J. Membrane Biol. 65, 197–204 (1982)

The Journal of
Membrane Biology

Analysis of Presteady-State Na^+ Fluxes across the Rabbit Corneal Endothelium

Jong J. Lim and Hans H. Ussing

Department of Ophthalmology, College of Physicians and Surgeons, Columbia University, New York, New York 10032, and Institute of Biological Chemistry A, University of Copenhagen, 2100 Copenhagen 0, Denmark

Summary. Instead of the conventional steady-state fluxes, the presteady-state fluxes of ^{22}Na across the rabbit corneal endothelium were measured. In contrast to reports that there is no net Na^+ movement across the corneal endothelium, we find a net transport of Na^+ across this tissue. The direction of net Na^+ flux is from the stromal to the aqueous side and the magnitude is 2.3 ± 0.4 $\mu eq/cm^2 \cdot hr$ ($n=11$, SEM). Net Na^+ transport is inhibited in the presence of ouabain (10^{-4} M). Acetazolamide (10^{-4} M) has only a slight inhibitory effect on the rate of Na^+ transport but decreases the transendothelial potential difference by about 30%. The passive component of the Na^+ transport has been estimated by analyzing the presteady-state influx and efflux curves and found to occur 10% via cellular and 90% via paracellular routes. The analysis for the separation of the pathways has been based on a recently proposed theory which holds that the flux ratio, regardless of its driving forces, is independent of time.

Key words presteady-state fluxes · cornea · endothelium · cellular and paracellular pathways · net flux

Introduction

The corneal endothelium is known to play a key role in maintaining corneal transparency through the pump-leak mechanism(s) presumed to be located mainly in this cell layer. In order to understand the inner workings of the mechanism, three parameters related to corneal transparency have been intensively investigated in our and other laboratories, *viz.*: (i) trans- and intracellular electrical measurements, (ii) the rate of trans-endothelial fluid transport, and (iii) trans-endothelial steady state isotopic fluxes. The trans-endothelial electrical measurements have shown that the preparation generates a very small potential difference (about 1 mV, aqueous negative) and that the resistance values range from 20 Ω to 70 $\Omega \cdot cm^2$ (Fischbarg, 1972; Barfort & Maurice, 1974; Fischbarg & Lim, 1974; Hodson, 1974; Lim & Fischbarg, 1981). It was also found that this small potential difference is dependent on the presence of Na^+, K^+, HCO_3^-, and H^+ in the bathing solution (Fischbarg & Lim, 1974). Although there are numerous reports on the transendothelial electrical properties as mentioned above, there are only a few reports dealing with intracellular electrical measurements. According to recent successful studies (Wiederholt & Koch, 1978; Lim & Fischbarg, 1979; Lim, 1980), the intracellular potential is high (about 80 mV, inside negative) and is comparable to values reported for similar leaky preparations (Frömter, 1979).

A second area of investigation has been centered on the rate of fluid transport across endothelial preparations. The magnitude of the fluid transport was reported to be about 4.5 $\mu l/hr \cdot cm^2$ when the preparation is bathed with a solution containing adenosine and oxydized glutathione (Fischbarg, Lim & Bourguet, 1977). When adenosine and oxydized glutathione (GSSG) are absent, the rate of fluid transport is 3.7 $\mu l/hr \cdot cm^2$ (Fischbarg et al., 1977). The direction of the fluid transport is from the stromal to the aqueous side. It was also reported that the fluid transport depends on the presence of Na^+, K^+, and HCO_3^- in the solution (Fischbarg & Lim, 1974; Hodson, 1974).

For the third area of the investigation, there are also only a few successful studies about the corneal endothelium (Hodson & Miller, 1976; Hull, Green, Boyd & Wynn, 1977). Hodson and Miller (1976) reported that there is a net movement of HCO_3^- from the stroma to the aqueous side. According to these authors, the magnitude of HCO_3^- flux is in agreement with one estimated from the short-circuit current (about 27 $\mu A/cm^2$ or 1 $\mu eq/hr \cdot cm^2$). A net HCO_3^- movement of about 2.51 $\mu eq/hr \cdot cm^2$ (for the chamber area) was reported by Hull et al. (1977) and confirmed by Kelly and Green (1980). The differences in the experimental conditions between these two groups may be responsible for the disparate values. Hodson and Miller used single corneas and measured the fluxes under a short-circuit condition, while Hull et al. employed paired corneas and measured fluxes under an open-circuit condition.

0022-2631/82/0065-0197 $01.60
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As for the transport of Na^+, K^+ and Cl^-, both groups have reported that these ionic movements are passive with no net transport. In this communication, however, we report that a net sodium transport across the rabbit corneal endothelium has been detected. The net Na^+ transport is abolished in the presence of ouabain (10^{-4} M) and, within the limits of the present experimental error, appears to decrease in the presence of acetazolomide (10^{-4} M). This finding indicates that Na^+ and HCO_3^- transport are coupled. A preliminary account of the present study has been reported elsewhere (Lim, 1981*a*, *b*).

Materials and Methods

Albino rabbits weighing 2.5 to 3 kg were used in these experiments. The rabbits were killed by injecting 5 ml of Na pentobarbitol through the marginal ear vein. The epithelium was scraped off and the mounting procedure for the preparation onto a chamber has been described in detail elsewhere (Fischbarg & Lim, 1974; Lim & Fischbarg, 1981).

Solution

The solution used for the control experiments was made of (in mM): NaCl, 110.4; $NaHCO_3$, 39.2; $KHCO_3$, 3.8; KH_2PO_4, 1.0; $MgSO_4 \cdot 7\ H_2O$, 0.78; $CaCl_2 \cdot 2H_2O$, 1.7; and glucose, 6.9. The pH of the solution was kept at 7.4 by bubbling it with a gas mixture of 95% O_2 and 5% CO_2 throughout the experiments.

Chamber

A modified Ussing and Zerahn (1951) type experimental chamber was used as shown in Fig. 1. An attempt was made in the design to increase the circulation of solution. The mixing process was checked with a dye, and it was found that the solution was well mixed in about 2 sec.

Temperature Control

The temperature of the solution in the chamber was maintained at 37 °C by circulation of warm water through the jackets of the funnels as shown in Fig. 1. The warm solution in the funnels was circulated by bubbling with the gas mixture mentioned above.

Electrical Measurements

The transendothelial potential difference was measured by an electrode system composed of polyethylene tubing filled with saline agar and calomel electrode. The polyethylene tubing had an OD and ID of 1.7 and 1.2 mm, respectively. One end of the tube made contact with the solution and the other end with the calomel electrode. The potential difference was measured by an electrometer (Keithley, model 610C).

Flux Measurements

Paired corneas from the same rabbit were used in this set of experiments. In order to maintain the de-epithelialized cornea in its natural concave shape, a pressure difference of about 5 cm of water column was imposed on the aqueous side. Thus, the volume of solution on the aqueous side was 15 ml while that on the stromal side was 11 ml. Immediately after the preparation was mounted in the chamber, the trans-endothelial potential difference (PD) was monitored for about 1 hr. A preparation which generated the PD lower than 400 μV (less than 5% of all corneas) was disregarded, under the assumption that irreversible damage had occurred. Only preparations having PDs stable and greater than 400 μV were used for the flux measurements. The isotope used was ^{22}Na obtained from New England Nuclear, Boston, Mass. (sp. act.: 248 mCi/g of Na); 50 μCi contained in 50 μl aqueous solution was added to the proper side (the stromal side for the efflux studies and the aqueous side for the influx studies). Following the addition of the isotope, 1 ml of solution was withdrawn from the opposite side of the chamber at 2-min intervals. The amount of solution withdrawn was replaced by an identical amount of fresh solution so that the total volume of the solution on either side remained constant throughout the experiment. A pipetman (model p1000, Rainin Co.), with an accuracy of better than 0.5% was used for withdrawal of the solution. Each experiment for ^{22}Na tracer measurement lasted only 32 min. The samples were counted by a gamma counter (Packard, Model 3001). Differences in the volumes of solution bathing the two surfaces were taken into account for the flux calculations.

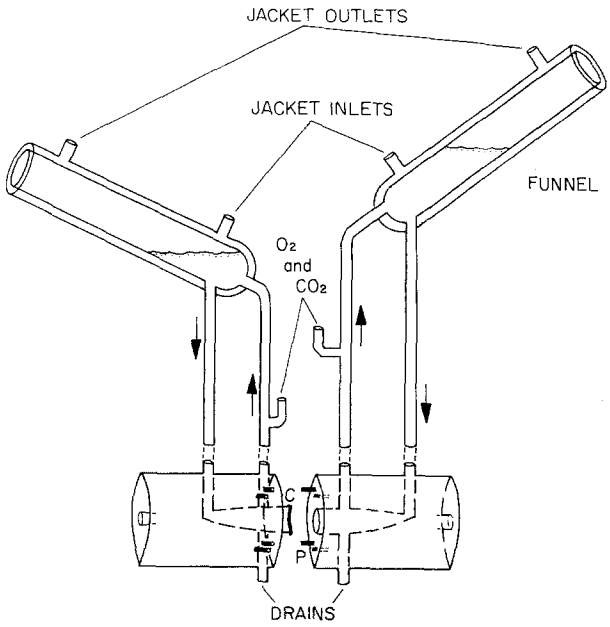

Fig. 1. A modified Ussing-type chamber used for the flux measurements. The temperature of the preparation was maintained at 37 °C by circulatory warm water through the jacketed funnels which contained the perfusing solutions circulated in turn by a gas mixture of 95% O_2 and 5% CO_2. *C* and *P* represent, respectively, the corneal endothelium and the guide pins. The arrows indicate the direction of fluid circulation

Ouabain Experiments

After mounting a cornea in the chamber, the preparation was equilibrated for about 1 hr as described in the flux measurements. When the PD was found to be in the expected range and stable during the monitoring period, 400 μl of solution containing 3.85 mM ouabain was added to both sides of the chamber. The potential difference was monitored until the PD was abolished; this period was usually about 5 min.

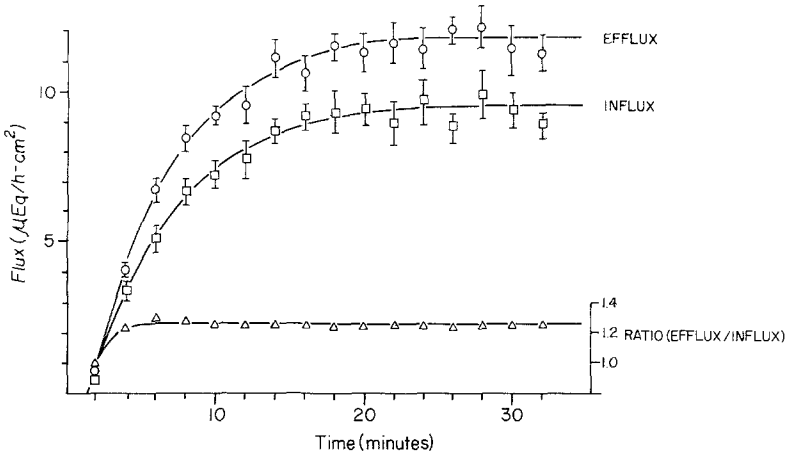

Fig. 2. Efflux, flux from stroma to aqueous (open circles), influx, flux from aqueous to stroma (open squares), and the ratio, efflux/influx (open triangles), for sodium ion as a function of time. The vertical bars represent the SEM ($n=11$)

Acetazolamide Experiments.

The procedure followed the normal protocol described for the flux measurements after which the concentration of acetazolamide in the bathing solutions on both sides of the chamber was brought to 10^{-4} M by the addition of suitable volumes of a 15-mM stock solution. Measurements of the fluxes were continued for an additional 20 min.

Flux Analysis

The equations used in this analysis are those for an epithelium and are detailed elsewhere (Ussing, Eskesen & Lim, 1981; Ussing, 1978; Sten-Knudsen & Ussing, 1981). Furthermore, the present analysis is based on the fact that the flux ratio is not dependent on time and that the flux ratio equation is also valid even in the case of an epithelium in series with the unstirred layers (Sten-Knudsen & Ussing, 1981; Ussing et al., 1981). The efflux (from the stromal to the aqueous side) and influx (from the aqueous to the stromal side) data were fitted by a computer to the general equation [see Eq. (1) in Ussing et al., 1981] using a PDP 11/34 minicomputer. Briefly stated, the equations describing the efflux and influx, respectively, are as follows:

$$J_e^T(t)=J_e^C(1-f(t-t_o))+J_e^P(1-g(t-t_o)) \quad (1)$$

$$J_i^T(t)=J_i^C(1-f(t-t_o))+J_i^P(1-g(t-t_o)) \quad (2)$$

where J's represent the fluxes, J_e^C, J_e^P, J_i^C, and J_i^P are the constant steady-state values and t_o is a constant that takes into account the time elapsed before the first appearance of the efflux and influx (*see* Fig. 2); the superscripts T, C, and P represent total, cellular and paracellular, respectively, while the subscripts e and i represent the efflux and influx, respectively; $f(t-t_o)$ and $g(t-t_o)$ are the time-dependent functions corresponding to the cellular and paracellular pathways, respectively. These functions must satisfy the following: at $t-t_o=0$, $f(0)=1$ and $g(0)=1$, and at $t-t_o=\infty$, $f(\infty)=g(\infty)=0$. It is not necessary, in principle, to define the functions $f(t-t_o)$ and $g(t-t_o)$ specifically as exponential functions in the analysis of the separation of the solute pathways for analytical analysis as shown by Ussing et al. (1981). In the present computer analysis, however, the following assumptions are made:

$$f(t-t_o)=e^{-\lambda_1(t-t_o)}$$

and

$$g(t-t_o)=e^{-\lambda_2(t-t_o)}$$

where λ_1 and λ_2 are constants and can be considered reciprocals of the time constants corresponding to the cellular and paracellular solute pathways, respectively. Values for these constants were determined from the theoretical curves obtained by least-squares fitting of the experimental data. As can easily be seen, these exponential functions satisfy the required conditions on f and g. The efflux and influx curves were fitted by a nonlinear method of least squares using either the subroutine ZXSSQ (International Mathematical and Statistical Libraries, Houston, Texas) or VA05A (Harwell Subroutine Library, Atomic Energy Research Establishment, Harwell, Berkshire). Both routines gave similar results. It should be mentioned that the present method of separating the pathways is based on the assumptions that the fast component is due solely to the paracellular pathway and that the passive cellular influx for Na^+ is independent of the mechanisms driving the net Na^+ efflux.

Results

Trans-endothelial Potential Difference

The average endothelial potential difference under controlled conditions was 0.76 ± 0.03 mV ($n=41$, and SEM) in 22 experiments. This average is slightly lower than the 1.02 ± 0.03 mV ($n=42$) recently reported by Lim and Fischbarg (1981) and may be the result of having eliminated adenosine and GSSG from the bathing solution in the present study.

Na^+ Fluxes

It can be seen in Fig. 2 that there is a net Na^+ flux across the corneal endothelial preparation. The Na^+ efflux and influx curves in Fig. 2 each represent the average of eleven separate data points. From the steady-state flux values (*cf.* Fig. 2), the flux difference estimated by eye was 2.3 μeq/hr·cm². When the net fluxes from each experiment were averaged, the mean value was 2.3 ± 0.4 μeq/hr·cm² ($n=11$, SEM). Figure 3 illustrates the typical raw data from one of the eleven experiments averaged and the computer-generated efflux and influx curves prepared by least-squares curve fitting of experimental data. The data fit by the subroutines (ZXSSQ and VA05A) was sensitively dependent on the initial values chosen. Two criteria were

used for best fit: lowest SSQ (sum of the squares) and best fit judged by eye.

Separation of Cellular and Paracellular Na^+ Pathways

From the presteady-state part of the fluxes, one can obtain cellular and paracellular pathways for the Na^+ transport. A detailed procedure for the separation of the pathways has been described in the Methods section and elsewhere (Ussing et al. 1981). Using that method, the cellular and paracellular contributions of a tight epithelium (frog skin) in normal Ringer were estimated to be 73 and 27%, respectively. With the proper modifications in the assumptions (specifically $J_i^p \neq 0$, f and g functions as given above in the present analysis), a similar procedure was followed to separate the cellular and paracellular contributions to the ionic flows across the corneal endothelium. The percentage distribution shown in Table 1 was calculated from the data used to obtain Fig. 2. The passive movement of solute through the cellular and paracellular pathways equal j_i^c plus j_i^p. One can not estimate the passive component of the flux by j_e^c plus j_e^p, because j_e^c contains the active component of Na^+ transport. As can be seen from Table 1, the cellular contribution to the passive solute transport is only about 10% while that of the paracellular pathway is about 90%. These values are comparable with those estimated from electrical measurements (Lim & Fischbarg, 1981). On the other hand, one must note that the present estimate is due only to Na^+ movement, whereas the electrical measurement includes the total ionic contributions to the respective two pathways.

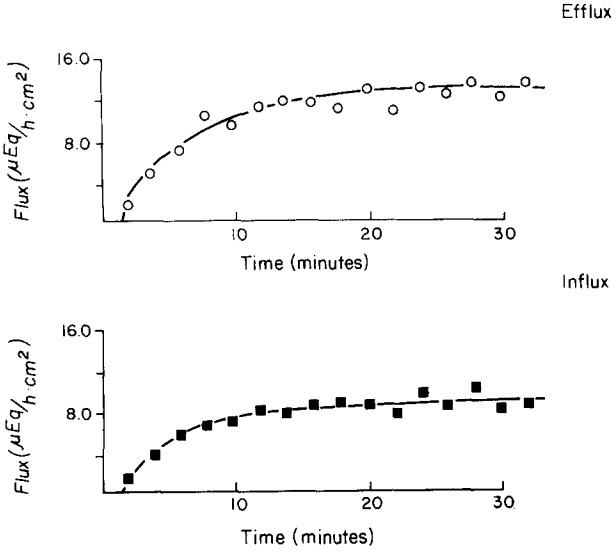

Fig. 3. Measured efflux (open circles) and influx (solid squares) for sodium ion from a typical experiment as a function of time were fitted to theoretical curves (solid lines) utilizing the computer subroutines. The parameters obtained were: $j_e^c = 0.51$, $j_e^p = 0.49$, $j_i^c = 0.12$, $j_i^p = 0.88$, $\lambda_1 = 0.06\ \text{min}^{-1}$, $\lambda_2 = 0.31\ \text{min}^{-1}$, $t_o = 1.62$ min. j's are the fractional values of the normalized fluxes

Diffusion Permeability

Since the measurements of the flux from stroma to aqueous include the active component of Na^+ transport as discussed above, the diffusion permeability for Na^+ can be computed only from the opposite flux (aqueous to stroma). The average value obtained

Table 1. Percentage distribution of Na^+ movement between cellular and paracellular solute pathways ($n = 11$)

j_e^c	j_e^p	j_i^c	j_i^p
52.0 ± 0.4	48.2 ± 0.5	10.0 ± 0.9	89.9 ± 0.9

j_e^c = percentage of total efflux transported through the cellular pathway.
j_e^p = percentage of total efflux moved through the paracellular pathway.
j_i^c = percentage of total influx transported through the cellular pathway.
j_i^p = percentage of total influx moved through the paracellular pathway.

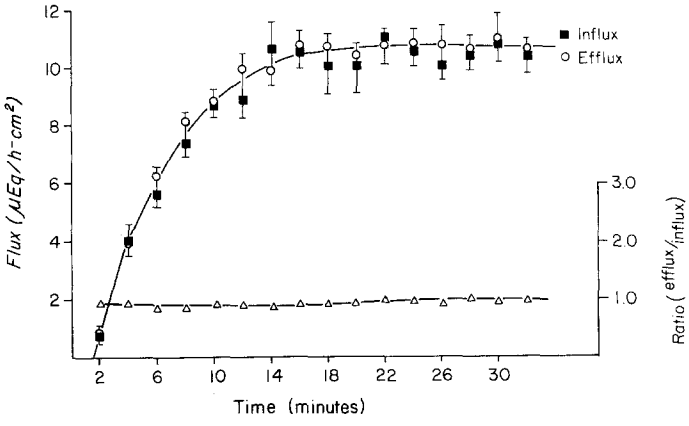

Fig. 4. Effect of 10^{-4} M ouabain on the efflux (open circles) and influx (solid squares) for sodium ion with time

for the Na^+ permeability was 0.064 ± 0.003 cm/hr ($n=11$, SEM) which is comparable to the values reported by Maurice (1951; 0.072 cm/hr) and Hodson and Miller (1976; 0.068 cm/hr).

Effects of Ouabain

Exposing the preparation to 10^{-4} M ouabain eliminates the net Na^+ flux. The data, shown in Fig. 4, represent the average of five experiments. The average values for the steady-state influx and efflux are, respectively, 11.5 ± 0.8 and 10.8 ± 0.3 $\mu eq/hr \cdot cm^2$, which are not statistically different from each other. The flux ratio is one and constant from the beginning, indicating that the Na^+ moves passively through a single pathway.

Effects of 10^{-4} M Acetazolamide

The effect of 10^{-4} M acetazolamide on the PD is shown in Table 2. The PD in the presence of acetazolamide is seen to decrease to about 70% of the control value. The effect of the carbonic anhydrase inhibitor on the net Na^+ is shown in Fig. 5. The data indicate a trend toward reduced Na^+ transport in the presence of the inhibitor.

Table 2. The effect of 10^{-4} M acetazolamide on the transendothelial PD (μV)

Exp. #	Control	Acetazolamide	Decrease
1	1040	820	220
2	870	520	350
3	770	600	170
4	810	580	230
5	990	840	150
6	860	600	260
7	770	400	370
8	500	420	80
Mean	826	598	228

Discussion

The role played by Na^+ in the maintenance of corneal transparency by the endothelium has been puzzling. Although the transendothelial potential difference and the rate of fluid transport across the preparation are crucially dependent on the presence of Na^+ in the bathing solution, no net Na^+ movement had been reported so far (Hodson & Miller, 1976; Hull et al., 1977). We have now detected, however, a net Na^+ transport across the rabbit corneal endothelium. The negative reports published thus far may be attributed to the leaky nature of the preparation which could have interfered with previous measurements. Additionally, although paired corneas in an open-circuit condition were used in both the present study and that of Hull et al. (1977), the initial conditions of pH and oxygen tension in the bathing solution may not have been maintained throughout their experiments as they were in the present study.

Our proposition that there are at least two major pathways[1] for the solute movement is based on the fact that the initial flux ratio as a function of time is not constant. The increase in the ratio implies (*see* Ussing et al., 1981) that either (i) the ions are traversing more than one pathway and/or (ii) such electrical parameters as the transendothelial resistance and potential difference do not remain constant. Since transendothelial PD and R_t are known to remain constant for up to about 6 hr in the experimental chamber, we feel justified in assuming that the electrical param-

[1] Fitting the experimental points to a single, rather than the double exponentials currently used, would necessitate that $f=g$ and also that $\lambda_1=\lambda_2$. This would yield a constant ratio which is not consistent with the theory upon which the present analysis is based.

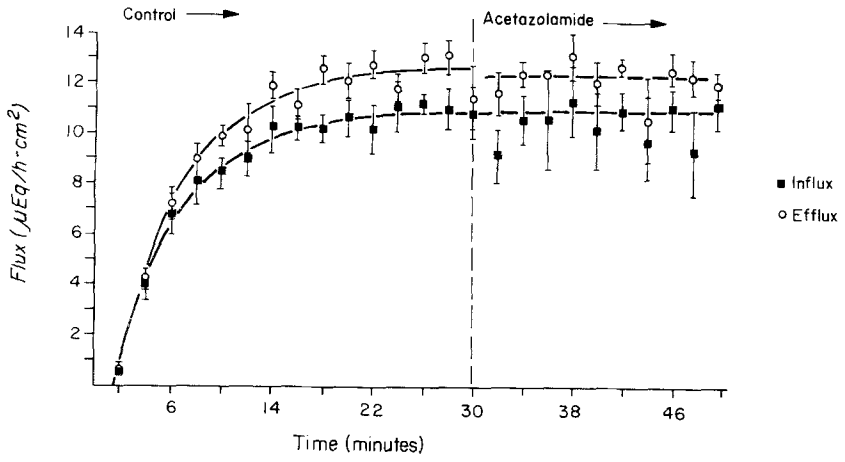

Fig. 5. Effect of 10^{-4} M acetazolamide on the efflux (open circles) and influx (solid squares) for sodium ion. At $t=30$ min, the solutions bathing both sides of the corneal preparation were made to contain 10^{-4} M acetazolamide by the addition of a concentrated solution of acetazolamide (*see* Methods) ($n=5$)

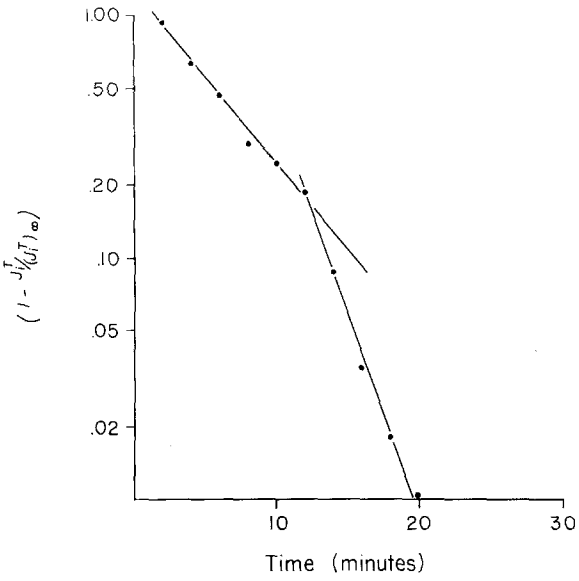

Fig. 6. Plot of $(1-J_i^T/(J_i^T)\infty)$ as a function of time on a semi-logarithmic scale. The initial and the latter slopes could be identified as the ones related to λ_2 and λ_1 corresponding to the reciprocals of the apparent time constants related to the paracellular and cellular pathways, respectively

eters remained constant for the 32-min experimental period. We therefore conclude that two pathways exist for the solute movement, which is consistent with electrical measurements previously made on this tissue layer (Lim & Fischbarg, 1981) and other epithelia (Frömter, 1972). The above conclusion is also supported by the fact that there are two slopes (*see* Fig. 6) on a plot of log $(1-J_i^T/(J_i^T)\infty)$ *vs.* time. Additional support comes from the differences in the residual sum of the squares between the experimentally obtained data fitted to the double [*see* Eqs. (1) and (2)] and the single exponential functions. The values for the residual sum of the squares of the double exponential functions fitted to the data points for the effluxes and influxes are, respectively, 8.28 and 5.53 $(\mu eq/hr \cdot cm^2)^2$, while the corresponding values for the single exponential functions are 28.94 and 15.92 $(\mu eq/hr \cdot cm^2)^2$. The standard F test yields that $p < 0.1$ which is thought to be significant in the present case. It should be emphasized that the cellular and paracellular pathways were separated by fitting the efflux and influx curves to Eqs. (1) and (2) and that the flux ratio was used as a parameter to judge the existence of the two pathways.

The validity of the flux measurements under open circuit, rather than short-circuit conditions, could be debated for the present case. The short-circuit technique (Ussing & Zerahn, 1951), so successful with tight epithelia, encounters difficulties when applied to such leaky tissues as the corneal endothelium. In this particular case, the supporting stroma is another leaky tissue having a resistance (R_s) of about $10\ \Omega \cdot cm^2$ which is in series with the R_t of about $30\ \Omega \cdot cm^2$. This implies that errors in the short-circuit current ($(I_{sc}/I_{meas})^\star = (R_t + R_s)/R_t$) and the fluxes determined under short-circuit condition could be as large as 30%. The interpretation of data obtained from short circuiting a leaky tissue is not only complicated but provides additional uncertainties because there usually exists a residual PD of a few hundred microvolts across the short circuited preparation due to drift in the saline bridges. Such PD is of the same order of magnitude as the PD generated by the leaky epithelium. Furthermore, since the PD is only 0.76 mV (aqueous negative) in our case, the expected flux ratio (efflux/influx) from the Ussing-Teorell equation (Ussing, 1949; Teorell, 1949) is only about 1.03. On the other hand, from the presently found average PD and the average R_t under similar experimental conditions ($32\ \Omega \cdot cm^2$, J.J. Lim, L.S. Liebovitch and J. Fischbarg, *in preparation*), a short-circuit current of about 24 $\mu A/cm^2$ or about 0.89 $\mu eq/hr \cdot cm^2$ can be estimated. This flux is appreciably less than the average net flux of $2.3 \pm 0.4\ \mu eq/cm^2$ presently measured. It could be argued, therefore, that the net Na^+ flux obtained under open circuit is not entirely related to the spontaneous electrical potential gradient across the endothelium. Furthermore, we have recently measured the Na^+ fluxes under short-circuit condition and found no difference in the net Na^+ flux measured ($2.2\ \mu eq/hr \cdot cm^2$ on the average, $n=3$). The validity of the present measurements is also supported by the fact that no Cl^- net flux is detected under either open or short-circuit conditions. The small magnitude of the PD measured and a large correction factors involved in the determination of the net flux led to the present preference of open-circuit conditions.

The similar magnitude of the presently reported net Na^+ flux ($2.3\ \mu eq/hr \cdot cm^2$) and that of the HCO_3^- flux obtained by Hull et al. (1977) suggests a very interesting prospect: the existence of a neutral Na^+ and HCO_3^- pump. Although no definitive conclusion was reached then, the notion of a neutral pump in the corneal endothelium has been explored previously (Fischbarg & Lim, 1974). In the framework of a neutral pump, the polarity of a small transendothelial potential difference observed could be better explained in terms of a diffusion potential. That is, $NaHCO_3$ would be transported by the pumps in a neutral manner across the preparation towards the aqueous and would leak back through the intercellular junctions into the intercellular spaces towards the

* I_{sc}: short-circuit current; I_{meas}: transendothelial current measured.

stroma (Fischbarg & Lim, 1974; Fischbarg, 1979). According to this conceptual scheme, the intercellular junction should be cation selective. This is, indeed, suggested by the values of the dilution, biionic and streaming potentials recently measured across the corneal endothelium (J.J. Lim, L.S. Liebovitch and J. Fischbarg, *in preparation*). The presently envisioned scheme is similar to that which has been advanced to explain a small potential difference observed across the gallbladder (Machen & Diamond, 1969).

If Na^+ and HCO_3^- are transported in a coupled manner, then the 30% reduction in the transendothelial PD due to the carbonic anhydrase inhibition should be reflected in some reduction of the rate of Na^+ transport. Indeed, the fact that acetazolamide reduced the net Na^+ flux is consistent with coupled transport of HCO_3^- and Na^+. One caveat, however, is that the values presently found are uncomfortably close to the limit of experimental resolution. Further studies to clarify this problem are continuing in our laboratory.

Whether or not Na^+ and HCO_3^- are transported across the corneal endothelium either coupled or uncoupled but in an electrically neutral manner, the magnitude of the measured flux suggests that the fluid transport is hypertonic. Given a rate of transendothelial fluid transport of about 4.0 $\mu l/hr \cdot cm^2$ (Fischbarg et al. 1977), and rate of $NaHCO_3$ transport of about 2.3 $\mu M/hr \cdot cm^2$ (Hull et al. 1977; present study), the ratio of the rate of solute flow to the rate of solvent flow yields an emergent osmolarity of about 1,150 mOsm/liter. Mayes and Hodson (1978) using a similar argument also estimated the emergent osmolarity to be hypertonic. If transport were isotonic, then the rate of fluid transported would have to be about 15 $\mu l/hr \cdot cm^2$, which is far greater than even the reported maximal rate of about 6 $\mu l/hr \cdot cm^2$ (Maurice, 1972; Fischbarg et al., 1977). Although physical damage to the tissue edges cannot be ruled out as a factor contributing to the discrepancy between measured and predicted rates of fluid transport, an alternative explanation is that the fluid transported would be hypertonic, rather than isotonic.

Among models to explain fluid transport across epithelia, the standing-gradient osmotic theory advanced by Diamond and Bossert (1967, 1968) is attractive in spite of certain reservations about the assumptions used in formulating this model (Hill, 1975; Sackin & Boulpaep, 1975; Lim & Fischbarg, 1976). One of the main criticisms is that, in order to predict an isotonic flow, a very large osmotic permeability in the order of 10 $cm^4/sec \cdot Osm$ (5,520 $\mu m/sec$**) must be invoked. It is, however, interesting to compare the emergent osmolarity presently estimated with the prediction by the standing-gradient theory. When one uses a permeability value in the order of 10^{-2} $cm^4/sec \cdot Osm$ (5.5 $\mu m/sec$), obtained by conventional methods, an osmolarity of two or three times isotonic (*cf.* Fig. 4 of Lim & Fischbarg, 1976) was predicted for the fluid transported. More recent evidence (Fischbarg, 1981, and *personal communication*), however, suggests that the osmotic permeability across the endothelium proper is about 700 $\mu m/sec$ and that across the apical cell membrane is 1,000 $\mu m/sec$. Even with such large permeability values, the standing-gradient model as applied to the endothelium (Lim & Fischbarg, 1976) would still predict that the osmolarity of the fluid transported will be about 1.5 times the isotonic level.

** Since 1 osmole = 18.1 grams of water = 18.1 cm^3, the conversion factor between units of $cm^4/sec \cdot Osm$ and $\mu m/sec$ is $5.52 \cdot 10^{-2}$.

The authors would like to express their utmost gratitude to Ms. Ottilie T. Garsen for her excellent technical assistance. We would also like to thank Drs. J. Fischbarg, E. Anderson, and L.S. Liebovitch for useful comments on this manuscript. The work was supported by U.S. Public Health Service Research Grants EY 02104 and EY 00105, and also in part by an Emergency Grant from Fight For Sight Inc., New York City.

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Received 30 March 1981; revised 7 July 1981;
revised again 29 October 1981