0.143

It is seen that from an amount of serum found to contain 0.148 mg. urea, together with 0.786 mg. of added urea, the amount actually recovered was 0.929 mg. as against a theoretical value of 0.934 mg. In view of these typical results, it is claimed that the values found for serum represent the true amounts present.

The results obtained are shown in Table III. The first two columns show values in mg. urea/100 ml., and the third the ratio $\frac{\text{aqueous}}{\text{serum}} \times 100$. By making a standard correction for the protein in the serum and aqueous humour, an approximate set of values for mg. urea/100 g. of protein-free solution is shown in columns 4 and 5, and the lower ratios thus obtained in the last column.

Table III

	Aqueous	Serum	$\frac{\text{Aqueous}}{\text{Serum}} \times 100$	Aqueous (corr. for 0.04 % protein)	Serum (corr. for 7.5 % protein)	$\frac{\text{Aqueous}}{\text{Serum}} \times 100$
Cat	28.17	27.45	102.6	28.19	29.68	94.95
Cat	33.75	34.47	97.88	33.77	37.28	90.58
Dog	44.63	43.72	102.1	44.65	47.28	94.45
Rabbit	13.20	12.80	103.2	13.30	13.83	95.48
*Rabbit	38.40	42.40	90.57	38.60	46.84	83.80
*Rabbit	26.16	30.02	87.15	26.18	32.46	80.64
Rabbit	16.98	17.40	97.63	16.99	18.81	90.33

* Pooled samples from three rabbits.

DISCUSSION

These findings indicate that such large differences as Walker obtained do not appear to be the general rule. Although the aqueous humour contained less urea than did the serum, namely between 80 and 96 %, the differences are very small.

It is therefore suggested that, although exact equality is not obtained, the tendency is towards an equal distribution of urea between the serum and aqueous humour. The nature of the membrane, about which so little is known, may affect the distribution, so that the relative concentrations at any one time do not necessarily determine in which direction urea will pass. It is therefore impossible to be at all definite about the interpretation of the urea ratios in the serum and aqueous humour. It is nevertheless the belief of the author that the existence of the Donnan equilibrium which has been established for Na^+ , K^+ and Cl^- ions, together with similar although not equal distribution of urea, as cited in this paper, present a strong case for the dialysis theory as regards the blood and aqueous humour.

I wish to record my indebtedness to Sir Stewart Duke-Elder and to Prof. Drummond for their help and interest; and to the Medical Research Council for defraying the expenses of this work and for a personal grant.

REFERENCES

- Anderson & Tompsett (1936). *Biochem. J.* **30**, 1572.
Benham, Davson & Duke-Elder (1937). *J. Physiol.* **89**, 61.
Davson, Duke-Elder & Benham (1936). *Biochem. J.* **30**, 773.
Duke-Elder (1927). *J. Physiol.* **62**, 315.
Ranganathan & Sastri (1936). *Biochem. J.* **30**, 2135.
Walker (1933). *J. biol. Chem.* **101**, 269.