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THE PERMEABILITY TO SODIUM IONS OF THE LIVING RABBIT'S CORNEA

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Interest in the permeability of the cornea centres around the transfer of drugs through it, and the swelling and loss of transparency that occurs in the stroma after death, or when the limiting membranes are damaged. Previous investigations have been reviewed by Cogan & Kinsey (1942*a*) and Grönvall (1937); very little of a quantitative nature has been attempted, and many of the qualitative conclusions are mutally contradictory. As far as osmotically important ions are concerned, Fischer (1928) concluded that the cornea is unidirectionally permeable to sodium chloride in the direction out-in; Cogan & Kinsey (1942*a*), however, in excised intact corneas, could detect no movement of sodium chloride in either direction. Cogan & Kinsey (1942*b*) advanced an explanation of the normal unswollen state of the cornea based on constant removal of water by osmotic attraction into the tears and aqueous humour. This theory has been criticized recently by Davson (1949*a*, *b*), who considered it necessary to postulate an active process to account for the absence of the swelling that should result from the free intercourse of all ions between the blood plasma and corneal interstitial fluid at the corneo-scleral boundary. This could permit the establishment of a Donnan equilibrium similar to that in the erythrocyte, and so the limiting membranes would accordingly exhibit impermeability to one of the osmotically important ions Na^+ or Cl'. The availability of radioactive isotopes has made possible the investigation of this problem under nearly normal conditions, and the work described here is directed towards defining the exchanges of the labelled ion Na^{24} between the cornea and the surrounding media. Rather than remove the limiting layers in order to investigate their individual contributions to the total resistance to transfer, as has been the normal method hitherto, it was thought preferable to derive this from an analysis of the variation with time of the concentration of the ion in the media on either side of these layers.

The experiments described here fall into three series. In the first the solution of Na^{24} was allowed to remain in contact with the corneal epithelium for various definite periods of time, at the end of which the cornea and aqueous humour were removed and their activities measured. In theory it is possible from these results to derive most of the permeability relationships between the cornea and its external media, but a more certain and simple analysis could be achieved if in addition the ion was injected into the blood and the concentration in the plasma, aqueous humour and cornea measured after various periods of time; this accordingly formed the second series of experiments. In the third series of experiments the radioactive salt was introduced directly into the anterior chamber and the activity changes with time in the cornea and aqueous humour followed. In what follows these experiments will be referred to as the E, B and A series respectively, the initial letters of the site of introduction of the Na^{24} in each case.

METHODS

Rabbits anaesthetized with nembutal were used throughout. Animals of medium size (5 lb.) were used when possible, but variations in the dimensions of the eyes were considerable, as reflected by those in the volume of the aqueous humour, for which the extremes were in the ratio of 2:1.

Series E. Bathing of the epithelial surface. Threads were stitched to the outer layers of the skin and knotted, at the inner and outer canthus, and on the upper and lower lids, centrally, and some 0·5 cm. from the margin. The rabbit was placed on its side and these threads attached to the edges of a frame 10 cm. square, held about 15 cm. above the eye. In this way the conjunctiva and nictitating membrane were lifted away from the cornea over its entire surface, and the conjunctival sac was drawn up to form a reservoir capable of holding 1–2 ml. of fluid. The test solution was warmed to body temperature and instilled into this reservoir. The level was maintained from time to time as necessary; only in one case was there any troublesome loss through the lacrimal duct. The fluid was renewed at about 20 min. intervals, the change in Na^{24} concentration having been found to be negligible over this period. After a determined interval, most of the fluid was removed in a syringe, the threads cut, and the eye luxated by an assistant gripping the superior rectus muscle with a pair of forceps. The corneal surface was then flushed with 10 ml. saline from a syringe and dried with the end of a rolled filter-paper. A needle on a syringe was pushed into the anterior chamber at the limbus, the aqueous humour mixed by aspirating a little and forcing it rapidly back, and the chamber evacuated as completely and quickly as possible.

Immediately after the final withdrawal of aqueous humour, the sharpened point of a blade of a small pair of scissors was introduced into the hole left by the needle and the cornea was cut round its outer margin. The excised cornea was then freed from any adhering iris and dried with filter-paper. Attached sclera was then trimmed off, and the cornea weighed and dissolved in a known volume of nitric acid. In some cases the cornea was divided into a central and outer part with a cork borer and the two parts analysed separately. The entire preparation of the cornea from its initial washing till its final drying rarely took more than a minute, and in the experiments of shortest duration was reduced to 30 sec. at the price of incomplete excision of the cornea. The aqueous samples were also weighed, and diluted with saline by weight to a sufficient volume to fill the counter (0·5 ml.), or if the activity was very high, to a greater convenient volume.

To determine the fraction of the Na^{24} which reached the aqueous humour through the general circulation, the aqueous was collected immediately from the other eye. This fraction was found to be of significance only in the experiments of shortest duration, and this control was discontinued in other cases in order to economize in experimental material. When experiments were done upon either eye in turn, however, the experimental period for the second eye was made at least twice as long as the first.

In the earlier experiments, a length of polythene tubing attached to a syringe dipped into the fluid in the conjunctival reservoir, which was stirred continuously by operating the plunger. When it became evident that the epithelial permeability was so low that the passage of ions through it caused a negligible drop in concentration in the contiguous layers of bathing solution, this precaution was neglected. As a result both the magnitude and variability of the permeability fell. It was thought the remaining variability could be reduced by using a blander solution, so finally, a 50:50 mixture of radioactive saline and defibrinated whole blood obtained by heart puncture from the same animal was used, with the further object of satisfying any metabolic needs of the epithelium. The aqueous fluid used previously was generally a mixture of isotonic $Na^{24}HCO_3$ and inert saline in the ratio 1:5 brought to pH 7–8 by addition of solid CO_2; in a few cases 1:10 v/v phosphate-citrate buffer pH 7·0 was added, and in some others, $Na^{24}Cl$ was used in isotonic solution, without making any noticeable difference to the results.

Series B. Injection into the blood. The animal was prepared as in the previous series of experiments, but inert saline introduced into the conjunctival reservoir. A few ml. of the Na^{24} solution were then injected intravenously and blood samples were collected from a cannula in the femoral artery at definite times. At the end of the experimental period for one eye, the aqueous and cornea were collected and arranged as described in series E, and the animal turned on its other side and the experiment continued for a further period of time with the eyelids of the second eye forming a reservoir.

To obtain equilibrium values the experiment was continued for many hours in some cases, and it was convenient to modify the procedure a little. The injection was given intraperitoneally, the blood samples were taken from the marginal ear vein, and in general, the eyelids were allowed to remain closed.

Series A. Injection into the anterior chamber. 0·03–0·1 ml. of the radioactive solution in the form of isotonic saline or saline-sodium bicarbonate in the ratio 1:5 at physiological pH, was initially held in a tuberculin syringe with a short fine needle (20-gauge). An assistant fixed the eye-ball and the point of the needle was pushed through the sclera some 3 mm. from the corneo-scleral junction. It was then pushed carefully upwards and forwards along the posterior surface of the iris until it could be seen in the centre of the pupil. The fluid in the syringe was then mixed with the aqueous humour by sucking into it some 0·1 ml. of the latter, and then expelling an equal volume, this action being repeated some 20 times during the course of ½–1 min. In the later experiments, it was found convenient to have a collar round the plunger of the syringe, ensuring a minimum capacity equal to that of the introduced radioactive fluid. The contents of the anterior chamber were gently stirred by this means throughout the experiment if it were of less than 5 min. duration, the fluid remaining in the syringe being kept for analysis at the conclusion. In longer experiments the syringe was withdrawn after the initial mixing was completed, and paracentesis performed at the end of the experimental time. After the aqueous had been removed, the cornea was excised, dried, weighed and analysed in the usual manner.

The absence of endothelial damage was checked by examination with a slit-lamp microscope during the course of a few of the experiments.

Dimensions of the cornea

To derive the absolute values of the permeability constants, certain average physical dimensions of the cornea and aqueous humour were required. These were in general obtained during the course of the main body of experiments.

(1) *Weight of aqueous humour.* The fluids were weighed by difference in a tared syringe, after as complete an aspiration through a 15-gauge needle as was practicable. Only the larger values from pairs of eyes in which the weight of the two fluids agreed within 15 % were accepted. The mean of sixteen such values was 0·253 g. $\pm$ 0·015, though a systematic error larger than the standard error of the mean must be assumed owing to the impossibility of complete collection with this technique.

(2) *Weight of cornea.* Paired corneas agreeing in weight within 10 % were selected. The larger values from twenty-two such pairs yielded a mean of 0·071 g. ± 0·003.

(3) *Superficial area of cornea.* The length of the arc of the meridional section of the cornea was measured with a length of cotton in a few eyes and compared with the length of the chord measured with callipers. This gave the angle subtended by the arc to be 134°; it is to be noted this figure is not very critical. In nine eyes, the area was calculated from this angular measurement and the length of the arc, giving a mean of 2·13 cm.^{2} ± 0·06.

(4) *Thickness of the stroma.* The mean thickness in six corneas calculated from their area weight and density (1·05), was 0·033 cm. ± 0·0014, which accords well with the mean of the values found on measuring the central thickness with a micrometer screw-gauge, 0·031 cm. Measurements on sections give the total thickness of the outer layers as about 50 μ., and so, calculated by difference, the stroma has a thickness of 0·028 cm.

All the Na^{24} estimations were made with the counter previously described (Maurice, 1948). Sufficient counts were taken normally to give 2–3 % probable error except for the weakest aqueous solutions in series E when it rose to 5–10 %, and the usual corrections for radioactive decay and counter resolving-time were applied. A probable error of 1 % or better was aimed at in the measurements for the aqueous-plasma equilibrium ratio; this was achieved by repeated alternate counting of the two solutions, which had a high activity adjusted in the dilution to be roughly equal for both. The plasma was diluted with saline by weight, a correction being applied for the difference in specific gravity of the liquids.

RESULTS

Theoretical

It is convenient at this stage to formulate in a simple manner some of the diffusion relationships between the relevant tissues and fluids in the eye, so as to establish a notation and to make evident some of the assumptions that have to be justified and numerical constants to be evaluated.

It is assumed that Na ions in the aqueous humour are only capable of exchanging either with those in the general vascular circulation, or those in the cornea across the endothelium, and the cornea can exchange its ions only with the aqueous humour across its endothelium, with the fluid in the conjunctival sac across the epithelium, and with the plasma at the corneo-scleral boundary. These assumptions imply that the only route of entry to the aqueous humour for Na^{24} ions in the conjunctival sac is through the general circulation or across the epithelium and endothelium, i.e. there is no local vascular pathway from the conjunctival sac to the aqueous humour. This proposition, though supported by Leber (1903) on anatomical grounds as far as readily diffusible substances are concerned, has been challenged implicitly through their experimental methods by most later workers, although no evidence has been brought forward to oppose it.

Now let m = total mass of Na ions in any tissue or fluid; m' = total mass of Na^{24} ions in any tissue or fluid; v = volume of tissue or fluid in which Na ions may diffuse freely; c = concentration m/v; M = total mass of tissue or fluid; a = activity of Na^{24} ions per unit weight of tissue or fluid = m'/M; A = superficial area of cornea; and the subscripts p, a, c and e refer to the blood plasma, the aqueous humour, the cornea and the external solution.

We may postulate that exchanges across the endothelial barrier can be represented by the formula:

$$\frac{dm_{ac}}{dt} = A(K_{ac}C_a - K_{ca}C_c), \tag{1}$$

where K_{ac} and K_{ca} are permeability constants for the endothelium in the direction aqueous to stroma, and stroma to aqueous respectively, a convention which (*mutatis mutandis*) is followed throughout. If further we make the assumption that the out-of-balance exchanges across the epithelium and the limbus are negligible compared with the total exchange across the endothelium, so that we have

$$\frac{dm_{ac}}{dt} = 0,$$

then

$$\frac{dm'_{ac}}{dt} = AK_{ac}\left(a_a - \frac{a_c}{r'_{ca}}\right), \tag{2}$$

where

$$r'_{ca} = \underset{t\to\infty}{\text{limit}}\ \frac{a_c}{a_a} = \frac{K_{ac}}{K_{ca}}\frac{v_c}{M_c},$$

as

$$\frac{v_a}{M_a} = 1 \text{ very nearly.}$$

For the epithelium there is a similar equation:

$$\frac{dm'_{ec}}{dt} = \text{An } K_{ec}\left(\frac{a_e}{n} - \frac{a_c}{r'_{ca}}\right), \tag{3}$$

where

$$n = \frac{K_{ce}}{K_{ec}}\frac{K_{ac}}{K_{ca}}.$$

For the blood-aqueous barrier the equivalent equation is

$$\frac{dm'_{ap}}{dt} = k_{ap}M_a(a_a - a_p r'_{ap}), \tag{4}$$

where k_{ap} is the commonly used permeability, or transfer, coefficient from aqueous humour to blood.

The activities of the fluids are related with the masses of radioactive ions entering them by a second series of equations:

$$\frac{dm'_a}{M_a} = da_a, \tag{5}$$

$$\frac{dm'_c}{M_c r'_{ca}} = \frac{da_c}{r'_{ca}}, \tag{6}$$

and so on.

Experiments of series B

After a single intravenous injection of Na^{24} the time course of the activity in the plasma was very similar in all the rabbits studied, and so the relative values for the activity of the aqueous humour and cornea in different animals could

be directly compared. The average values of the plasma activity that were adopted as standard, in terms of that at 30 min. are shown in Table 1.

TABLE 1

Time (min.)	a_p	$\int a_p dt$
0		
		7
2	1·9	
		5·2
5	1·50	
		7·0
10	1·25	
		11·8
20	1·07	
		10·5
30	1·00	
		9·9
40	0·97	
		19·0
60	0·94	
		27·6
90	0·90	
		26·7
120	0·86	

After about 4 hr. the plasma activity remains virtually constant for a period of an hour or two at a time. In a few cases, however, a blood sample taken an hour previously to that taken at the time of paracentesis showed a small rise or fall had occurred.

The value of k_{ap}, the transfer coefficient between aqueous humour and plasma, is important in the interpretation of the results obtained from these experiments and those in series E, and was obtained from the numerical integration of equation (4) in the form:

$$\Delta a_a = k_{ap} \int (a_p r'_{ap} - a_a) dt. \tag{7}$$

In Fig. 1 the points represent values of a_a/a_p found experimentally at various times; the value of a_p being calculated on the basis of a plasma water volume of 93 %.

The value of this ratio when the steady-state has been achieved, r'_{ap}, must be known in order to carry out the integration, and the points corresponding to the longer times in Fig. 1 show the measurements that were made to determine its value. When the blood activity was found to have changed in the hour before the collection of the aqueous, a correction to the ratio was made according to the formula of Palm (1947); this only once exceeded 3 %. It is evident that the value of the ratio continues to increase long after equilibrium would have been expected to have been established on the basis of equation (4). This is very probably a result of the influence of structures, particularly the vitreous humour, which were ignored in the theoretical treatment. Whatever the explanation of this rise, it is evident that the value chosen for the numerical

integration is the steady-state value obtained by extrapolating the curve formed by the points back to the initial 2 hr. period. This suggests the proper value of r'_{ap} to be used is about 0·80, and accordingly the lines shown in the left half of the figure were constructed, corresponding to values of k_{ap} of 0·015 and 0·025 min.$^{-1}$. Most of the experimental points are seen to lie between these lines, and these were accepted as the limiting values of k_{ap} under the particular experimental conditions.

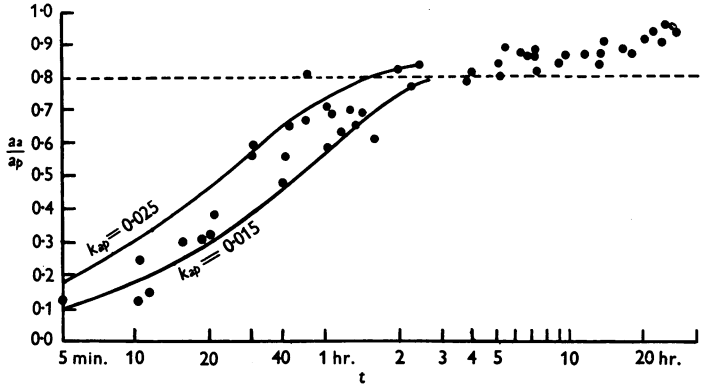


Fig. 1. Variation of the ratio of activity in the aqueous humour to that in the plasma with time, after intravenous injection of Na^{24}. Curves obtained by numerical integration of plasma activity according to equation (7).

The exchange between the cornea and aqueous humour was treated in a way similar to that between aqueous humour and plasma, the numerical integration being carried out according to equation (2) in the form

$$\frac{\Delta a_c}{r'_{ca}} = \frac{AK_{ac}}{r'_{ca}M_c}\int\left(a_a - \frac{a_c}{r'_{ca}}\right)dt, \tag{8}$$

taking the variation in time of the aqueous humour activity to be that corresponding to an intermediate value of $k_{ap}=0·0175$ min.$^{-1}$.

As will be explained in a later section, this equation is only applicable to a region of the cornea remote from its scleral boundary, and the appropriate equation for the cornea as a whole will be considered at the same time.

A knowledge of the value of r'_{ca} is evidently required, and measurements of this ratio were carried out conjointly with some of the determinations of r'_{ap}, the results being shown on the right side of Fig. 2. Here again there is an indication, though not so certain as in the case of r'_{ap}, that the ratio is increasing with time, and it is possible that some of the Na in the cornea is not freely diffusible, and is associated perhaps with the cells which constitute some 15 % of its volume. These results suggest that a value of $r'_{ca}=0·72$ should hold over the first hour or two, and this accords well with the limiting value approached during this early period by the central portions of the cornea shown

as open circles, and the whole corneas shown as filled circles, on the left of the figure. Using this value, and putting $\frac{AK_{ac}}{r'_{ca}Mc} = 0{\cdot}050$ min.$^{-1}$, the values of a_c/a_a obtained by the integration of equation (8) fell on the broken line in Fig. 2, which is seen to fit the experimental points adequately.

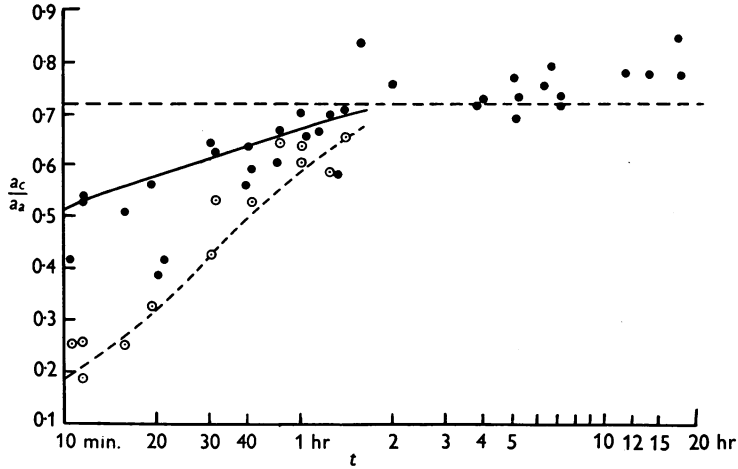


Fig. 2. Variation of the ratio of the activity in the cornea to that in the aqueous humour with time, after intravenous injection of Na^{24}. Filled circles represent entire corneas, open circles central portions of cornea only. Curves derived by numerical integration as described in text.

Inserting the numerical values of the constants a value for the permeability constant of the endothelial barrier, $K_{ac} = 0{\cdot}072$ cm.hr.$^{-1}$ results.

Experiments in series E

Fig. 3 shows the concentration of Na^{24} attained by the corneas in all the experiments of this series, the ratio $\frac{a_c}{r'_{ca}a_e} \times 100$ being plotted against the time the active solution remained in contact with the epithelium, both for convenience on logarithmic scales. The results with the stirred solutions are represented by triangles, those with unstirred aqueous solutions, filled circles, and unstirred 50 % blood solutions, open circles. It is evident that in general the activity of the cornea is considerably raised when the fluid in the conjunctival reservoir is continuously stirred, and also that the nature of the solution is not of great significance as long as it remains still, the range of variation with the 50 % blood solution being perhaps smaller.

The lines drawn on the figure are obtained from an electrical solution, described later, of the equations derived in the theoretical section, and were chosen to include between them nearly all the points obtained with the blood-saline mixture and the majority of those with the unstirred saline. The values

of epithelial permeability included by these lines, a range of 3:1, can be calculated from their initial gradients, for in the first minutes $a_a << a_c << a_e$, and hence equation (3) may be rewritten

$$\frac{da_c}{dt} = \frac{AK_{ec}}{M_c} a_e. \tag{9}$$

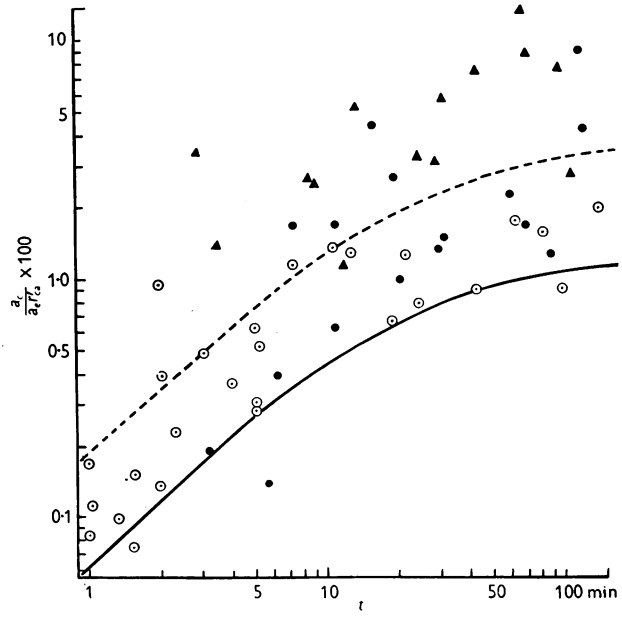


Fig. 3. Ratio of activity of cornea to activity of external solution, as percentage of equilibrium activity, plotted against time solution containing Na^{24} remained in contact with cornea. Open circles, 50 % whole blood solution; filled circles, saline; triangles, continuously stirred saline. Curves obtained from electrical analogue.

Inserting the numerical values of the constants we obtain the corresponding range of values for the permeability constant of the epithelium

$$K_{ec} = 0{\cdot}00080 - 0{\cdot}0024 \text{ cm.hr.}^{-1}.$$

In Fig. 4, the results of the aqueous humour analyses in these experiments are plotted against the time from the instillation of the radioactive fluid into the conjunctival reservoir until the withdrawal of aqueous humour. The ordinate is the ratio of the aqueous activity to that in the corresponding cornea, expressed as a percentage of the equilibrium ratio r'_{ac}. Allowance for entry via the general vascular system was made for those experimental times in which it was found to be greater than 5 %, by measuring the activity in the aqueous humour of the control eye and subtracting it from that of the experimental aqueous humour. Here filled circles represent values of the ratio appropriate to points above the upper line in Fig. 3, and open circles to those below this line.

As the corneal concentration is always low compared with that in the external solution, the rate of inflow of Na^{24} across the epithelium should be virtually independent of time, in any particular eye. Hence this aqueous humour/cornea activity ratio should be independent of its individual epithelial permeability, if the assumption made previously that the trans-corneal route is the only important way of entry into the aqueous humour be true. The fact that the

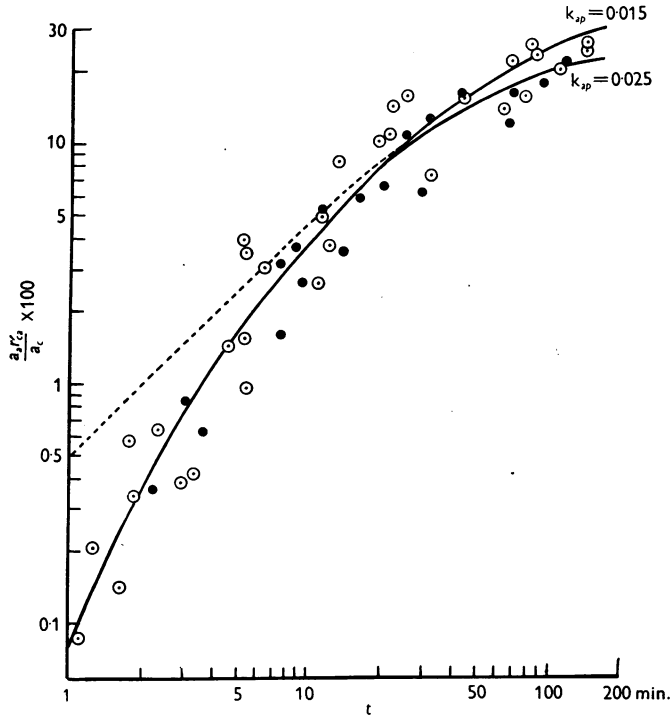


Fig. 4. Ratio of activity of aqueous humour to activity in cornea, as percentage of equilibrium activity, plotted against time solution containing Na^{24} remained in contact with cornea. Open circles low, filled circles high, epithelial permeabilities. Curves obtained from electrical analogue; full lines correspond to two curves in Fig. 1, broken line to shorting R_2 in analogue, i.e. ignoring diffusion time through cornea.

filled and open circles, representing values for the permeability of the epithelium having an average ratio of 5:1 are on the whole concurrent, lends a great deal of support to this assumption, though there is a tendency of uncertain significance for the filled circles to lie below the open for the longer times.

A more direct method of showing that the trans-corneal route of entry is the only significant one has been attempted by many workers previously. This makes use of a mask of some material—vaseline, collodion or plasticine—to protect all but the corneal epithelium from contact with the test fluid. Apart

from the difficulties encountered in making an effective seal with the moist surface of the cornea, the results of such experiments are considered to be equivocal as a demonstration that no significant alternative route exists. As the corneal epithelium is damaged by flushing with a saline solution, contact with a foreign body is likely to damage it even more, and a long series of experiments would be necessary to confirm that the presence of the test substance in the aqueous humour under these conditions is not merely a reflexion of the resultant increased epithelial permeability. On this occasion an attempt was made to obtain further evidence as to the relative unimportance of the extra-corneal pathway, by carrying out some experiments with the corneal surface largely protected by means of a thin collodion cap lightly greased with vaseline. As this could be fixed firmly in position only by means of stitches passed through the conjunctiva, close to the corneo-scleral boundary, it is probable that the normal circulation in this region is disturbed and the conclusions are open to objection on these grounds. These reservations made, however, the results confirm the hypothesis adopted above, for as Table 2 shows, the activity of the centre of the cornea is reduced below the value it would normally attain, while the aqueous humour/cornea activity ratio maintains its proper value.

TABLE 2

Time		$\frac{a_c}{a_e} \times 100$			$\frac{a_a}{a_c} \times 100$	
		Protected				
min.	sec.	Central	Peripheral	Normal range	Protected	Normal
4	50	0·12	0·36	0·18–0·55	6·5	2·2
9	40	0·073	0·46	0·31–0·92	6·5	5·3
16	40	0·19	0·80	0·42–1·26	9·0	8·5
18		0·05	1·4	0·44–1·32	—	—
22	50	0·23	0·46	0·50–1·50	14	15

Exchanges at the corneo-scleral junction

Those corneas in this series of experiments that were divided into two parts showed a rather lower activity in the peripheral compared with the central portion. On the contrary, those corneas in series B which were divided showed a relatively higher activity in the peripheral portion, which rose so as to remain approximately in equilibrium with the aqueous humour, whilst the activity of the central portion lagged behind, as seen from Fig. 2. If the cause is postulated to be lower endothelial resistance at the periphery, a hypothesis which has some experimental support (Ridley, 1930), the permeability of this region would need to be similar to that of the centre to account for the results of the series E, but very large for those of series B. A lower central epithelial resistance could likewise not be consistent with these experimental findings. The possibility of exchange with the blood across the corneo-scleral boundary remains, and a simple mathematical treatment was attempted to see if such an exchange was

capable by itself of explaining the numerical data, and, if so, of estimating its magnitude. Here the subscripts i and o refer to the inner and outer portions of the cornea, the other symbols retaining their previous significance.

We shall assume interchanges between the plasma and the outer portion of the cornea across the limbus may be described by:

$$\frac{da_{po}}{dt}=k_{po}(a_p r'_{cp}-a_o), \tag{10}$$

the inner portion being unaffected by the plasma.

This equation is certainly only a very rough approximation, but the variability of the experimental results does not encourage a more exact formulation.

Writing $$k_{ac}=\frac{AK_{ac}}{r'_{ca}M_c} \quad \text{and} \quad k_{ce}=\frac{AK_{ec}}{M_c}$$

we obtain for the inner portion

$$\frac{da_i}{dt}=k_{ce}a_e+k_{ac}(a_a r'_{ca}-a_i) \text{ from equations (2) and (3),} \tag{11}$$

and for the outer

$$\frac{da_o}{dt}=k_{ce}a_e+k_{po}(a_p r'_{cp}-a_o)+k_{ac}(a_a r'_{ca}-a_o) \text{ from equations (2), (3), (10) and (12).}$$

For the experiments in series E it is nearly true that

$$\frac{da_i}{dt}=\frac{da_o}{dt}, \quad \text{and } a_p \ll a_o$$

and so $$\frac{k_{po}}{k_{ac}}=\frac{a_i-a_o}{a_o}.$$

In this series twenty-three corneas were divided, of which nineteen had a median value of 0·30 for this ratio, with a standard deviation of 0·3, the remaining four values being disallowed as too far beyond these limits. The variability is much higher than that which could be caused by the errors of counting, a deviation of 0·1. Although in excising the corneas speed rather than completeness was the objective, there is no correlation between k_{po}/k_{ac} and M_o as might be expected if there was an error from this cause. A factor which is possibly influencing the results is a regional variability of the epithelial permeability either normally or consequent to the experimental conditions.

In the application to the conditions of the B series of experiments equation (12) was taken with $a_e=0$ and k_{ac} and k_{po}/k_{ac} having the values assigned to them previously, and a numerical integration was carried out in the same way as for equation (8) to give the variation with time of the activity of the outer portion. An average curve for the activity of the entire cornea was then obtained by combining the curve obtained in this way and that already obtained

for the central portion, making use of the median value 0·64 for the ratio M_o/M_c. The full line drawn on Fig. 2 resulted, and it is seen to pass closely enough through the experimental points, ignoring some clearly aberrant values at 20 min. and after 1 hr., to demonstrate the approximate validity of the assumption made in formulating equation (10), that there is a balanced exchange of Na ions between the blood and the cornea at its periphery.

The exchange between the blood-plasma and the cornea as a whole may be symbolized by a constant k_{pc}, and if total exchanges of Na are being considered we obtain

$$\frac{k_{pc}}{k_{po}}=\frac{M_o}{M_c}, \quad \text{hence} \quad k_{pc}=0{\cdot}19\ k_{ac},$$

that is, the interchange across the limbus is 19 % of that across the endothelium.

It is now possible to provide some justification for the assumption made at the beginning of this section, that the out-of-balance exchanges across the other boundaries of the cornea are negligible compared with the total exchange across the endothelium. For the limbus it has just been shown that there is a fair agreement in the calculated movement inwards and outwards across the boundary, and so the out-of-balance movement must be a small fraction of 19 % of the endothelial flux. As far as the epithelium is concerned K_{ac} is 30 to 90 times K_{ec}. Even if K_{ce} does differ from K_{ec} it seems very unlikely that $K_{ec} \sim K_{ce}$ will be significant when compared with K_{uc}.

Diffusion through the corneal stroma

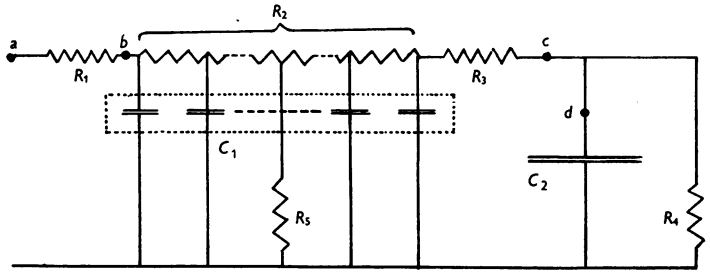
Although it may not be immediately evident from Fig. 4, a delayed entry of Na^{24} into the aqueous humour is shown in these experiments. The broken line is drawn, however, to show how the aqueous humour/cornea ratio would vary if the maximum rate of entry was initiated immediately, and it is clear that there is a deficit of Na^{24} found in the aqueous humour during the first few minutes. The obvious explanation is that it is a result of the time taken for the Na ions to diffuse through the tissues of the cornea, and though the experiments were not sufficiently accurate to enable the delay to be located either in the stroma or the combination of endothelium and Descemet's membrane, it would seem reasonable to suppose the former is the more important on account of its much greater thickness.

From the equations given previously and the diffusion equation for the corneal stroma

$$\frac{da_s}{dt}=\frac{D_s d^2 a_s}{dx_s^2} \tag{13}$$

it is possible to derive mathematically a theoretical relation for the aqueous humour/cornea ratio and time, which on solution would evaluate all the unknown constants including D_s, the diffusion constant of the sodium ion in

the corneal stroma. In order to evade the labour involved it was thought preferable to obtain these results with the help of an analogous electrical circuit.



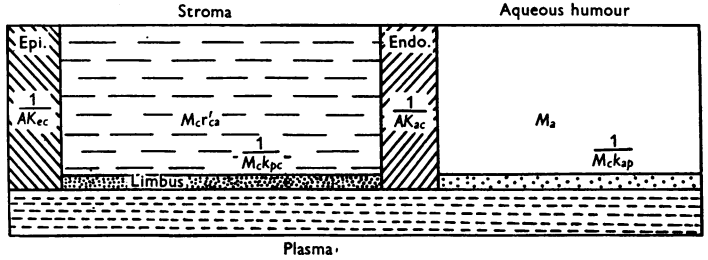


Fig. 5. Schematic cornea and its relationships with surrounding media compared with electrical analogue. Diffusion and electrical resistances are in corresponding places, as also Na^{24} and electrical capacities. Nominal values of electrical components corresponding to experimental series E. Values chosen as described in text. $C_1 = 9\,\mu F.$, $C_2 = 40\,\mu F.$, $R_1 = 100\ M\Omega.$, $R_2 = 8 \times 0{\cdot}39\ M\Omega.$, $R_3 = 12\ M\Omega.$, $R_4 = 4{\cdot}5$ or $7{\cdot}7\ M\Omega.$, $R_5 = 70\ M\Omega.$

Evaluation of the diffusion constants by means of an electrical analogue

It can be seen that equations (2), (3) and (4) are formally identical with Ohm's Law:

$$\frac{dq}{dT} = \frac{1}{R}(V_1 - V_2),$$

and equations (5) and (6) with the law connecting the charge and potential of a condenser $\frac{dq}{c} = dV$.

The permeability relationships of the cornea can therefore be represented by an electrical circuit as shown in Fig. 5, where equivalent parts hold the same relative positions. The capacities C_1 and C_2 correspond to the corneal and aqueous Na volumes, and must have the ratio

$$\frac{C_1}{C_2} = \frac{M_c}{M_a} r'_{ca}.$$

They were in the form of banks of paper condensers of capacities about 9 and 45 μF.; as their nominal values did not hold for static charges their values had to be adjusted to the correct ratio in separate experiments by observing how such charges were shared between them.

R_1 representing the epithelial barrier was in common with all the other resistors of the carbon composition type normally used in radio. Its value was not measured, but was such that the voltage acquired by condenser C_1 remained very small compared with that applied to the end 'a' of the resistor. A value of 100 MΩ. was found convenient for all but the shortest experimental times when 10 MΩ. was substituted.

R_3, the endothelial resistance analogue, was chosen so that in combination with C_1 it had a half period of 1 min. 23 sec. This derived from the value 0·050 min.$^{-1}$ found for AK_{ac} previously, a ratio of the time scales $t/T = 10$ having been decided on.

R_4, equivalent to the blood-aqueous barrier, was chosen similarly so that in combination with C_2 it had a half period of either 4 min. 37 sec. or 2 min. 46 sec. corresponding to the values 0·015 and 0·025 min.$^{-1}$ found as the extremes for k_{ap}.

R_5, the equivalent diffusion resistance between cornea and sclera, was chosen so that $R_3/R_5 = 0{\cdot}17$ roughly, this being the ratio between k_{pc} and k_{ac} for Na^{24} exchanges under the conditions of the experiments in series E.

For the stroma the analogous equation to equation (13) is

$$\frac{dV}{dT} = \frac{dR_2}{dC_1}\frac{d^2V}{dR_2^2}, \tag{14}$$

it being assumed the resistance and capacity R_2 and C_1 are split into an infinite number of identical sections so that

$$\int dR_2 = R_2,$$

and

$$\frac{dR_2}{dC_1} = \frac{R_1}{C_1}.$$

Again $\int dx_s = x_s$, the total thickness of the stroma, and hence

$$\frac{dx_s}{dR_2} = \frac{x_s}{R_2},$$

and so dividing equation (13) by equation (14) and substituting

$$D_s = \frac{x_s^2}{C_1 R_2}\frac{T}{t}$$

$$= \frac{x_s^2}{C_1 R_3}\frac{T}{t}\frac{R_3}{R_2}, \tag{15}$$

where all the quantities are known except R_3/R_2 which is easily determinable. R_2 and C_1 could not be divided indefinitely and nine sections were taken as

being sufficient. The value of R_2 was then chosen to give the best agreement with the points in the lower part of Fig. 4.

The experiments of series E were simulated by discharging both condensers and applying a convenient d.c. potential at 'a' for a given length of time after which the circuit was broken at 'a', 'c' and 'd'. The potential difference across C_1 when the charge was uniformly distributed, and that across C_2 (equivalent to a_c/r'_{ca} and a_a) were then measured with a high impedance valve-millivoltmeter.

Hysteresis effects were at first very troublesome with the paper condensers but were very largely overcome by equilibrating them before the experiment at the voltage they were expected to assume ultimately, and then quickly charging them to their initial voltages and starting the experiment. As a result readings were attained which were consistent, and close to those theoretically calculated where possible; the error was thought not to exceed 5 % at any point.

In this way the curves drawn on Fig. 4 were achieved. The division in the full lines correspond to the two values of R_4 and hence to the extreme values found for k_{ap}. The broken line shows the effect of shorting out the resistor R_2, that is, equivalent to ignoring the time taken for the ions to diffuse across the cornea. As the full lines lie well within the points, the values found previously for k_{ap} and k_{ac} are confirmed, if not very precisely owing to the large scatter of the experimental points. It was at first believed that this scatter could be accounted for by the variations in k_{ap}, but this is evidently not so; other causes, apart from individual variations between animals easily suggest themselves, one of the most potent could be variations of the epithelial permeability during the course of the experiment.

The line was obtained with a value of R_2 which gave a ratio $R_2/R_3 = 0{\cdot}275$ on measurement, and on substituting in equation (15) one arrives at a value for the diffusion constant of the Na ion across the corneal stroma $D_s = 2{\cdot}4 \times 10^{-6}$ cm.^{2} sec.$^{-1}$. The temperature in the conjunctival reservoir was measured with a thermocouple during the course of an experiment and found to be within a degree of that in the rectum. At body temperature the diffusion constant of Na in water is $2{\cdot}0 \times 10^{-5}$ cm.^{2} sec.$^{-1}$ and therefore the resistance to diffusion across the stroma is about 8 times the value in free solution. The difficulties encountered by the experiments of very short duration are such, however, that this figure is only considered to represent the order of magnitude of the ratio.

One assumption implicit in the use of the electrical model chosen, that the resistance to diffusion in the aqueous humour and the solutions in the conjunctival reservoir causes only a negligible concentration gradient in these fluids, must now be considered. Translated into the more easily comprehensible electrical terms, the diffusion properties of the Na ion in free solution may be represented by a network of 35 KΩ. resistances and 1 μF. capacities in series. As the epithelial resistance analogue is 400–1200 MΩ., it is clear the current it will pass will not cause the neighbouring condensers to become appreciably discharged within practical time limits. It follows that stirring of the solution in the reservoir is unnecessary, for the activity loss of contiguous layers of solution by diffusion across the epithelium is negligible as assumed earlier. The influence of the diffusion resistance in the aqueous is more important but cannot be exactly analysed, as it will be diminished through mixing of the aqueous by its normal flow, the intra-ocular pulse, and any thermal circulation. The aqueous humour analogue will on the average be represented by forty-

five such resistance capacity sections, and therefore a total resistance of 1·6 MΩ. will be introduced between R_3 and R_4, and may very roughly be treated as an addition of 0·80 MΩ. to each of them. The influence of a resistance change 4 times this figure in R_4 is seen in Fig. 4 as the lines corresponding to $k_{ap}=0{\cdot}015$ and 0·025 min.$^{-1}$ and as the additions to R_3 and R_4 will, to some extent, be compensatory it is unlikely that the effects of diffusion in the aqueous humour will be significant under these conditions.

Experiments of series A

In Fig. 6 a filled circle represents the activity of a sample of aqueous humour obtained at the end of an experiment compared with that found in the sample obtained from the same eye, when the initial mixing was completed. It is seen that the points fall on a curve with a half period of about 14 min. As the actual rate of loss of Na^{24} in each eye is different, to make the values of the corneal activity more nearly comparable the following procedure was adopted.

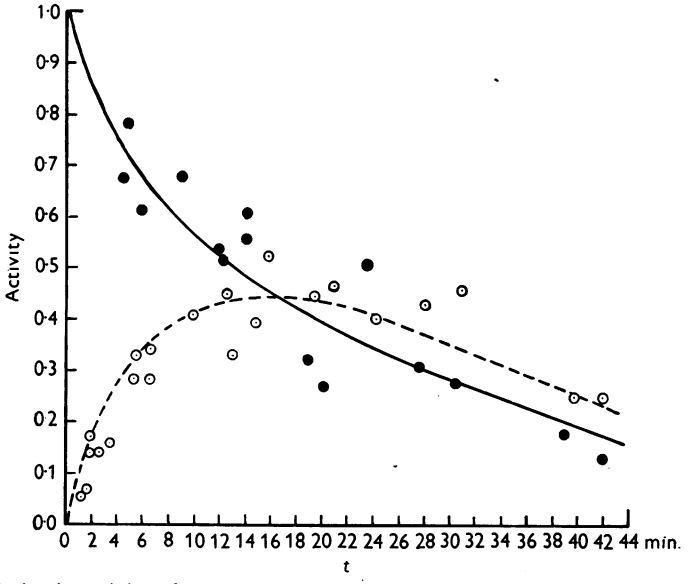


Fig. 6. Variation in activity of cornea, open circles, and aqueous humour, filled circles, with time, after injections of Na^{24} into anterior chamber. Curves from electrical analogue.

An initial value of the aqueous humour activity was calculated from the sample collected at the end of the experiment on the basis that the rate of loss from the anterior chamber had a half period of 14 min. The mean of the value obtained in this way, and of the initial aqueous humour activity as measured, was taken as a_a, and $\frac{a_c}{a_a r'_{ca}}$ is plotted against the time from the injection until the cornea was dried after excision as the unfilled circles in Fig. 6. Where, in the experiments of short duration, only one aqueous sample was taken, a correction was made for the loss of activity during the few minutes between the injection and collection in adopting the value of a_a.

These experiments were likewise simulated on the electrical model. With the circuit open at '*d*', C_2 was charged to a known level and C_1 discharged. The circuit was then completed at '*d*', and after a determined time disconnected at '*c*' and '*d*', and the potentials across C_1 and C_2 measured. In this way the curves drawn on Fig. 6 were obtained; it was found necessary to reduce both R_3 and R_4 from the values that were used previously. The reduction in R_3 was 2·3 times, and half life of the C_2R_4 combination was equivalent to a value of $k_{ap} = 0{\cdot}050$ min.$^{-1}$.

DISCUSSION

These investigations were directed primarily towards adding to our knowledge of the mechanism by which the normal thickness of the cornea is maintained. This point will be the chief concern of what follows here, though some other points that arose will be discussed later.

After the death of an animal or when the corneal epithelium or endothelium is damaged, the corneal stroma increases in thickness. It is natural to assume in the first place that the properties of the corneal stroma *in vivo* are the same as those it shows *in vitro*, and that its tendency to swell is countered by the properties of the limiting membranes in regard to the transfer of the constituents of the body fluids across them. This viewpoint was adopted by Cogan & Kinsey who have carried out the most extensive investigation of the problem. They found (Kinsey & Cogan, 1942*a*) that in the cat, the excised stroma swells in aqueous solutions of many salts, notably NaCl, and over a large range of tonicity and pH on either side of the normal. The cornea exhibited a swelling pressure of about 80 g.cm.$^{-2}$ in normal saline, though this apparently fell after 20 hr., a result which might be due to irreversible changes taking place. There is no reason to suppose that these properties are essentially different in the rabbit.

The stroma will be maintained in its normal state of hydration if it is separated by a semi-permeable membrane from a solution having a colloid osmotic pressure equal to the swelling pressure; this follows at once from energy considerations if an infinitesimal volume of water is moved from one side of the membrane to the other. Hence, where osmotic relationships are concerned, the stroma may be considered as equivalent to such a solution, regardless of the actual state of the water it contains. The concentration of colloid corresponding to the value adopted for the swelling pressure is about 4 m.osmol. l.$^{-1}$.

As far as the properties of the limiting membranes are concerned, most of the literature describes experiments carried out under very abnormal conditions, or with drugs or other substances not normally present in the body, and much of this is only roughly quantitative. The only results that could be found, which were under conditions comparable with those reported here and with a normal constituent of the plasma, were those of Grönvall (1937) on the citrate ion.

Here, the increase in concentration in the aqueous humour only, after instilling fluid into the conjunctival sac, was measured, but the findings run so closely parallel to those for Na^{24} that they are quoted *in extenso* in Table 3. The increase

TABLE 3

Time (min.)	Citrate $\frac{c_a}{c_e} \times 10{,}000$	Na^{24} $\frac{a_a}{a_e} \times 10{,}000$
7·5	4	1·0–3·0
15	3	3·0–9·0
20	20	4·8–15
30	25	7–21
60	60	16–48

in citrate concentration of the aqueous humour over that in the control eye, relative to the concentration in the external solution, is placed alongside the comparable figure for Na^{24}, the values for a_c/a_e corresponding to the limiting curves in Fig. 3 being taken in conjunction with the value for a_a/a_c given by the curve in Fig. 4. It is seen that the permeability of the cornea as a whole to the citrate ion is in general a little above the higher of the values obtained for Na. If it were indeed true that the ratio of the figures for Na and citrate remained constant with time, it would follow that the epithelial permeability was higher for the citrate ion and that the endothelial permeabilities were equal; though this cannot be asserted on the basis of so few figures, there is a fair indication that the permeability of the epithelium and endothelium has at least the same order of magnitude for both substances. The fact that ions so dissimilar as Na and citrate behave in this way suggests very strongly that at least the greater part of the transfer across these membranes is through extracellular spaces, and it may well be that all ions will behave similarly.

Cogan & Kinsey (1942*b*) advanced a theory to account for the normal unswollen state of the cornea based on the semi-permeability of the limiting membranes they had demonstrated, and what other workers had shown to be a slight hypertonicity of the aqueous humour and tears with respect to the plasma. They supposed that water entered the cornea at its scleral boundary from the plasma, but that the stroma would be prevented from swelling as this water would be withdrawn by osmosis across the epithelium and endothelium.

The inadequacy of this theory as it stands was pointed out by Davson (1949*a*, *b*) as not only water but plasma solutes would pass into the stroma at the corneal boundary. There is no reason to suppose the capillaries here are different from those in any other part of the body, in that they are impermeable to any plasma constituent which is present to the extent of 4 m.osmol. $l.^{-1}$. As water only is removed across the limiting membranes there should be a tendency to swell at the expense of salts and water entering at the periphery. As Davson (1949*a*) suggested, the high salt concentration that is produced in

the stroma might stabilize the mechanism, and in Fig. 7*a* this possibility is developed a stage further.

In this figure the three divisions represent the limbal capillaries, the stroma and the aqueous humour, and the figures against the symbols their osmotic activities, which are assumed to be close to their concentrations in value. For

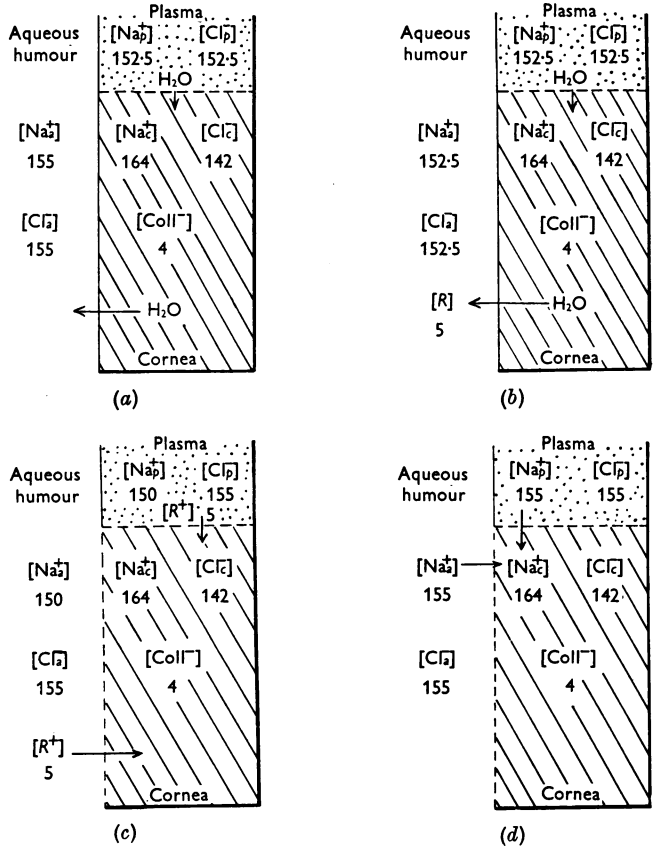


Fig. 7. Osmotic activities of constituents of aqueous humour, corneal interstitial fluid and plasma (as dialysate) according to various hypotheses. (*a*) Cogan & Kinsey's theory, aqueous humour hypertonic to plasma in NaCl. (*b*) Same theory, aqueous humour hypertonic in neutral substance *R*. (*c*) Active transfer of cation *R* out of cornea. (*d*) active transfer of Na out of cornea. Arrows indicate directions of passive transfer.

simplicity all the cations are shown as Na, and all the diffusible anions as Cl. The symbol Coll refers to the collagen fibres of the cornea which are supposed to be responsible for its swelling pressure. The plasma concentrations are represented by those in its dialysate with which it is in osmotic equilibrium, the concentration of the aqueous is taken as 310 m.mol. l.$^{-1}$, and the ratio of the concentrations of Na and Cl ions in the stromal interstitial fluid roughly those

found by Davson. The contribution of the tears to the water equilibrium is ignored; their tonicity is a variable quantity and if the permeability of the epithelium and endothelium for water at all resembles that for Na their influence will be small. The tonicity of the aqueous humour, on the other hand, is considered all important in determining that of the stroma which will accordingly have an osmotic concentration of 310 m.osmol. $l.^{-1}$. Water can scarcely fail to equilibrate between two fluids separated over a large area by a relatively thin membrane, and the tonicity of the blood in the peripheral vessels can be expected to have only a local influence. The suggestion advanced tentatively by Davson (1949*a*) that the stromal fluid is hypertonic to the aqueous, is probably based on a wrong estimate of the volume of intercellular water, a change from 15 to 12 % would rectify the balance; furthermore, there is a possibility that part of the Na in the cornea is not in a freely diffusible state.

Now in Fig. 7*a*, equilibrium will be established between aqueous and stroma with the concentrations shown, if the endothelium is impermeable to NaCl. For this, impermeability to Cl alone is sufficient. Considering now exchanges between the blood and cornea it may be recalled that it is the basis of the Donnan equilibrium that no energy is required to move an infinitesimal quantity of NaCl from one point to another as long as the product [Na] × [Cl] is the same in both places. If we thus choose $[Na_p] \times [Cl_p] = [Na_c] \times [Cl_c]$ as has been done in the figure, there would be no gain in energy if NaCl entered the cornea from the limbus, and hence no swelling would be expected. It is seen that the aqueous humour would need to be 2·5 m.mol. $l.^{-1}$ hypertonic to the plasma in NaCl, a figure comparable to the 3·4 m.mol. $l.^{-1}$ found for Cl and 3·8 m.mol. $l.^{-1}$ for Na found to be the excess in the aqueous humour over the plasma in the cat (Davson, Duke-Elder & Maurice, 1949). There would be a constant movement of water from limbus to aqueous humour and it is possible, but not evident in which way, that the NaCl equilibrium would be affected by this.

An equilibrium such as shown in Fig. 7*b*, is conceivable in which the endothelium is permeable to NaCl but impermeable to some other substance *R*, which is in excess in the aqueous humour. *R* is shown as a neutral substance and the Na and Cl in plasma and aqueous humour will be in equilibrium. If these two fluids were dialysed against one another, *R* would pass into the plasma, and NaCl would pass from the plasma into the aqueous humour. Davson *et al.* (1949) showed that on dialysis the movement of Na and Cl was in the other direction, hence this hypothesis does not appear to be tenable. If *R* was an ion the movement of the ion of the same charge, Na or Cl, in the same direction would be even greater. The theory of Kinsey & Cogan necessitates, therefore, that the endothelial barrier be impermeable to Cl ions and this would appear unlikely in view of the findings in respect to Na and citrate.

Some independent evidence that Cl does, in fact, move across the endothelial barrier at roughly the same rate as Na can be derived from the observed rate of

swelling of the cornea after death. As soon as active transport ceases, the maximum rate of swelling should be determined by the rate at which NaCl can cross this barrier, and if this is the same as that which is calculated later for Na, the maximum rate of swelling at body temperature would be 3 % of the weight of the cornea in an hour. This is of the same order of magnitude as the rate of swelling in enucleated cat's eyes at room temperature (Kinsey & Cogan, 1942*b*) which was found to be 20 % in 20 hr.

If a secretory action in the endothelium or epithelium is postulated, a wide variety of hypotheses are tenable, since the transfer out of the cornea of water, Na or Cl, or any substance present to the extent of 4 m.mol. $l.^{-1}$, could be sufficient to maintain its normal thickness.

The excretion of water directly from the stroma would be the least efficient energetically of all methods of keeping it in its relatively dehydrated state, as the water could certainly diffuse back with great readiness (Cogan & Kinsey, 1942*a*; von Bahr, 1948). In the absence of any information as to the diffusion constant of water across the endothelium *in vivo*, this possibility cannot be pursued any further.

Among the less concentrated ions HCO_3, K, Ca and perhaps Mg and H_2PO_4, are all possible candidates for active transfer out of the cornea. A concentration deficiency of 5 m.mol. $l.^{-1}$ would need to be created in the stromal interstitial fluid and Fig. 7*c* illustrates a possible arrangement with R as a transferred cation, Na as before representing all the other cations including any quantity of R above 5 m.mol. $l.^{-1}$. It is seen that the relation

$$[Na_a] \times [Cl_a] = [Na_c] \times [Cl_c]$$

is satisfied, as also the osmotic equality across the endothelium.

If the actively transported ion were Na or Cl, the state of affairs would be something similar to that shown in Fig. 7*d*. Less energy would be required to secrete these ions across the barrier than those present in greater dilution, as the concentration ratio through which they must be moved is less. It is worthy of note that frog's skin has a permeability under the conditions of 0·9 %NaCl on either side closely approximating to that found here for the corneal epithelium (Katzin, 1940). As both are epithelial systems and Na ions are known to be actively transported in the case of frog's skin (Ussing, 1949), in the absence of any other evidence preference should be given to the assumption that the same mechanism is at work in the cornea.

Assuming then that Na is the actively transported ion, it is possible to calculate the energy requirement to maintain the equilibrium of the cornea. A comparison of Fig. 7*d* with Fig. 7*b* or *c* will show in this case that there is an excess of NaCl in the aqueous humour of 2·5 m.mol. $l.^{-1}$ over that which is required for equilibrium with the cornea, and an equal excess in the plasma if we neglect the possibility of a hypertonic aqueous humour. Na will move down

this concentration gradient across the endothelium and corneo-scleral boundary at a rate determined by the permeability of these barriers and must be transferred out at the same rate. The rate of removal for the whole cornea will then be

$$\frac{2{\cdot}5}{1000\times 1000}(k_{ac}+k_{pc})M_c r'_{ca} \text{ mol.min.}^{-1} = 7{\cdot}5\times 10^{-9} \text{ mol.min.}^{-1},$$

and the energy requirement imposed by the concentration step at the endothelium

$$7{\cdot}5\times 10^{-9}\, RT \log_e \frac{155}{152{\cdot}5} \text{ cal.min.}^{-1} = 7{\cdot}5\times 10^{-8} \text{ cal.min.}^{-1}.$$

The Cl ions, not being actively transported, must be in equilibrium between cornea and aqueous humour. As there is a relative deficiency of 2·5 m.mol. $l.^{-1}$ in the fluid of the former, it must of necessity be at a corresponding negative potential in respect to the aqueous humour, over and above the negative potential caused by the indiffusible anions of the corneal stroma. As a result, both the net flux of Na ions across the endothelium and the potential step up which they must be transported back again, are roughly doubled, and so the total energy used in the Na transfer will be $3{\cdot}0\times 10^{-7}$ cal.$min.^{-1}$.

The oxygen consumption equivalent of this energy requirement is only $6{\cdot}0\times 10^{-11}$ l. $min.^{-1}$. The overall oxygen usage for the isolated cornea can be derived from the results of Robbie, Leinfelder & Duane (1947) and turns out to be 1×10^{-7} l. $min.^{-1}$, thus relatively only a very small fraction of the available energy need be applied to the maintenance of the normal state of corneal hydration, in comparison with that applied by workers in this field to the same problem.

The calculated active transfer of Na is of the same order as the inward flux of Na across the epithelium, and 60 times less than the flux across the endothelium. If this transfer took place outwards across the epithelium, this membrane and the cornea as a whole would display a high degree of unidirectional permeability whilst if it took place across the endothelium, the unidirectional permeability of the cornea as a whole would be slight. Unfortunately owing to the extreme sensitivity of the epithelium to damage, it was not found possible to devise a satisfactory technique to measure its permeability in the in-out direction with any certainty. Fischer (1928) came to the conclusion that there was an irreciprocal movement of NaCl across the cornea in the direction out-in, the opposite of what would be expected from the mechanism suggested here. His experiments involved, however, the placing of a hypertonic salt solution on the cornea and showing a resultant increase in the Cl content of the aqueous humour; apart from the reservations that must be made on account of the damage to the epithelium that such treatment could cause, this phenomenon can be explained more convincingly on the basis of the osmotic withdrawal of water from the anterior chamber.

It is interesting that the epithelium, endothelium and the blood-aqueous barrier all have their permeability raised, presumably as a result of damage, by

treatment little more violent than stirring the solution in contact with them. What is more striking, however, is that the permeability of neither the endothelium nor the blood-aqueous barrier rose without limit under these conditions, indeed the results of the experiments in series A were the most consistent found. Again, the points in Fig. 3, corresponding to a raised epithelial permeability, could be interpreted in the same sense, there being a tendency for the triangles to group themselves round a line separated from the main body of points, but conforming to their contour. The ratio of the artificially raised permeability to the average normal value is of the same order in each case, being 2·0–3·3 times for the blood-aqueous barrier, 2·3 times for the endothelium, and about 5 times in the case of the epithelium. These phenomena point very definitely towards a double mechanism being involved in the exchange of Na across these boundaries, a more delicate one which reduces the value of the permeability below that imposed by an underlying more rugged invariable barrier.

The diffusion resistance of the corneal stroma may be compared with that found for Na across frog's muscle by Harris & Burn (1949). Though the resistance to diffusion is 8 times the value for the ion in free solution in the former substance compared to 4 times in the latter, the corresponding volumes occupied by interstitial fluid are 85 and 13 % of the total volume. This high resistance to diffusion offered by the stroma is perhaps associated with its good transparency, which could depend on a tightly packed arrangement of its fibres.

SUMMARY

1. In the rabbit the penetration into the cornea and aqueous humour of solutions of Na^{24} brought into contact with the corneal epithelium was studied, and also the distribution of Na^{24} between the plasma, aqueous humour and cornea, after intravenous injection.

2. Simple relationships formulated for movements of Na between the media permitted them to be represented by an electrical analogue, which was of value in evaluating the experimental results.

3. The permeability constant for the corneal epithelium in the direction in-out was found to be 0·00080–0·0024 cm. $hr.^{-1}$, and for the endothelial barrier from aqueous to cornea 0·072 $cm.hr.^{-1}$.

4. There is a balanced exchange of Na with the blood stream at the periphery of the cornea, amounting to about one-fifth of the exchange across the endothelium.

5. The resistance to diffusion of Na across the corneal stroma appears to be 8 times its value in free solution.

6. The permeability of the blood-aqueous humour and corneal endothelium barriers rises to a fixed value 2–3 times normal if the Na^{24} is injected directly into the anterior chamber and mixed with the aqueous humour. The permeability of the epithelium rises about 5 times if the solution in contact with it is stirred.

7. The stability of the corneal thickness is discussed in terms of a mechanism involving a hypertonic aqueous humour as proposed by Cogan & Kinsey, and of one requiring active transfer of substances across the limiting membranes of the cornea.

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APPENDIX

Since the preparation of this paper the author has become aware of the work of Potts & Johnson (1950), who have carried out similar experiments with the ions P^{32}, Na^{24}, I^{131} and Cs^{134}. Insufficient data is given by these authors to allow an analysis on the lines presented here except in the case of the penetration of I^{131} into the cornea after intravenous injection. These results lead to a value of $r'_{ca} = 1{\cdot}6$ and a value of K_{ac} some ten times that found above for Na^{24}. At a rough estimate an even higher figure for K_{ac} might be expected for Cs^{134}, but for P^{32} it would appear that K_{ac} is lower than for Na^{24}.

These authors also show that the four ions studied all enter the cornea most rapidly at the periphery after intravenous injection, and are led to the same conclusion as is reached here, that there is an exchange with the plasma in this region. The results quoted, however, do not justify their statement that this is the predominant supply route for the cornea.

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