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THE PERMEABILITY OF THE BLOOD-AQUEOUS HUMOUR BARRIER TO POTASSIUM, SODIUM, AND CHLORIDE IN THE SURVIVING EYE

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There is a great deal of evidence that the aqueous humour is a filtrate from the blood plasma, molecules of the size of serum albumen or larger being retained whilst crystalloids are distributed between the two fluids in a manner characteristic of ultra-filtrates *in vitro*. If this is so, then the intra-ocular pressure will be determined by the capillary pressure and the colloid osmotic pressure of the blood; the cause of the raised intra-ocular pressure in glaucoma may be sought, possibly, in a derangement of the normal hydrostatic or osmotic relationships.

Against this view the early idea of Seidel [1920], that the aqueous humour is a secretion, has been revived. Its extreme form has been put forward by Robertson [1939*a*, *b*] and by Robertson & Williams [1939]. They claim that the formation of the fluid is virtually independent of the composition of the plasma and that its pressure is independent of the relative osmotic pressures of the plasma and the aqueous. Such a theory is difficult to reconcile with the classical researches of Henderson & Starling [1904] who showed that the intra-ocular pressure is directly connected with the arterial pressure, and the more recent investigations [Duke-Elder, 1927; Duke-Elder & Duke-Elder, 1931] on the influence of the composition of the blood and of the vascular pressures on the intra-ocular pressure. Recently, Duke-Elder [1938] has put forward the view that the aqueous behaves in a general way as a dialysate, but that its composition and osmotic pressure are modified by an activity of the epithelial cells of the ciliary body. This view promises to reconcile opposing standpoints. The present work is designed to show to what

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extent the membranes lining the living eye can, by a cumulative activity, modify the rate of exchange of ions between the plasma and the aqueous humour, either qualitatively or quantitatively. In this paper we shall refer to the barrier between the blood and the aqueous humour simply as "the membrane"; such a barrier will obviously include such membranes as the endothelial capillary membrane, Bruch's membrane, etc.

Perhaps the only really strong argument made by Robertson in favour of his view is that the membrane of the eye shows uni-directional permeability, crystalloids and water being able to penetrate into the eye but not out of it. Unfortunately this author neither presents experimental evidence nor quotes any reference in support of this claim. If this were so a membrane with this property would, from analogy with other systems, be expected to show secretory functions, although uni-directional permeability *per se* would not necessarily preclude an equilibrium distribution of crystalloids indistinguishable from that in a dialysate. Gaedertz & Wittgenstein [1927], on the basis of experiments carried out chiefly with dyes, claim that the membrane is specifically permeable to anions and impermeable to cations; this fact itself would not justify the postulation of a secretory mechanism, since specific ionic permeability is associated with cells which show no secretory activity, e.g. the erythrocyte [Davson & Danielli, 1938]. The demonstration of a specific anion permeability of the membrane would also provide useful theoretical evidence for secretory activity, whereas a membrane incapable of distinguishing between negative and positive ions would give greater difficulties.

In the present work three main problems have been investigated:

(*a*) Does the membrane, as claimed by Robertson, show uni-directional permeability to crystalloids?

(*b*) Is the membrane, as claimed by Gaedertz & Wittgenstein, specifically anion permeable?

(*c*) Can the membrane secrete ions?

By using the isolated head preparation and varying the concentrations of potassium, sodium and chloride in the perfusing fluid, unequivocal answers to the problems (*a*) and (*b*) were obtained, showing that the claims of Robertson and of Gaedertz & Wittgenstein are without foundation. To test (*c*) a similar preparation was used. Secretion is associated with an oxidative activity of the cells which is normally poisoned by cyanide (see Höber *et al.* [1927–30] in respect of kidney and liver, and Huf [1936] in respect of frog's skin). If, therefore, the cells secrete, then the rate of penetration of an ion, such as potassium, across the mem-

brane, should vary according as the eye is alive or poisoned with cyanide. If this argument is correct, then the results shown here do not indicate that there is a secretion of the potassium ion by the membrane.

The rate of penetration of potassium was measured in the direction from blood to aqueous humour, since it is possible to maintain a reasonably constant high level of potassium in the blood. An attempt was made to measure the rate of penetration of potassium from the eye into the blood. To do this it was necessary to reduce the potassium content of the perfusing blood, and this was done by dialysing the blood against isotonic NaCl-$NaHCO_3$. It was found, however, that the level of potassium in the blood rises so rapidly to its normal value during the perfusion that it was impossible to maintain a concentration difference between the inside and the outside of the eye for long enough to make accurate measurements. Presumably the rise in blood potassium was due to an escape from the muscle [Fenn, 1936] and/or nerve cells.

The rates of penetration of sodium and chloride were measured in the direction of eye to blood, since it is possible to reduce their concentration in the blood by diluting with isotonic solutions containing a colloid, thereby maintaining the relative osmotic pressures reasonably constant. One experiment was carried out with a raised sodium content, i.e. sodium penetrating from blood to aqueous humour, but the result was uncertain, since the large differences in osmotic pressure created by the addition of sodium chloride caused water to pass out of the eye.

Experimental

The essentials of the procedure were to perfuse an isolated cat's head with cat's blood containing either an excess or deficiency of one of the ions, potassium, sodium or chloride. The aqueous of one eye was used to obtain the initial value of the concentration of the ion considered, and after a definite interval of time the aqueous of the other eye was withdrawn for analysis. Determinations of the concentration of the ion in the blood serum during the perfusion gave the concentration difference between the serum and the aqueous, and from these values a permeability constant could be calculated.

When it was desired to compare the rates of penetration of a given ion into the living and dead eyes of the same head, a determination of the concentration of the ion in the animal's own blood at the time of severance of the head enabled us to make an approximate calculation of the concentration initially present in the aqueous. Consequently the with-

drawal of the aqueous from the one eye was made after 1 hr. of perfusion, and from the other after a further hour during which the head was poisoned with cyanide. In this way two permeability constants were obtained, one for the penetration into the living eye, and one into the poisoned eye.

Details of perfusion. The cat was anaesthetized with ether and subsequently with chloralose. The dorsum of the second cervical vertebra was exposed and cleaned, and a dissection was then made to expose the common carotids for about $1\frac{1}{2}$ in. to their bifurcation. Portions of the larynx and trachea were removed. The common carotids were clipped, cannulated and connected with the perfusion circuit. Just before the occlusion of the vertebral circulation with an écraseur the clips on the carotids were removed, thereby establishing the perfusion of the head. As a result the head was not deprived of a fully oxygenated blood supply at any time during the procedure. A Dale-Schuster pump was used to propel the blood through the perfusion circuit which included a resistance and an oxygenator, the latter being of the type used by Gregory [1939] and Chute & Smyth [1939]. A current of 95% O_2 and 5% CO_2 was passed through it. The temperature of the circulating blood was maintained at 37° C. by immersing most of the circuit in a water-bath, the temperature of which was controlled by a thermoregulator of the type used by Lythgoe & Quilliam [1938]. Two cats were usually bled under ether anaesthesia to provide defibrinated blood for the perfusion apparatus before the experiment. For further details of the procedure the reader is referred to a paper by Chute & Smyth [1939]. The perfusion pressure was maintained as constant as possible at 180 mm. Hg. The flow varied with different animals between 40 and 90 ml./min. and invariably increased after poisoning the preparation with cyanide.

The isolated head thus prepared exhibits a blink reflex in response to a puff in the eye, or to a movement of the vibrissae, or to a direct stimulation of the cornea or the inner canthus of the eye. A tap on the nose elicited a jaw jerk and a blink, and often in raised blood potassium experiments a series of jaw movements. The pupil of the eye was constricted, and spontaneous eye, ear and jaw movements were occasionally seen. These reflex movements were invariably more marked in preparations perfused with blood of high potassium content. When the isolated head is deprived of an oxygenated blood supply, or is poisoned with cyanide, the reflexes vanish and the pupils dilate widely. In the dying head, gasping movements of the jaws and a series of movements of the alae of the nostrils were observed. The constriction of the pupil was taken

as an indication that the preparation was alive. Eye fluids for analysis were withdrawn with a clean dry syringe.

Chemical methods of analysis. Davson [1939] has recently described an exceptionally accurate method for the determination of sodium in serum and aqueous humour involving the Barber-Kolthoff [1928] gravimetric precipitation. This method was used in this work for both sodium and potassium, the latter being precipitated by the Kramer [1920] sodium cobaltinitrite procedure and estimated volumetrically. The chloride was determined by the method of Sendroy [1937].

Theoretical

The rate of penetration of a substance into the eye will be given by the following equation:

$$\frac{dx}{dt} = kA\,(C_S - C_{Aq});$$

x is the amount of the substance penetrating into the aqueous humour, C_S and C_{Aq} are the concentrations of the substance in the serum and aqueous humour respectively, A is the area of the membrane through which diffusion occurs, k is a permeability constant, and t is the time in min.

This assumes that the rate of passage across the membrane is slow compared with the rate of diffusion in the eye and blood.

Since the concentration of the substance at any moment is given by $C_{Aq} = (x+I)/V$, where I is the amount initially present in the eye and V is the volume of the fluid in the eye, which remains virtually constant, we get

$$\frac{dC_{Aq}}{dt} = \frac{kA}{V}\,(C_S - C_{Aq}),$$

which gives

$$\frac{1}{(t_2 - t_1)} \log \frac{(C_S - C_{Aq})_{t_1}}{(C_S - C_{Aq})_{t_2}} = \frac{kA}{V}\,\frac{1}{2{\cdot}303} = K,$$

assuming that the serum concentration remains constant (actually the serum concentrations did vary slightly so that a mean value for the whole period was used), t_1 is the time at the beginning and t_2 the time at the end of the experimental period.

Thus if it is assumed that A/V, the ratio of the area of the blood-aqueous barrier to the volume of the eye is constant for different eyes, the logarithmic ratio may be considered as a measure for comparison of the rates of penetration of a given substance into the eye. So that the

greater the rate of penetration, the greater will be the value of K. Such a treatment is not strictly correct for ionic permeability, since potential differences will be set up owing to the unequal rates of diffusion of the positive and negative ions. Since many of the basic assumptions of the equation are first approximations only, and further, since the experiments show that the relative rates of penetration of anions and cations are not greatly different, a more exact treatment would be supererogatory. It should be noted that K, the measure of the rate of penetration of any given ion, is independent of the concentration units used so that for the convenience of appreciating the relative magnitude of the changes in concentration, the actual concentrations have been scaled so that A_1, the initial concentration in the aqueous humour, is equal to 100.

Results

In Table I the initial concentration of the potassium ion in the aqueous humour (A_1) is made equal to 100, and its concentration in the aqueous humour after a definite interval of perfusion (A_2) and its mean value in the serum (S) are scaled up appropriately.

Inspection of the values of K, the measure of the rate of penetration of potassium, shows a variability between the extremes of 17 and 36, with a mean value of 24 for the rate of penetration into the living eye,

Table I. Penetration of potassium into the aqueous humour. The concentrations of potassium are scaled so that A_1, the initial aqueous concentration, is made equal to 100. A_2 is the aqueous concentration after perfusion with a serum potassium of S. The time period in all cases was 1 hr. except in the experiment 12. i. 39, where it was 75 min.

Exp.	A_1	A_2	S	$100\left\{K=\frac{1}{t_2-t_1}\log\frac{(S-A_1)\,t_1}{(S-A_2)\,t_2}\right\}$	Remarks
3. i. 39	100	134	160	36	Alive
21. xii. 38	100	163	256	23	Alive
14. xii. 38	100	148	253	16	Alive
7. xii. 38	100	155	220	27	Alive
15. xi. 38	100	115	143	20	Alive
18. i. 39	100	160	227	28	Alive
5. v. 39	100	121·5	149·5	25	Alive
11. v. 39	100	122·5	160	20	Alive
15. v. 39	100	123·5	174	17	Alive
				Mean = 24	
12. i. 39	100	176	258	23	Poisoned
24. iii. 39	100	124·5	166	20	Poisoned
31. iii. 39	100	134	195	19	Poisoned
18. v. 39	100	147	206	25	Poisoned
25. v. 39	100	138·5	185	26	Poisoned
28. vi. 39	100	116	146	19	Poisoned
				Mean = 22	

Mean value of K for all experiments on live and poisoned heads = 23.

against a mean value of 22 for the poisoned eye. In view of the individual variations a difference between the means of only two units is without significance. Thus, so far as these experiments go, there is no great difference in behaviour between a living and a dead eye in respect to the rate of penetration of potassium. Whether or not the variability of the preparations is due to different permeabilities of the membrane, or merely to variations in A/V, cannot be decided at present. The fact that, out of the nine experiments on living eyes, six values of K differ by not more than four units from the mean, indicates a regularity in behaviour in response to a raised blood potassium that is reconcilable with a mechanical diffusion process.

In an endeavour to obtain more accurate evidence as to the possible difference in behaviour of living and dead eyes, experiments were carried out in which the head was perfused with a blood containing a raised potassium content for two successive hours, 1 hr. alive and 1 hr. either alive or poisoned with cyanide. In this way a direct comparison on the same animal could be made, provided a value for the initial concentration in the aqueous humours of the eyes before the perfusion began could be obtained without withdrawing the aqueous humour. An approximate value can be obtained by estimating the potassium content of the serum of the animal whose head was used in the experiment, and multiplying by the factor 1/1·07, which is the average ratio of the concentrations of potassium in the aqueous humour and serum at the end of the operative procedure. The high value is due to a gradual increase in the potassium content of the blood under anaesthesia, the concentration in the aqueous humour lagging behind. This unfortunately may introduce a rather large error into the calculation, perhaps in an extreme instance of approximately six units. It is not sufficient, however, to mask any large change in permeability which would be expected if cyanide suppresses a secretory mechanism. The results of this series of experiments are shown in Table II, where "A_1" is the calculated value of the initial concentration of potassium in the aqueous humour, A_2 is the value after 1 hr. of perfusion and A_3 after a further hour. S_1 and S_2 are the mean values of the serum potassium concentration during the first and second hours respectively. K_1 and K_2 refer to the values of

$$\frac{1}{t_2 - t_1} \log \frac{(S - A_1)_{t_1}}{(S - A_2)_{t_2}}$$

calculated for penetration during the first and second hours.

In the first three experiments shown in Table II, the head was alive for both periods of 1 hr., and it is seen that the values of K_1 and K_2 are

TABLE II. Penetration of potassium into the aqueous humour of both eyes. The concentrations of potassium are scaled so that "A_1", the calculated initial aqueous concentration before the perfusion, is made equal to 100. A_2 and A_3 are the observed aqueous concentrations after 1 and 2 hours' perfusion with a blood containing potassium in the mean concentrations of S_1 and S_2 respectively.

Exp.	"A_1"	A_2	A_3	S_1	S_2	$K_1 \times 100$	$K_2 \times 100$	Remarks
15. v. 39	100	144·5	179	244	252	16	17	Alive
11. v. 39	100	161	197	258	252	22	20	Alive
5. v. 39	100	151	184	226	226	23	25	Alive
25. v. 39	100	171	237	298	317	19	26	Poisoned
31. iii. 39	100	140	188	248	275	14	19	Poisoned
24. iii. 39	100	140	174	206	233	21	20	Poisoned
28. vi. 39	100	134·5	156	187	196·5	22	19	Poisoned

reasonably constant. In the last four experiments the head was poisoned with cyanide at the beginning of the second hour, and it is seen that although there is a slightly greater variation in the relative values of K_1 and K_2 the differences in any given experiment fall within the limits of experimental error. A slight increase in the value of K during the second hour might be expected owing to the increased flow that occurs after poisoning with cyanide.

In Table III are shown some results on the diffusion of sodium and chloride.

TABLE III. Permeability of the aqueous humour-blood barrier to sodium and chloride. The concentrations are scaled so that A_1, the initial aqueous concentration, is made equal to 100. A_2 is the aqueous concentration after perfusion for one hour with a serum concentration of S. In experiment 25. i. 39 penetration is in the direction blood to aqueous humour, in the remaining experiments the direction is from aqueous humour to blood.

Exp.	A_1	A_2	S	$K \times 100$	Remarks
		Sodium			
17. iii. 39	100	94	75·5	10	Alive
22. ii. 39	100	90·5	70	17	Alive
19. v. 39	100	97·5	74·5	5	Poisoned
26. v. 39	100	96·5	80	8	Poisoned
31. v. 39	100	94·5	60	6	Poisoned
				Mean = 9	
25. i. 39	100	111	119	38	Alive
		Chloride			
19. vi. 39	100	94·5	80	14	Poisoned
14. vi. 39	100	94	78	14	Alive

In the first five sodium experiments the concentration of sodium in the serum was reduced by the addition of a diluting mixture consisting of isotonic glucose and gelatine, or gum arabic; in this way the osmotic pressure was maintained as constant as possible. It is very difficult to make viable preparations using such a perfusion fluid, and a number died soon after the change over. When this occurred, cyanide was added

to ensure complete suppression of any secretory activity and the values of K so obtained are presented in Table III. It should be emphasized here that we do not wish to make a close comparison between the permeabilities of living and dead eyes to sodium and chloride owing to the small number of experiments on these ions. Our main desire is to show that sodium and chloride can diffuse out of the eye. If the suppression of a secretory activity could be expected to produce a marked alteration in the permeability to sodium and chloride then of course a comparison of the figures presented would certainly indicate the absence of a secretory activity. The results, however, show beyond doubt that sodium can penetrate the membrane between the blood and the aqueous humour. In the sixth experiment the sodium content of the blood was raised by the addition of M NaCl. This increased the osmotic pressure and therefore caused a loss of water from the eye; this was apparent by the marked decrease in the intra-ocular pressure[1] as soon as the sodium chloride was added. It is to be noted that the value of K in this instance is 38 against values of 5–17 for the rate of penetration in the opposite direction, and this difference is doubtless due to the migration of water producing an apparent penetration of sodium. Whether any sodium actually penetrates cannot be proved.

In the same table two experiments are shown on the rate of loss of chloride from the eye, one on a live head and the other on a poisoned one. In these cases the blood was diluted with isotonic $NaNO_3$-$NaHCO_3$ solution. The results show no difference in behaviour between the living and dead eye. The close agreement between the two values is accidental. The point that we wish to emphasize from the results in Table III is that sodium and chloride are able to migrate across the membrane.

The mean value of K for the penetration of sodium from the aqueous humour to the blood is 9 compared with a value of 23 for potassium penetrating in the reverse direction. This difference is significant, and is what one would expect from the comparative sizes of the two ions. Chloride seems to diffuse at about the same rate as sodium, thereby showing that Gaedertz and Wittgenstein's claim of specific anionic permeability is both qualitatively and quantitatively unfounded.

[1] This observation confirms the earlier work of Duke-Elder [1927], showing that the intra-ocular pressure is an inverse function of the osmotic pressure of the blood. It may also be noted that reduction of the colloid osmotic pressure of the blood by diluting it with isotonic sodium chloride solution causes a large increase in intra-ocular pressure. Consequently Robertson's [1938] claim, based on measurements involving the use of the inaccurate tonometer, that a reduced colloid osmotic pressure has no influence on the intra-ocular pressure is not borne out by experiments on the surviving eye.

Discussion

The results described in this paper prove that potassium and sodium may penetrate into the eye and that sodium and chloride may pass out; consequently, any secretory theory of the formation of the aqueous humour, which assumes an irreciprocal permeability of the membrane or a specific ionic permeability, is without foundation.

It should be noted that the experiments of Gaedertz & Wittgenstein, which led them to conclude that the membrane is specifically permeable to anions, were based essentially upon studies of the penetration of dyestuffs into the eye. Acid dyestuffs were found to penetrate, whereas basic dyes, e.g. neutral red, did not. Most dyestuffs are weakly acidic or weakly basic, and it is now generally accepted that the mechanism of penetration of salts of weak acids and bases through a membrane is essentially different from that of salts of strong acids and bases, such as NaCl; the former being brought about by the penetration of undissociated acid or base produced by hydrolysis [see Tyler & Horowitz, 1937; Krahl & Clowes, 1938], followed later by ionic exchanges; the latter by ionic exchanges alone. Thus salts of the weak base ammonium penetrate readily into the erythrocyte, which is impermeable to cations, the mechanism being most probably the initial penetration of ammonia followed by an exchange of anions [Jacobs, 1927; Jacobs & Parpart, 1938]. The demonstration of the rapid penetration of salts of weak bases such as ammonium into the erythrocyte does not prove that its membrane is specifically cation permeable, but rather supports the opposite view that it is specifically anion permeable.

There are many other objections to the use of dyes in permeability studies which need not be entered into here. It is enough to say that the apparent failure of a dye to penetrate into a given cell is not only due to an impermeability of the cell membrane to molecules of this dye, but involves the problem of vital staining, a much more complicated subject which has been exhaustively reviewed by Gicklhorn [1931].

An interesting investigation based on the use of dyes was made by Friedenwald & Stiehler [1938]. They claim that the epithelium of the ciliary body shows a selective permeability in that water and basic dyes are preferentially transferred in the direction of blood to aqueous humour, and acidic dyes in the reverse direction. This selectivity is said to be due to the concentration of Warberg's yellow enzyme in the ciliary epithelial cells. In view of the fallacy in arguing from the behaviour of dyestuffs to the behaviour of ions in general, it is perhaps unfortunate

that these striking claims rely essentially on observations of selective staining. In addition Rose Bengal which was principally used in this investigation is a strong haemolytic agent in concentrations above 1×10^{-6} *M* in the presence of light and above about 1×10^{-4} *M* in the dark [Blum, Pace & Garrett, 1937]. Friedenwald & Stiehler used a concentration of 1×10^{-3} *M*. The action of lysins of this class is not confined to the erythrocyte [Lillie, Hinrichs & Kosman, 1935].

Proponents of the secretory theory argue that the distribution of diffusible substances between the aqueous humour and the plasma is not in accordance with that required by the Donnan equilibrium. The evidence from chemical analyses is certainly conflicting. To quote only the more recent publications, Davson, Duke-Elder & Benham [1936] find that the sodium, potassium and chloride ions are distributed approximately in accordance with theory, whereas Hodgson [1938] finds the excess of chloride in the aqueous humour to be too great. More recently, Davson [1939] has carried out analyses of sodium in the aqueous humour and the plasma of cats, avoiding disturbances due to anaesthesia and using a method which gave a mean error of about 1 in 500. He found that although individual cats showed variations in their distributions from a theoretical ratio of 1·04, the variations were no larger than those in experimental ultra-filtrates and dialysates. Similarly, Stary & Winternitz [1932] showed that although the concentration of calcium in the aqueous humour is 7·4 mg.% compared with a value of 12·1 mg.% in serum, the concentration in an ultra-filtrate of serum was 7·4 mg.%, i.e. the same as that in the aqueous humour. The explanation of the discrepancy from the theoretical value is that some of the calcium in serum is indiffusible. Deductions from estimations of the relative concentrations of non-electrolytes in serum and aqueous humour present some difficulty, since (*a*) the chemical methods may not be sufficiently specific, so that comparison between a protein-free and a protein-rich fluid is not justified, and (*b*) where the non-electrolyte is a metabolite, its concentration in the aqueous humour and plasma will vary continuously. The results of Walker [1933] and Adler [1933] on non-electrolyte distribution conflict with the dialysis theory. It would not be fair to dismiss them on the above grounds alone. The decision as to whether there is an active secretion of urea, glucose, etc., will depend on investigations of the sort described here. Nevertheless, it would be very unsafe to argue that, because some non-electrolytes are not equally distributed between the two fluids, there is therefore an active secretion of these substances, either into or out of the eye. This point is well exemplified by the work of

Fischer [1930], who found that the distribution of lactic acid between blood and aqueous humour was different in normal and aphakic eyes, the concentration in the aqueous in the former being larger, presumably owing to the continuous addition of metabolites from the lens which would be absent in the aphakic eye.

Attention should be drawn to the recent observation of Benham, Duke-Elder & Hodgson [1938] that the osmotic pressure of the aqueous humour is higher than that of the blood; i.e. the osmotic pressure relations are the opposite to those required by the Donnan equilibrium. The method of measurement depended on the relative rates of evaporation of two fluids [Hill, 1930], and there is a possibility that the proteins in serum are capable of modifying the rate of evaporation without modifying the osmotic activities of the other molecules in serum [Rideal, personal communication]. Until such an acceleration in the rate of evaporation can be proved the results just quoted present a stumbling block to the acceptance of a simple ultra-filtration mechanism for the formation of the aqueous humour.

A novel argument in favour of a secretory mechanism is that of Robertson [1939*a*]. This author has followed the penetration of glucose, urea, etc., into the lymph, the gastric juice, the cerebrospinal fluid and the aqueous humour after an intravenous injection of the substances. He argues that, because the course of penetration into the aqueous humour follows that into the gastric juice more closely than that into the lymph, the aqueous humour is a secretion. Reference to the equation derived to suit the conditions of penetration into the eye will show that the rate of variation of the concentration of the penetrating substance at any given moment is a function of A/V, the ratio of the area of the membrane to the volume of fluid in the eye. For comparison between rates of penetration into two different systems, this ratio must be known, otherwise differences in the rate may simply be attributable to differences in this ratio and not to any special characteristic of the membrane. It is clear that in two such anatomically different systems as the optic and lymphatic even an approximate equality of A/V is unlikely, so that the comparisons are without value. Quite apart from this consideration the argument ignores certain obvious dissimilarities of the circulation of the fluids compared.

This brief review of the problem of the origin and conditions of maintenance of the aqueous humour shows that there are many points to be settled. Clearly, then, a decisive answer to the question "Is the aqueous humour a dialysate?" cannot be given until more experimental

work is forthcoming. The results described in this paper, however, indicate that the influence of a secretory activity in respect to the potassium, sodium and chloride ions is negligible.

Summary

1. The surviving isolated head was perfused from a pump oxygenator circuit, the concentrations of the potassium, sodium and chloride ions in the perfusing fluid were varied and the rate of penetration of the ions into the aqueous humour was determined chemically.

2. In response to a raised potassium content of the perfusing fluid potassium penetrated into the eye; the rate varied from animal to animal, but eleven out of fifteen experiments gave values of a "permeability constant" (K) not differing from the mean by more than 16%. The mean (K) of a group of experiments on living eyes was not significantly different from that of a group of eyes poisoned with cyanide.

3. In response to a decreased sodium content of the perfusing fluid sodium penetrates from the eye into the blood; the rate of penetration is about one-third of the rate for potassium.

4. In response to a decreased chloride content of the perfusing fluid chloride diffuses out of the eye; the rate is about the same as that of sodium.

5. The results show that the membrane separating the eye fluids from the blood is not specifically impermeable to cations, as claimed by Gaedertz & Wittgenstein, nor does it show unidirectional permeability as claimed by Robertson. The experiments comparing the behaviour of living and poisoned eyes indicate that the membrane does not secrete potassium and probably not sodium or chloride.

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