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THE EXCHANGE OF SODIUM BETWEEN THE VITREOUS BODY AND THE BLOOD AND AQUEOUS HUMOUR

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The exchange of diffusible substances between the aqueous humour on one hand, and its surrounding tissues and the blood on the other, has recently been the subject of many investigations. As a result the magnitude of the exchange across the various boundaries has been fairly well established. Interest in the corresponding properties of the vitreous body has been slight. This lack is the more important since it is now realized that diffusion into the vitreous body exercises a considerable influence on the analysis of the exchange of substances across the blood-aqueous barrier. This paper reports an investigation into the passage to the blood and aqueous humour of ^{24}Na injected into the vitreous body. In the discussion attention is concentrated particularly on the latter exchange and on its effect on blood-aqueous dynamics.

METHODS

Tracer studies. A sterile 0·9% solution of $^{24}NaCl$, generally with the addition of a little concentrated fluorescein solution, was taken into a tuberculin syringe. The injection was made through a needle 12 mm long and 0·3 mm in external diameter. Rabbits, of mixed stock, were operated upon under general anaesthesia; ethyl chloride was used in the first experiments, but pentothal was later found to be preferable. The upper lid was retracted and the point of the needle was pressed against the sclera beside the superior rectus muscle and as near the equator as possible. The eyeball was rolled downwards by means of the needle point until the needle was perpendicular to its surface. The syringe was rotated in the fingers, while the point was held with a gentle pressure against the eye until the sclera was penetrated. The needle was then directed a little backwards and inserted to its full length so that its tip lay centrally in the vitreous between the retina and the posterior pole of the lens. According to the proposed duration of the experiment 0·01–0·02 ml. of solution was injected, and the syringe was rapidly withdrawn. In some experiments, particularly those which continued long enough for an appreciable re-entry of ^{24}Na from the blood into the eye to take place, a control injection of inert NaCl into the animal's other eye was made.

The experiments were of two kinds. The first were designed to measure the rate of passage of ^{24}Na from the vitreous body into the anterior chamber. The animal was killed at the end of a determined period and the aqueous and vitreous humours were collected from the experimental and the

control eyes. The aqueous humour was first withdrawn through a narrow-gauge needle introduced at the limbus. A large serum needle was then introduced through the sclera and the vitreous body was sucked out into a syringe until 1–1½ ml. were collected. The aqueous humour was weighed and diluted with trichloroacetic acid, and the vitreous humour was centrifuged and diluted with water if necessary. The radioactivity of the samples was then assayed in an F10 liquid counter (20th Century Electronics). To allow for the re-entry of ^{24}Na from the blood, the activities of the aqueous and vitreous humours of the control eye were subtracted from those of the experimental eye.

In the second group of experiments the amount of ^{24}Na remaining in the eye at various times after its injection was estimated by means of an external Geiger-Muller counter. An end-window counter was used (G.E.C. GM4), mounted with its axis horizontal. The conscious animal was held with its eye open about 3 cm directly in front of the counter window. A mirror bearing an index mark against which the cornea and its reflexion could be aligned served to locate the eye (Fig. 1).

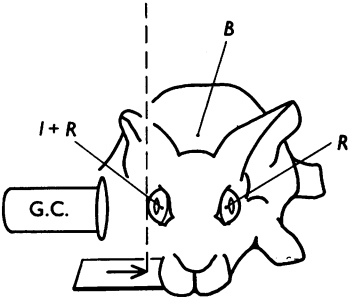

Fig. 1. Experimental arrangement for measuring activity in eye of rabbit, showing method of aligning eye against arrow on mirror at a fixed distance from Geiger counter (G.C.).

A count was taken for ½–3 min according to the strength of the radiation. The animal was then turned so that its control eye was opposite the window and a count was taken for an equal period. The eyes were counted alternately several times; at least sufficient counts were accumulated from each eye to reduce to less than 5% the standard error of the statistical variation of the difference between them. Each time the activity of the animal's eye was assayed, a count was also taken from a drop of the injected material which had dried on filter paper and was held a fixed distance from the window. This acted as a reference to allow for the decay of the active material and variations in counter sensitivity.

At any time, let

I = the amount of the originally injected ^{24}Na remaining in the eye;
B = the amount that has passed into the blood;
R = the amount that has entered each eye *from* the blood;
n = the relative efficiency for producing counts of the material in the eye nearer the counter;
f = that of the material in the further eye;
b = that of the material in the body.

Then, if the experimental eye is nearer the counter, the counting rate will be proportional to

$$n(I+R)+fR+bB;$$

and if the control eye is nearer, to

$$nR+f(I+R)+bB \text{ (Fig. 1).}$$

The difference between these rates is $(n-f)I$; this difference divided by the activity of the dried sample of injected solution is, therefore, a measure of I, the amount of ^{24}Na remaining in the eye.

Conductivity. The conductivity of the aqueous humour was compared with that of the intact vitreous body by means of a 2·9 kc/s bridge (Mullard E7566). The electrodes were made of platinum wire, varnished except for 2–3 mm at the tip which was flattened and blacked.

The electrodes were inserted into the vitreous body through windows cut in the sclera so as to cause as little disturbance as possible to the contents of the globe. In some experiments with rabbit's eyes the electrodes were mounted side by side and lowered into the vitreous body together. In the majority of experiments, which were performed on ox eyes, the electrodes were pushed in from different directions at an angle of about 90° to one another. They were located in holders which were fixed so that there was a predetermined distance between the electrode tips of 2–10 mm. Comparative measurements were made on the aqueous humour from the two eyes of the same animal with the electrodes located in the identical position. Rabbit aqueous humours were held in a small glass cylindrical vessel, and those of the ox in an ellipsoidal cavity in a block of paraffin wax containing 2 ml. of fluid.

RESULTS

When fluorescein is present in the test solution, the injected volume can be clearly seen through the pupil. It does not mix by circulation with the mass of the vitreous body but spreads diffusely from its edges. The eyes showed little or no reaction to the injection; the conjunctiva was not inflamed, the pupil was mobile, and the aqueous free from flare. In those animals where the control eye was untouched no difference between the two eyes was observed in any of these respects shortly after the injection. The amount of protein precipitated by the addition of trichloroacetic acid to the withdrawn aqueous humour was generally small and about equal for the two eyes; in two cases, however, there was a heavy precipitate on the injected side and these were rejected. In two animals fluorescein was given intravenously and its penetration into the aqueous humour on the injected and control side compared by an objective technique (Langham & Wybar, 1954) and found to be equal.

The rise of pressure within the eye when given volumes of fluid are injected into it has been investigated by Perkins & Gloster (1957). They found that, on the average, a rabbit with an intra-ocular pressure of 20 mm Hg would have raised this to 32 mm on the injection of 0·01 ml., and to 79 mm on the injection of 0·02 ml. It is worthy of note that injections of 0·05 or 0·1 ml. will raise the intra-ocular pressure above the systolic arterial pressure, so that apart from any mechanical injury that might result, blood would be prevented from circulating in the eye for some minutes and the cells be damaged by anoxia. Larsson (1930), in fact, found that after injections of this volume of saline into the vitreous body of rabbits the pressure was very much raised for 10–20 min, and that permanent changes in the fundus of the eye were frequently observed.

Vitreous-aqueous exchange. The results of these experiments are shown in Fig. 2, where the ratio of the concentration of ^{24}Na found in the aqueous humour to that found in the vitreous is plotted against the duration of the experiment. The points representing these ratios rise with remarkable regularity after a delay of 1 hr. From 6 hr onwards they attain a constant value of $0{\cdot}20 \pm 0{\cdot}010$ (S.E.M. 15 eyes).

The concentration of ^{24}Na in the aqueous humour of the control eye was about 5% of that of the experimental eye after 24 hr, and about 30% after

40 hr. Thereafter, the activities of the fluids were too low to give an acceptable standard error to their difference.

Vitreous loss. The rate of loss of ^{24}Na from the eyes of eight animals in which it was injected into the vitreous humour is shown in Fig. 3. After a period of less than 5 hr, during which a steady state is established, the rate of loss is exponential in each eye. The straight lines drawn in the figure were estimated visually to be the best fit to the points. Their average slope corresponds to a turnover rate of $1{\cdot}56 \times 10^{-3} \pm 0{\cdot}06 \times 10^{-3}$ (S.E.M.) min^{-1} in excellent agreement with the value of $1{\cdot}55 \times 10^{-3}$ min^{-1} found by Friedenwald & Becker (1955) for the rate of penetration of ^{24}Na into the vitreous humour from the blood.

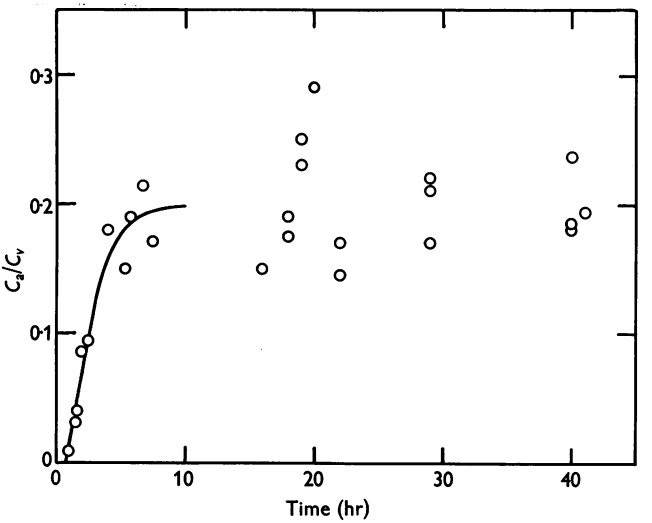

Fig. 2. Rise in concentration of ^{24}Na in aqueous humour after its injection into vitreous body. Ordinate, ratio of concentration in aqueous to that in vitreous after subtraction of values from control eye; abscissa, time after injection. Each point from one animal; curve calculated as explained in text.

Conductivity. The measurements of the conductivity of both vitreous and aqueous humours were disturbed by the presence of the boundaries of the fluid, the sclera and lens in one case and the glass or wax receptacle in the other. The magnitude of this effect could be estimated by comparing the conductivity of saline when contained in the receptacle and when in a large beaker; the proximity of the walls generally caused a reduction of about 5%. However, the measured conductivity of both the vitreous and aqueous humours fell detectably below a maximum value only when the electrodes were very close to a limiting surface. The value of this conductivity in the vitreous was from 3 to 20% below that of the aqueous with a median value of 10% below. It is difficult to assess the accuracy of this figure, but it is probably correct to within 10% which is all that is required in the discussion that follows.

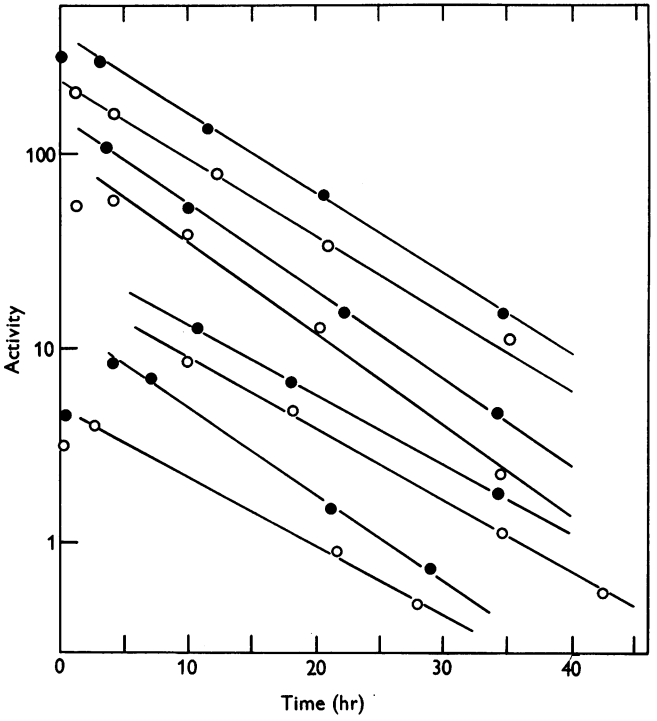

Fig. 3. Rate of loss of ^{24}Na from eye after injection into vitreous humour, measured with external counter. Ordinate, activity of experimental less control eye divided by standard source, in arbitrary units; abscissa, time after injection. Each group of points from one animal; lines fitted visually.

DISCUSSION

Geometrical and diffusional relationships

Later in the discussion it will be necessary to assume values for some of the geometrical characteristics of the intra-ocular fluids. Fig. 4*a* is re-drawn from a photograph of a meridional section of a frozen rabbit's eye (Davson, 1953). By mensuration in the original, the volume of the vitreous body, V_v, is 1·7 ml., its area of contact with the posterior segment of the globe up to the base of the ciliary body, A_{pv}, is 6·1 cm^2, and the radius of curvature of this segment, r_v, is 0·85 cm. The boundary between the posterior chamber and the vitreous body, $A_{\pi v}$, taken as the strip between the base of the ciliary body and the edge of the lens, is 2·5 mm wide and 1·3 cm in average diameter, or 1·0 cm^2 in area. The volume of the anterior chamber, V_a, is 0·29 ml. and that of the posterior chamber, V_π, is about one-fifth of this, 57 μl. (Copeland & Kinsey, 1950).

At this stage, also, it will be helpful to give a short account of the present-day concept of the diffusional exchanges within the eye; these are shown

schematically in Fig. 4*b*. Every fluid or tissue exchanges with each of the others with which it is in contact. In addition, fluid is secreted at a rate F by the ciliary body into the posterior chamber. This fluid flows round the border of the pupil preventing the backward diffusion of ions from the anterior to the posterior chamber, and drains out of the eye at the angle of the anterior

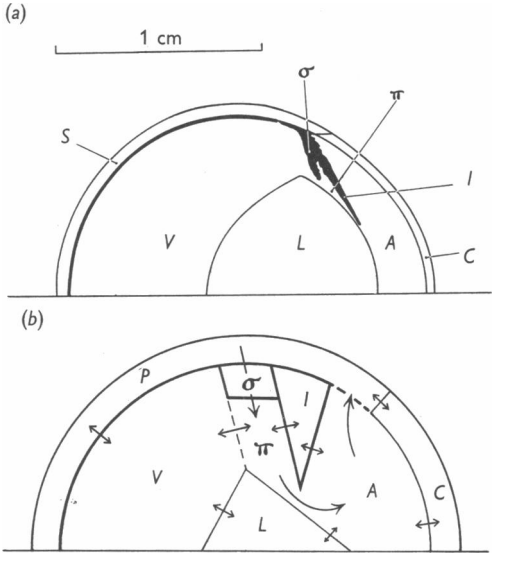

Fig. 4. (*a*) Geometrical and (*b*) diffusional relationships in eye of rabbit. *A*, anterior chamber; π, posterior chamber; *V*, vitreous body; *C*, cornea; *L*, lens; *I*, iris; σ, ciliary body; *S*, sclera; *P*, plasma. Double arrows represent balanced, diffusional exchanges; single arrows, secretion and flow of the aqueous humour.

chamber. The loss of a substance from the anterior chamber is divided into two parts, that resulting from the outflow and that from diffusion across the iris and, to a lesser extent, the cornea. The total outflux, and its flow and diffusional components are conventionally expressed by transfer coefficients k_o, k_f and k_d, respectively, so that:

$$k_o = k_f + k_d, \tag{1}$$

$$dC_a/dt = k_f (C_\pi - C_a) + k_d (C_p - C_a), \tag{2}$$

and

$$k_f = F/V_a \tag{3}$$

where C_a, C_π and C_p are the concentrations of the substance in the anterior and posterior chambers and the plasma respectively.

Both k_f and k_d may be calculated by means of equation (2) if the variations of C_a, C_π and C_p with time are known. These concentrations were determined experimentally by Kinsey & Palm (1955) after the intraperitoneal injection of the ^{24}Na and thiocyanate ions. According to the calculations of Friedenwald & Becker (1955) a value of 0·0175 min^{-1} for k_f and about 0·027 min^{-1} for k_o resulted in both cases. From equation (3), then, the flow rate, F, is 5μl. min^{-1}.

Vitreous-blood exchange

Just as Friedenwald & Becker (1955) found a remarkable consistency in the rate of penetration of ^{24}Na into the vitreous body from the blood, in these experiments its rate of loss from the vitreous body to the blood shows little variation between animals. The extreme values found for the transfer coefficients have a ratio of 1·3, compared to about 2 for the blood-aqueous barrier (Kinsey & Palm, 1955) and 5 for the endothelium and epithelium of the cornea (Maurice, 1955). This consistency suggests that there is a purely physical mechanism restricting the exchange, and there is the possibility that the mechanism is the slowness of the diffusion of ^{24}Na in the vitreous body itself.

This hypothesis may be checked theoretically, by supposing that the shape of the vitreous body is roughly that of a sphere with a concentric sector removed. If, then, all the resistance to the ionic exchange between the vitreous body and the blood lies in the former there will be no resistance at the vitreous-choroid surface and this surface will take up the concentration of the plasma. The rate at which the average concentration within a sphere comes into equilibrium with a constant concentration maintained at its surface is governed after a short initial period, by the exponential of $(-\pi^2 D/r^2)t$, when D is the diffusion constant in the sphere, r its radius and t the time (Carslaw & Jaeger, 1947, p. 201). Inserting the value of r_v, 0·85 cm, and for D_v, the rate of diffusion in the vitreous body, a value 10% below that of ^{24}Na in free solution at 37° C, $1{\cdot}05 \times 10^{-3}$ cm^2 min^{-1} (Adamson, Cobble & Nielsen, 1949), the exponential constant takes the value 0·013 min^{-1}. This is about nine times greater than the experimentally found value of the transfer coefficient from the vitreous humour to blood.

This discrepancy could only be resolved if the resistance to diffusion of Na in the vitreous body was about ten times more than in free solution. This possibility is suggested by the high relative viscosity of vitreous homogenates found by Woodin & Boruchoff (1955). However, if this were so, the conductivity of the intact vitreous body should be at least five and probably ten times less than in the aqueous humour. No evidence of such low values was found experimentally and it must be concluded that the resistance to the diffusion of Na in the vitreous body does not limit its exchange with the blood.

Before an estimate can be made of the magnitude of the blood-vitreous barrier it is necessary to determine what proportion of the ^{24}Na leaves the vitreous body through the alternative pathway of the aqueous humour. It will be shown later that the exchange of ^{24}Na by diffusion between the posterior chamber and the blood may be ignored. All the ions that leave the eye by this route, then, must pass through the anterior chamber, and their amount is equal to that which passes from the anterior chamber into the blood in unit

time, $k_o C_a V_a$. This rate of loss from the vitreous body is equivalent to a transfer coefficient, k_{va}, given by

$$k_{va} = k_o \frac{C_a V_a}{C_v V_v}.$$

Inserting the value found experimentally for C_a/C_v, 0·20, and the values of k_o, V_a and V_v quoted earlier, a value of $0{\cdot}92 \times 10^{-3}$ min^{-1} results for k_{va}. Comparing this with the over-all transfer coefficient for the vitreous body, $1{\cdot}56 \times 10^{-3}$ min^{-1}, it is seen that over half the ^{24}Na leaves by way of the anterior chamber, and that which exchanges directly with the blood at the surface of the vitreous body is accounted for by a transfer coefficient, k_{vp}, of $0{\cdot}64 \times 10^{-3}$ min^{-1}. On the assumption that it is regulated by a membrane which has a uniform permeability, K_{vp} over the entire posterior surface, of the vitreous body

$$K_{vp} = k_{vp} \frac{V_v}{A_{pv}}.$$

From this a comparatively low value of $1{\cdot}8 \times 10^{-4}$ cm min^{-1} for K_{vp} is obtained, about six times less than that of the corneal endothelium, for example (Maurice, 1955).

Evidence as to which structure corresponds to the blood-vitreous barrier is provided by the experiments of Brindley (1956) on the electrical resistance of the coats of the eye. He concluded that in the frog the greater part of the resistance between the inside and outside of the eye lay in the retina and, in particular, its external limiting membrane. Extending this method to the rat (Brindley, private communication), he estimated that the retina as a whole had a resistance of 180 Ω cm^2 of which 130 Ω cm^2 lay in the limiting membrane. The pigment epithelium, choroid and sclera together had a resistance of only 50 Ω cm^2. If the assumption is made that the resistance of the retina is the same to all ions, the value 180 Ω cm^2 corresponds to a permeability of $2{\cdot}9 \times 10^{-4}$ cm min^{-1}. That this is of the same order as the value of K_{vp} for the rabbit, derived above, suggests that the blood-vitreous barrier lies in the retina and mainly in its external limiting membrane. It should be noted, however that the retinal vessels in the rabbit are restricted to an area of 0·2–0·25 cm^2, less than 5% of the total posterior surface of the vitreous body. In animals with a more extensive retinal vascularization it may well be that the exchange across its capillaries is more important.

Among previous investigations of the blood-vitreous exchange, that of Friedenwald & Becker (1955) has already been mentioned. Duguid, Ginsberg, Fraser, Macaskill, Michaelson & Robson (1947) have reported experiments, similar to those in this paper, in which sulphacetamide was injected into the vitreous body and the animal killed after a variable period of time and the amounts in the vitreous and aqueous humours estimated. A volume of test

solution of 0·1 ml. was injected which most probably caused a lasting disturbance to the eyes. They found an over-all transfer coefficient out of the vitreous body of $1{\cdot}1 \times 10^{-3}$ min^{-1}, and a ratio of C_a/C_v which 6–48 hr after the injection varied between 0·10 and 0·45 with a median of 0·19. These figures are close to those found for ^{24}Na.

The rate of exchange of a variety of substances between blood and vitreous humour has been measured by Davson (1955*a*). This rate was similar for the ions Na, Br and K, but rather lower for I and higher for thiocyanate. One substance, ethyl thiourea, had a transfer coefficient of 0·013 min^{-1}, and it is probable that its exchange must be almost entirely limited by the resistance to diffusion in the vitreous body itself.

Vitreous–aqueous exchanges

The delay in the appearance of ^{24}Na in the anterior chamber after its injection, shown by the points in Fig. 2, is undoubtedly due to the slowness of its diffusion in the vitreous body. An exact calculation of the appearance time is not feasible, but as an approximation the vitreous body may be taken as a flat cylinder with a radius, 1·2 cm, equal to the distance between the site of injection in its middle and its border with the posterior chamber. As before, the diffusion constant of ^{24}Na is taken to be $0{\cdot}95 \times 10^{-3}$ cm^2 min^{-1}, and it is assumed it passes into the aqueous humour at a rate proportional to the concentration at the cylindrical surface and finally attains to the steady state value of 0·20. The result of this calculation (Carslaw & Jaeger, 1947, p. 306) is shown as the curve in Fig. 2. By its agreement with the experimental points it supports the above assumptions, and, in particular, the value of the diffusion constant that has been adopted.

The exchange between the aqueous and vitreous humour when the steady state is achieved will now be analysed in some detail. The quantity of the test ion moving from the vitreous body into the posterior chamber will be called $m_{v\pi}$ and it will be assumed to follow an equation

$$\frac{\mathrm{d}m_{v\pi}}{\mathrm{d}t} = V_a \mathrm{g}\,(C_v - C_\pi), \tag{4}$$

where g is a constant. If diffusion from the posterior chamber into the blood is relatively small

$$C_\pi = \frac{1}{\mathrm{F}} \frac{\mathrm{d}m_{v\pi}}{\mathrm{d}t},$$

or, by equations (3, 4),

$$C_\pi = \frac{\mathrm{g}}{k_f}(C_v - C_\pi)$$

$$= \frac{\mathrm{g}}{\mathrm{g} + k_f} C_v.$$

Also, since the aqueous concentration of the test ion is virtually constant,

$$F C_\pi = V_a C_a k_o,$$

and so

$$C_a = \frac{g k_f}{(g + k_f) k_o} C_v.$$

Since k_f and k_o are constants and C_a/C_v is found experimentally to remain constant it follows that g also must be a constant, as previously assumed. Insertion of values into the equation leads to a value of 0·0078 min^{-1} for g.

This treatment depends on the adequacy of equation (4), the form of which suggests that the exchange of the ion between the vitreous humour and the posterior chamber is limited either by a membrane or by the establishment of a diffusional steady state across a thicker layer of fluid. Zonular fibres of the lens are found in this region but no true membrane has been distinguished, and it is probable that a layer of vitreous is resisting the movement of Na. If it is assumed that there is a uniform concentration gradient across the layer which has a thickness d and a constant cross-sectional area $A_{\pi v}$, it follows that:

$$\frac{dm_{v\pi}}{dt} = \frac{A_{\pi v} D_v}{d} (C_v - C_\pi),$$

so that by comparison with equation (4)

$$d = \frac{A_{\pi v} D_v}{V_a g}.$$

Substituting the appropriate values for the right-hand quantities, a figure of 4 mm results for d. Since the cross-sectional area of the vitreous body has increased by about 50 % at a level 2 mm from its interface with the posterior chamber, a proportionally larger value for d should be more correct. The concentration gradient will, in fact, be felt to some extent at all points in the vitreous body and it will not be strictly linear in its anterior region. A more exact calculation is not feasible, however, on account of the complex shape of the vitreous body and the lack of detailed information as to the exchanges that take place at its boundaries. Nevertheless, it might be expected intuitively, that the greater part of the diffusion gradient would be in the anterior half of the thickness of the vitreous body in accordance with the conclusion of the calculations above. The result is, therefore, consistent with the resistance to the exchange being in the vitreous mass itself.

Blood–aqueous dynamics

In an earlier section the influence of the diffusion of Na between the chambers of the eye on its exchange between the blood and the vitreous humour was considered; attention will now be turned to its influence on the exchange between the blood and the aqueous humour. There is a complementary relation

between experiments in which ^{24}Na is introduced systemically and passes into the eye and those in which it is injected into the vitreous body and is lost to the blood. A sufficiently long time after the injection of the test ion, the diffusion of the unlabelled Na from the vitreous body into the anterior chamber in the first case should be identical with that of the labelled ^{24}Na from the vitreous body into the anterior chamber in the second.

This can be illustrated by a comparison of the experimental results given in this paper, and those of Kinsey & Palm (1955) in which ^{24}Na was injected intraperitoneally. For example, 6 hr after the injection they found that the amount of unlabelled Na remaining in the anterior chamber was 10% of the total Na (here, and in what follows, Friedenwald & Becker's (1955) averages of Kinsey & Palm's data will be adopted). From the experiments in this paper it may be estimated that after 6 hr. the proportion of Na in the vitreous humour which has not exchanged with the blood would be 55% of that originally present, and the ratio of the concentration in the aqueous to that in the vitreous humour would be 20%. Accordingly, the amount of unexchanged Na in the anterior chamber will be the product of these figures, 11%, in agreement with Kinsey & Palm's figure. A long time after its systemic injection, then, the rate at which ^{24}Na in the anterior chamber approaches equilibrium with the blood will be its over-all transfer coefficient in the vitreous body, 0·00156 min^{-1}.

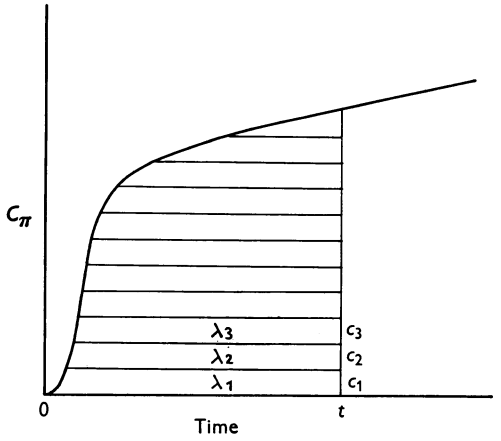

Fig. 5. Principle of calculation of diffusion from posterior chamber into vitreous body. Ordinate, concentration in posterior chamber; abscissa, time after injection.

It is of more interest in aqueous humour dynamics to consider the exchanges that take place in the early stages after the injection. These can be estimated if it is assumed, again, that the test ion diffuses freely into the vitreous body across its border with the posterior chamber. Fig. 5 shows the rise in concentration of ^{24}Na in the posterior chamber. At any time t the concentration

present is considered as being divided up into a number of smaller concentrations, c_1, c_2, etc., which have been present for periods of time λ_1, λ_2, etc. After contact with a constant concentration c_1 in the posterior chamber for a time λ_1 a quantity $2A_{\pi v}c_1\sqrt{(D_v\lambda_1)}$ of ^{24}Na will have entered the vitreous body. Similarly, a quantity $2A_{\pi v}c_2\sqrt{(D_v\lambda_2)}$ will have entered in time λ_2, and so on. Since the ions diffuse independently of one another the concentrations may be considered to act separately in this manner, and the total amount which will have entered in time t is given by

$$m_{\pi v}=2A_{\pi v}\sqrt{D_v}\int\sqrt{\lambda}\,\mathrm{d}C_\pi, \tag{5}$$

which may be obtained by graphical integration.

The validity of this treatment can be tested using the experimental results of Kinsey & Palm. The rate at which ^{24}Na enters the posterior chamber from the blood should at all times equal the sum of its rate of accumulation, its rate of loss to the vitreous body, and its rate of loss to the anterior chamber. Neglecting any diffusional exchange between the blood and the posterior chamber, this may be written

$$\mathrm{F}C_s=V_\pi\frac{\mathrm{d}C_\pi}{\mathrm{d}t}+\frac{\mathrm{d}m_{\pi v}}{\mathrm{d}t}+\mathrm{F}C_\pi, \tag{6}$$

where C_s is the ^{24}Na concentration of the fluid which is secreted into the posterior chamber by the ciliary body. Na is very nearly in equilibrium between the plasma and the posterior aqueous humour (Kinsey, 1953); the value of C_s cannot, then, be far different from that in a dialysate of plasma. The term on the left-hand side of equation (6) can be derived from the curve given by Kinsey & Palm showing the change of the average plasma ^{24}Na concentration with time and the accepted value for F. The terms on the right-hand side can be calculated from the graph of the average value of C_π found by Kinsey & Palm. These are plotted together in Fig. 6, where the sum of the right-hand terms is also shown. Taking into consideration the uncertainties of some of the assumptions and the variability of the experimental results, the agreement of the two sides of the equation is very satisfactory. This supports, then, the hypothesis that there is a free exchange of Na over the interface between the vitreous body and the posterior chamber, and also the assumption that the exchange by diffusion between the blood and the posterior chamber is small compared with the unidirectional entry of Na in the secretion of the ciliary body.

Applying this treatment to Kinsey & Palm's data for thiocyanate leads, again, to a level curve for the sum of the right-hand terms of equation (6). This level corresponds to a value of C_s about 70% of C_p, and, according to Davson (1955*b*), this is close to that of a dialysate of thiocyanate in plasma. As Davson suggested, this indicates that thiocyanate enters by the same mechanism as Na. Kinsey & Palm have shown, on the other hand, that when thiocyanate

was repeatedly injected over 19 hr its concentration in the posterior chamber rose to only 61 % of that in the plasma. If this were true it would imply that it entered at a lower concentration than that of a plasma dialysate. It would be desirable to check their result before drawing any conclusions, however, for a steadily rising blood concentration over the hours preceding the collection of the sample could also lead to a relatively low concentration in the posterior chamber.

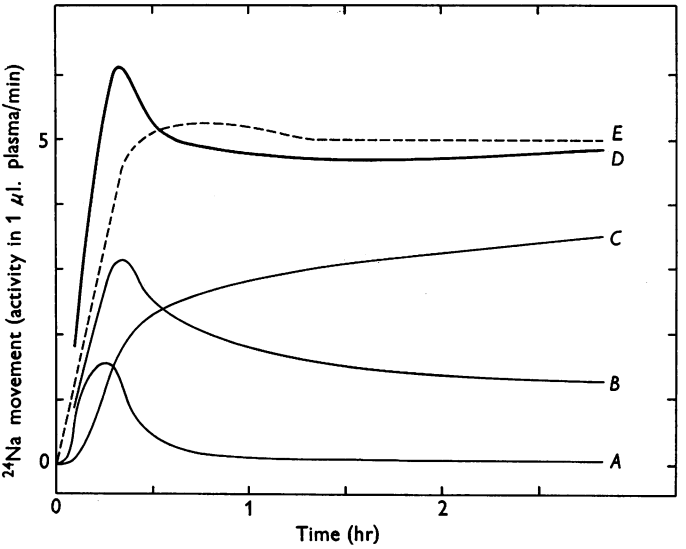

Fig. 6. Balance of ^{24}Na movement in posterior chamber. Ordinate, rate of movement of ^{24}Na in unit/min, assuming plasma concentration 1 unit/µl.; abscissa, time after injection of ^{24}Na intraperitoneally. Curve *A*, rate of accumulation in posterior chamber; *B*, rate of loss to vitreous body; *C*, rate of loss to anterior chamber; *D*, sum of *A*, *B* and *C*; *E*, rate of entry from blood. (Calculated from results of Kinsey & Palm, 1955).

Friedenwald and Becker's treatment

It is clear that by including curves representing the rate of accumulation of the test ion in the anterior chamber, the outflow from it and the diffusion across the iris into it, the methods illustrated in Fig. 6 can also be applied to the anterior aqueous humour. Essentially, this is the same treatment employed by Kinsey & Palm (1955) and Friedenwald & Becker (1955) in analysing the combined dynamics of the posterior and anterior chambers, and would lead to the same results.

In the same paper, the latter authors have put forward also an elegant mathematical theory which makes it possible to formulate the changes in concentration of the test ion in the anterior or posterior chamber separately. They showed that the changes in the posterior chamber can be represented by an expression containing two negative exponential terms. These terms cor-

responded closely to the transfer coefficients of the test substance in the posterior chamber and the 'posterior reservoir'—principally the vitreous body in the case of ions such as Na which penetrate the lens very slowly (Langham & Davson, 1949). A third exponential term corresponding to the transfer coefficient in the anterior chamber, k_o, was needed to formulate the concentration change in the anterior aqueous humour. The experimental data of Kinsey & Palm for both ^{24}Na and thiocyanate were fitted adequately by these expressions.

This treatment was based on the assumptions of a constant concentration of the test ion in the plasma after its injection, and of an exchange between the posterior chamber and the vitreous body which follows an equation of the type of equation (4) for short experimental periods. The authors were fully aware that the latter assumption could only be an approximation to the truth over limited experimental times. Nevertheless, the more correct expression for this exchange, given by equation (5) and shown in curve *B*, Fig. 6, closely follows an exponential course from 50 to 300 min. This is a necessary result of Friedenwald & Becker's treatment, and justifies their use of a constant transfer coefficient between the posterior reservoir and posterior chamber over this period. In the first half hour, however, neither of their assumptions is valid and it must be considered fortuitous that the posterior chamber data are fitted by a double exponential at this time. Accordingly, it does not follow that the more rapid exponential coefficient corresponds to the transfer coefficient of the test ion in the posterior chamber.

Their exponential analysis for the concentration in the anterior chamber rests on a sounder basis and provides a valuable method of determining the true value of k_o from the analysis of aqueous humour samples alone. These authors pointed out a difficulty in its application, however, as a result of the exchange between the aqueous and vitreous humours not declining according to a true exponential. The value of the transfer coefficient for the exchange between the posterior reservoir and the posterior chamber must be derived from the rate at which the concentration in the aqueous humour approaches equilibrium 3–5 hr after the injection. This value is rather different from that which applies from $\frac{1}{2}$–2 hr, when the exponential term containing k_o is operative. This introduced an appreciable error in the estimate of k_o which they were able to overcome only by using the value of the transfer coefficient obtained from the posterior chamber analysis.

The treatment given in this paper suggests a way round the difficulty. Except for substances such as the K ion or ethyl thiourea, which penetrate rapidly into the lens and vitreous body respectively, the exchange between the aqueous and vitreous humours should, by equation (5), be proportional to $\sqrt{D_v}$; the value of the transfer coefficient at any time should then also be proportional to $\sqrt{D_v}$. The appropriate value for ^{24}Na, 0·0044 min^{-1}, is established from the analysis of Friedenwald & Becker and the diffusion constant for this

ion is also known. It should, therefore, be possible to calculate a value of the coefficient for any other substance whose diffusion rate is known, and, by applying Friedenwald & Becker's treatment, obtain a true value of k_o.

SUMMARY

1. ^{24}Na was injected into the vitreous body of rabbits, and its movement into the anterior chamber and its rate of loss from the eye was determined.

2. After a few hours the ratio of the concentration of ^{24}Na in the aqueous to that in the vitreous humour reaches a steady value of 0·20.

3. Nine-tenths of the ^{24}Na injected into the vitreous body is lost from the eye in 24 hr. Of this, 60% leaves by way of the anterior chamber.

4. All exchanges between the aqueous humour and the vitreous body can be explained on the basis of free diffusion across their surface of separation, and of almost free diffusion in the vitreous body itself.

5. The conductance of the intact vitreous body is about 90% of that of the aqueous humour. This suggests that the Na ion can diffuse almost freely within it.

6. The direct loss of ^{24}Na to the blood is not limited by the rate of diffusion in the vitreous body, but by a membrane of low permeability on its surface; this is probably the external limiting membrane of the retina.

7. The influence of the vitreous body on aqueous humour dynamics is calculated. It is concluded that both Na and thiocyanate enter in the secretion of the ciliary body in a concentration close to that of a plasma dialysate, and that there is little diffusional exchange between the blood and the posterior chamber.

8. The validity of Friedenwald and Becker's mathematical treatment of aqueous humour dynamics is examined and an extension of its usefulness suggested.

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