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INTRACELLULAR ACTIVITIES OF CHLORIDE, POTASSIUM AND SODIUM IONS IN RABBIT CORNEAL EPITHELIUM

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The mechanism of ion transport in the epithelium of rabbit cornea was studied by determining the intracellular ion activity of Cl^-, Na^+ and K^+ under various conditions. Ionic activities were measured by means of microelectrodes containing liquid ion-exchangers selective for Cl^-, Na^+ or K^+. The Cl^- activity in basal cells of the epithelium in Na^+ containing bathing solutions amounts to 28 ± 2 mM ($n = 11$). This value is 1.9-times greater than expected on the basis of passive distribution across the tear side membrane. This finding suggests the existence of a Cl^- accumulating process. Replacement of Na^+ in the aqueous bathing solution by choline or tetraethylammonium results in a reversible decrease in Cl^- activity to 22 ± 1 mM ($n = 11$, $P < 0.025$). The ratio of observed and predicted Cl^- activity decreased significantly from 1.9 to 1.4 ($P < 0.05$). The decrease in Cl^- activity due to Na^+ replacement was rather slow. In contrast, after readmittance of Na^+ to the aqueous bathing solution, Cl^- activity rose to a stable level within 30 min. These results indicate involvement of Na^+ in Cl^- accumulation into the basal cells of the epithelium. The K^+ and Na^+ activities of the basal cells of rabbit corneal epithelium in control bathing solutions were 75 ± 4 mM ($n = 13$) and 24 ± 3 mM ($n = 12$), respectively. The results can be summarized in the following model for Cl^- transport across corneal epithelium. Cl^- is accumulated in the basal cells across the aqueous side membrane, energized by a favourable Na^+ gradient. Cl^- will subsequently leak out across the tear side membranes. Na^+ is extruded again across the aqueous side membrane of the epithelium by the ($Na^+ + K^+$)-ATPase.

Introduction

The important role of ($Na^+ + K^+$)-ATPase in corneal epithelial Na^+ transport is indicated by the inhibition of active Na^+ transport by ouabain in amphibian [1] and rabbit cornea [2]. The role of active Na^+ transport in maintaining corneal transparency is still a matter of dispute. The active Cl^- transport process in amphibian corneal epithelium contributes to the maintenance of corneal transparency [3] and the same has been found for Cl^- transport in rabbit corneal epithelium [4]. The process of active Cl^- transport in amphibian corneal epithelium was described to be dependent on Na^+ at the aqueous side (AS) of the epithelium [5,6]. Frizzell et al. [7] proposed a general model of active Cl^- transport, which consists of an uphill entry of Cl^- into the cells energized by a downhill entry of Na^+ across the same membrane. This entry step would take place via a neutral NaCl co-transport mechanism, sensitive to furosemide. Candia [8] reported that furosemide is able to inhibit net Cl^- transport in amphibian corneal epithelium.

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Abbreviation: DIDS, 4,4'-diisothiocyanostilbene-2,2'-disulfonate.

In Cl^- absorbing epithelia like rabbit gallbladder [9] and the intestinal epithelium of the winter flounder [10] the measured intracellular Cl^- activity exceeds the Cl^- activity predicted for passive Cl^- distribution across the apical membrane by a factor of 2.3 and 3.4, respectively. A similar excess of Cl^- has been described in preliminary reports for Cl^- secreting epithelia [11] and in the bullfrog corneal epithelium [12]. The Na^+ activity in the basal cells of the amphibian corneal epithelium has not been determined sofar. Thus, it is not known whether the Na^+ gradient across the aqueous membrane of the epithelium is sufficient to energize the uphill Cl^- movement into the cells.

In rabbit corneal epithelium Klyce and Wong [13] arrived at an intracellular Cl^- concentration of 40 mM by means of an indirect method. In a model for Cl^- transport across rabbit corneal epithelium, they pointed out that Cl^- is accumulated in the cell, and that it can leak out across the tear side membrane on the basis of a slightly favourable electrochemical gradient (4 mV). Oteri [14] found in scraped-off rabbit corneal epithelium intracellular concentrations of Na^+, K^+ and Cl^- of 57, 109 and 23 mEq/l, respectively. It is difficult to transform these results to intracellular ion activities. Therefore we set out to measure Cl^-, Na^+ and K^+ activities and intracellular potentials in rabbit corneal epithelium. The results have been used to construct a model of epithelial chloride transport of rabbit cornea that is in agreement with the one proposed for amphibian corneal epithelium [7].

Materials and Methods

Experimental set-up

New Zealand white rabbits weighing 2–3 kg were used. The rabbits were killed by a blow on the neck followed by exsanguination. The eyes were enucleated and kept in a moist atmosphere at 4°C. The cornea, including a scleral ring of 2 mm was excised and care was taken not to damage it by wrinkling. Prior storage of the eyes for several hours did not influence the electrophysiological parameters of the cornea.

For the experiments an Ussing type chamber was used as described previously [15]. The temperature of the bathing solutions was controlled by a water jacket around the upper chamber and was kept constant at 35°C.

Transcorneal potential difference (PD) and resistance (R) were measured by means of a quadrupole Ag/AgCl electrode system. Electrical contact between the bathing solutions and the Ag/AgCl electrodes was made by means of microelectrodes with broken tips (diameter of approx. 50 μm) filled with 3 M KCl in agar. Liquid junction potentials between the experimental bathing solutions connected and 3 M KCl agar bridges never exceeded 1 mV. The PD measuring electrodes were connected via high input amplifiers (52K, Analog Devices) to a differential amplifier (52K, Analog Devices) and the signal was recorded with a pen-writer (Rikadenki). Resistance was determined by sending current pulses in the order of seconds. Pulses were obtained by applying a constant voltage to a voltage controlled current source. The resulting current and corneal PD responses were recorded with a pen-writer. Current-voltage relationships were measured by varying the magnitude of the voltage pulses. The measurement of an I/V relationship was completed within 6 min without effecting the spontaneous corneal PD.

The position of the microelectrode was such that it could be lowered into the corneal epithelium perpendicularly by means of a micromanipulator (Leitz). The electrical signal of the conventional and the ion-selective microelectrodes were fed to a Keithley amplifier with a high input impedance ($> 10^{14}\ \Omega$). The output of the Keithley amplifier and the signal of the PD measuring macroelectrodes in the bathing solutions were fed to differential amplifiers (52K, Analog Devices). By this procedure we obtained values of epithelial potentials across the tear side ($\psi_{TS,C}$) and the aqueous side ($\psi_{C,AS}$) membranes.

Construction of conventional and ion selective microelectrodes

Boro-silicate glass tubing (1 mm outer diameter) with an inner filament (Clark electromedical instruments) was used to pull microelectrodes on a Narishige horizontal electrode puller. It was found that proper functioning of the ion selective microelectrodes required a tip opening of 0.5–0.7 μm diameter. Therefore, also conventional electrodes

were made with similar tip openings, which is reflected in their low tip impedance (~ 5 MΩ). It was suggested before [17] that microelectrodes filled with 3 M KCl give rise to artifacts. It was found that 3 M KCl filled microelectrodes (tip resistance 2 MΩ) resulted in potential responses of up to 25 mV negative with respect to the aqueous side after impalement of the stroma, while Ringer filled microelectrodes gave a zero potential difference. A similar finding was reported by Nelson et al. [18] using 3 M KCl filled microelectrodes with relatively low tip resistance in experiments in frog skin. These artifacts can be avoided by either using 3 M KCl microelectrodes with a high tip resistance (> 30 MΩ) or using a filling solution isotonic to the cell interior.

In order to make the proper decision and to exclude artifacts [17,18] the following experiment was carried out: the cornea was incubated with ouabain (10^{-3} M, aqueous side) and amphotericin B ($2 \cdot 10^{-6}$ M, tear side). Ouabain inhibits active Na^+ transport [1,19] and prolonged incubation with amphotericin B induces leaky membranes [2]. When the transcorneal PD was zero, the epithelial cells were impaled with microelectrodes filled either with 3 M KCl or with 150 mM KCl. In the case of 150 mM KCl filled microelectrodes, the intracellular potential was essentially zero. However, with 3 M KCl filled microelectrodes, intracellular potentials of 18 mV (cell interior negative) were obtained which value became even larger after penetration of the stromal layer. For this reason we have used 150 mM KCl filled microelectrodes with a tip diameter up to 0.7 μm in all further experiments. With these electrodes potential recordings could be obtained for a period of up to 10 min. Tip resistances amounted to 45 MΩ and tip potentials were less than 3 mV.

Ion selective microelectrodes were prepared by dipping the tip of dry microelectrodes into a siliconizing fluid for 15 s to allow this fluid to enter the tip. Subsequently the silicone was baked onto the glass in an oven at 150°C for 90 min. The siliconized microelectrodes could be stored in a dry atmosphere. Prior to the experiments the siliconized microelectrodes were injected from the rear with a small volume of liquid ion exchange solution for Na^+ (Clark electromedical instruments), K^+ or Cl^- (Corning Scientific Instruments 477317 and 477315, respectively). This ion exchange solution entered the tip of the microelectrode by capillary force. Subsequently, the microelectrode was filled with 0.1 M KCl in the case of a K^+ selective and Cl^- selective microelectrode and with 0.1 M NaCl in the case of a Na^+ selective microelectrode. The ion selective microelectrodes were tested for their selectivity by measuring the electrical signal of the electrode in test solutions with various concentrations. One of the test solutions for each ion selective microelectrode also contained a high concentration of the ion most likely to interfere. Table I shows the test solutions used. The electrical response of the Cl^- ion selective microelectrode is described by

$$E_{Cl} = E_0 + S \log a_{Cl^-} \quad (1)$$

where E_0 is a constant independent of the Cl^- activity (a_{Cl^-}) but depending on other experimental conditions, S is the change in electrode potential for a ten-fold change in Cl^- activity. In the presence of an interfering ion (e.g HCO_3^-) in the calibration solution, Eqn. 1 expands to

$$E_{Cl} = E_0 + S \log(a_{Cl^-} + k \cdot a_{HCO_3^-}) \quad (2)$$

TABLE I

IONIC COMPOSITION OF THE TEST SOLUTIONS FOR Na^+, K^+ AND Cl^- SELECTIVE MICROELECTRODES

Microelectrode selective for:	Composition of the test solutions (mM)		
	Cl^-	K^+	Na^+
	(a) 10 KCl	10 KCl	10 NaCl
	(b) 100 KCl	100 KCl	100 NaCl
	(c) 10 KCl + 100 $KHCO_3$	10 KCl + 100 NaCl	10 NaCl + 100 KCl

TABLE II

SOME CHARACTERISTICS OF Na^+, K^+ AND Cl^- SELECTIVE MICROELECTRODES

The activity coefficient of HCO_3^- has been assumed to be similar to that of Cl^-. Mean ± S.E. at 20°C, $n = 6$.

	Selectivity constant (k)	$1/k$	Microelectrode resistance (Ω)	Slope (mV/decade)
Na^+	$k_{KNa} = 0.36 \pm 0.02$	2.8 : 1	$4 \cdot 10^{10}$	60.0 ± 0.5
K^+	$k_{NaK} = 0.020 \pm 0.001$	50 : 1	$7 \cdot 10^9$	59.0 ± 0.05
Cl^-	$K_{HCO_3Cl} = 0.091 \pm 0.002$	11 : 1	$1 \cdot 10^{10}$	56.5 ± 0.4

where K is the selectivity constant, indicating the selectivity of HCO_3^- over Cl^-. Combination of Eqns. 1 and 2 and calculation of the ion activities then yields the selectivity constant k. Some characteristics of the ion selective microelectrodes are given in Table II. It is obvious that the measurement of the intracellular Na^+ activity will be greatly influenced by the K^+ present. The obtained value of the intracellular Na^+ activity must therefore be corrected for the K^+ activity by means of the selectivity constant for K^+ over Na^+ of the Na^+ selective electrode. In Fig. 1 tracings of typical impalements with K^+, Cl^- and Na^+ selective microelectrodes are shown. Stable potential recordings could be obtained for several minutes with these electrodes. This may indicate [20] that the cells of corneal epithelium are coupled to a great extent. The blocks imposed on the recording of Fig. 1 of the ion selective microelectrodes are the voltage responses of the TS membrane of the epithelium due to current pulses. The voltage deflections imposed on the recordings of the Na^+ and Cl^- microelectrodes show capacitive effects due to the high electrode impedance. The magnitude of the blocks are an indication of the tear-side membrane resistance (R_{TS}).

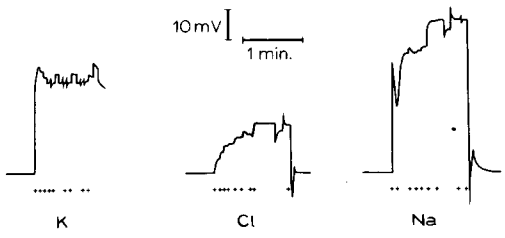

Fig. 1. Tracings of typical impalements of the epithelial cells with K^+, Cl^- and Na^+ selective microelectrodes. At + the microelectrode was advanced 5 μm further into the epithelial layer. In particular with Cl^- and Na^+ selective microelectrodes capacitive effects were observed. At the last + the microelectrode tip penetrated the stromal layer. Withdrawal of the microelectrode to the tear side bathing solution caused the potential to return to the level measured before impalement.

The response of the ion selective electrodes after impalement of the epithelium is composed of a contribution of the membrane potential and a contribution resulting from the change in ion activity between the bathing solution and the basal cells of the epithelium, i.e.

$$\Delta\tilde{\mu}_{Cl} = \psi_{TS,C} + S \log(a_{Cl^-,C}/a_{Cl^-,TS}) \quad (3)$$

where $\Delta\tilde{\mu}_{Cl}$ is the electrochemical potential for Cl^- ions, $\psi_{TS,C}$ is the potential across the tear side membrane and $a_{Cl^-,C}$ and $a_{Cl^-,TS}$ are the Cl^- activities of the cell and the tear side bathing solution, respectively. Eqn. 3 can also be used for the Na^+ and K^+ electrodes.

In order to find out whether the Cl^- selective microelectrode was sensing other anions, an experiment was carried out in a solution with low Cl^- activity ($a_{Cl^-} < 0.1$ mM). This solution contained 25 mM HCO_3 which is the major interfering anion in measuring intracellular Cl^- activity [10,21]. After stabilization of the transcorneal PD, the intracellular recordings of the conventional and the Cl^- selective microelectrodes were compared. The tear side membrane potential was 56.5 ± 0.5 mV ($n = 5$), cell interior negative. The Cl^- selective microelectrode yielded a value which was not significantly different from this potential, i.e. 58.1 ± 0.4 mV ($n = 4$). This finding indicates the absence of interfering anions inside the cell and suggests that the activity of HCO_3^- inside the cell is at least smaller than in the tear side bathing solution.

Bathing solutions

The bathing solution had the following composition (mM): 110 NaCl, 21.2 $NaHCO_3$, 8.9 Na_2SO_4, 3.8 $KHCO_3$, 1.0 KH_2PO_4, 1.5 $CaCl_2$, 1.0 $MgSO_4 \cdot 7H_2O$ and 25 glucose. Changes in ion composition of the bathing solution were established on an equimolar basis, except for chloride which was substituted by sulphate. Ion activity coefficients of the bathing solutions were taken from Robinson and Stokes [16]. The osmolality of the bathing solutions was measured with an Advanced Osmometer and adjusted to 310 mosM ($\pm 1\%$) by adding mannitol. The bathing solutions were adjusted to pH 7.4 prior to each experiment.

Results

The mean transmural PD and resistance, measured with current pulses, are not significantly different from the values previously reported [2,15]. When we plot the intracellular potential measured across the tear side membrane ($\psi_{TS,C}$) against the transmural PD, we find an inverse relation as shown in Fig. 2. This is in agreement with the results of Klyce [22]. The inverse relation indicates that variations in the PD across corneal epithelium originate from variations in the tear side membrane potential. The ratio of the resistance of both cell membranes (the voltage devider ratio) is

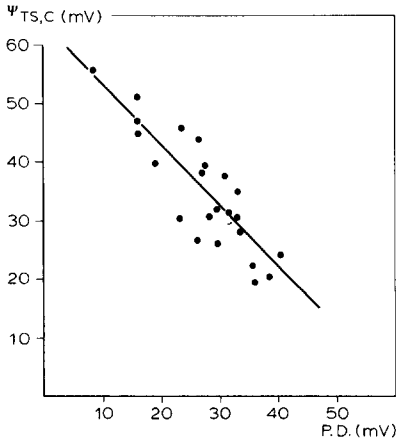

Fig. 2. Relation between the potential difference across the tear side membrane $\psi_{TS,C}$ and the potential difference across the whole tissue (PD). The slope of the regression line is -1.06 ($r = 0.82$).

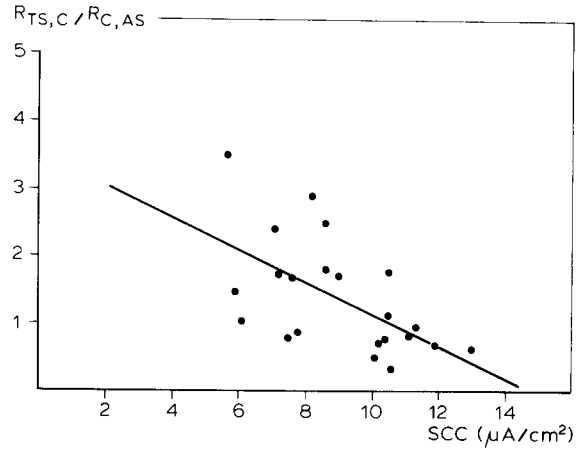

Fig. 3. Relation between the voltage divider ratio of the tear side aqueous membrane, $R_{TS,C}/R_{C,AS}$, and the short circuit current, I_{sc}, of rabbit corneal epithelium. The slope of the regression line is -0.24 ($r = 0.60$).

plotted against the short circuit current, I_{sc} ($I_{sc} = V/R$), in Fig. 3. The inverse correlation shown in Fig. 3 suggests that the resistance of the tear side membrane determines the overall transport rate expressed as I_{sc}. Therefore, the tear side membrane is the rate limiting barrier in corneal epithelial Na^+ transport. The inverse relations in Fig. 2 as well as in Fig. 3 indicate that puncturing the epithelial cells with microelectrodes with relatively large tips (0.5–0.7 μm), does not influence the potential profile across the epithelium.

Chloride activity in the basal cells of corneal epithelium

The Cl^- activity in the basal cells of the corneal epithelium is determined as described above. The results are summarized in Table III. The mean Cl^- activity observed in the basal cells of the epithelium is found to be $a_{Cl^-,o} = 28 \pm 2$ mM (11 tissues).

In the case of a passive distribution across the TS membrane, the measured $\psi_{TS,C}$ of 41.0 ± 2.6 mV leads to an expected Cl^- activity $a_{Cl^-,e} = 15$ mM, according to the equation:

$$\psi_{TS,C} = -(RT/F)\ln(a_{Cl^-,cell}/a_{Cl^-,bath})$$

where R, T and F have their usual meaning, $a_{Cl^-,bath}$ is the Cl^- activity in the bathing solution (i.e 89 mM) and $a_{Cl^-,cell}$ is the Cl^- activity in the

TABLE III

INTRACELLULAR Cl^- ACTIVITIES OF THE EPITHELIUM IN Na^+ CONTAINING BATHING SOLUTIONS

ΔE_{Cl} is the intracellular potential measured with the Cl^- selective microelectrode. The numbers in parentheses indicate number of impalements.

Experiment	PD (mV)	R ($k\Omega \cdot cm^2$)	$\psi_{TS,C}$ (mV)	ΔE_{Cl^-} (mV)	a_{Cl^-} (mM)	$a_{Cl^-,0}/a_{Cl^-,e}$
1	8.5	1.8	55.8 ± 0.3 (4)	29.4 ± 0.5 (5)	28	3.4
2	23.5	3.3	46.2 ± 0.3 (5)	15.2 ± 0.7 (5)	23	1.9
3	31.0	3.6	37.6 ± 0.6 (4)	9.4 ± 0.4 (6)	26	1.5
4	27.5	3.9	39.6 ± 0.8 (5)	9.9 ± 0.5 (9)	25	1.6
5	18.0	2.0	51.2 ± 0.5 (5)	20.8 ± 0.4 (6)	24	2.4
6	29.5	2.8	31.9 ± 0.7 (9)	8.6 ± 0.4 (4)	32	1.5
7	31.5	3.5	31.5 ± 0.8 (3)	10.8 ± 0.4 (6)	36	1.6
8	33.0	3.5	30.7 ± 1.1 (6)	12.9 ± 0.5 (5)	41	1.7
9	33.5	3.3	35.1 ± 0.6 (6)	9.5 ± 0.3 (5)	30	1.5
10	16.0	2.6	47.2 ± 0.6 (6)	13.1 ± 0.4 (4)	21	1.7
11	26.5	3.5	44.1 ± 0.4 (6)	12.1 ± 0.3 (5)	23	1.6
Mean	25.1	3.0	41.0	13.8	28	1.9
S.E.	2.5	0.2	2.6	1.9	2	0.2

cell. The ratio $a_{Cl^-,0}/a_{Cl^-,e}$ is a measure for the accumulation of Cl^- in the cells, and in control conditions this value amounts to 1.9 ± 0.2 (cf. Table III, 11 tissues).

In a review on Cl^- activities in epithelial tissues [23] it was suggested that the increased Cl^- content of the cells is due to a neutral NaCl co-transport mechanism driven by the Na^+ gradient across the membrane. In order to investigate whether a similar mechanism operates in the rabbit corneal epithelium, the aqueous bathing solution of the cornea was replaced by a bathing solution lacking Na^+. Intracellular potentials and Cl^- activity in the epithelial cells were measured after stabilization of the PD. Because the Cl^- activity of the epithelial cells slowly decreased after Na^+ substitution, the Cl^- activity was measured in the period 60–80 min after changing to a Na^+-free bathing solution. The results of these experiments are summarized in Table IV.

Substitution of Na^+ at the aqueous side caused a depolarization to 20.2 ± 1.8 mV ($n = 11$), while $\psi_{TS,C}$ did not change significantly. The change in PD is probably not related with a Na^+ permeability in the aqueous membrane of the epithelium since the epithelial resistance did not increase. The observed Cl^- activity in the cells decreased significantly to 22 ± 1 mM ($P < 0.025$, $n = 11$) after sodium replacement. The ratio $a_{Cl^-,0}/a_{Cl^-,e}$ decreased to $1.4 \pm .1$ ($n = 11$), a value significantly lower than the control value ($P < 0.05$).

The reversibility of the effects of Na^+ omission on Cl^- activity was studied by restoring the Na^+ concentration in the aqueous bathing solution to control values. The effects of re-admittance of Na^+ to the aqueous side of the cornea on $\psi_{TS,C}$, a_{Cl^-}, PD and R are given in Table V. After a transient depolarization the PD recovered to the same value as during the Na^+-free period within 30 min. The value $\psi_{TS,C}$ again did not change significantly. The observed a_{Cl^-} in the basal cells of corneal epithelium increased to 30 ± 2 mM ($n = 11$), which is not significantly different from the control value (Table III). However, the measured a_{Cl^-} was significantly larger than during the Na^+-free incubation period ($P < 0.005$). The ratio $a_{Cl^-,0}/a_{Cl^-,e}$ in the basal cells of the epithelium increased to a value of 1.9 ± 0.1 ($n = 11$), significantly higher than in the Na^+-free period ($P < 0.005$).

Determination of the sodium and potassium activities in the basal cells of corneal epithelium

As shown in Table II, the Na^+ selective microelectrode senses Na^+ only 2.8-times better than K^+. This property of the Na^+ selective microelec-

TABLE IV

INTRACELLULAR Cl^- ACTIVITIES OF THE EPITHELIUM AFTER 60–80 MIN OF INCUBATION IN Na^+-FREE AQUEOUS SIDE BATHING SOLUTION

ΔE_{Cl} is the intracellular potential measured with the Cl^- selective microelectrodes. The number in parenthesis indicate the number of impalements.

Experiment	PD (mV)	R ($k\Omega \cdot cm^2$)	$\psi_{TS,C}$ (mV)	ΔE_{Cl^-} (mV)	a_{Cl^-} (mM)	$a_{Cl^-,0}/a_{Cl^-,e}$
1	10.0	2.0	53.6 ± 0.5 (5)	17.3 ± 0.3 (4)	19	2.1
2	15.0	2.1	49.4 ± 1.2 (5)	10.4 ± 0.1 (4)	16	1.5
3	27.5	4.0	28.9 ± 0.6 (5)	0.6 ± 0.7 (4)	26	1.0
4	19.0	3.4	44.0 ± 1.0 (3)	6.9 ± 0.7 (4)	18	1.4
5	15.0	2.0	47.2 ± 1.0 (5)	10.1 ± 0.2 (5)	18	1.5
6	21.0	2.8	35.0 ± 0.3 (5)	5.7 ± 0.5 (5)	25	1.3
7	22.0	3.1	30.8 ± 0.3 (6)	5.3 ± 0.2 (5)	30	1.3
8	27.0	3.7	37.9 ± 0.4 (5)	9.6 ± 0.5 (4)	27	1.5
9	27.0	3.2	38.4 ± 0.4 (4)	6.6 ± 0.6 (5)	23	1.3
10	16.0	2.6	39.8 ± 0.7 (6)	7.8 ± 0.2 (5)	26	1.5
11	23.0	3.0	40.8 ± 0.9 (6)	5.6 ± 0.4 (5)	20	1.3
Mean	20.2	2.9	40.5	7.8	22	1.4
S.E.	1.8	0.2	2.3	1.3	1	0.1

trodes presents a problem in determining the Na^+ activity in the epithelial cells. By means of K^+ selective microelectrodes and normal 150 mM KCl containing microelectrodes the intracellular K^+ activity was found to be $a_{K^+} = 75 \pm 4$ mM ($n = 13$, cf. Table VI). The high a_{K^+} value thus interferes with the measurement of Na^+ in the basal cells. The measured Na^+ activity of the basal cells should be corrected for a_{K^+} and the result of this correction is given in Table VI. The mean Na^+ activity in the basal cells of the epithelium amounted to 24 ± 3 mM ($n = 12$). A consequence of this indi-

TABLE V

INTRACELLULAR Cl^- ACTIVITIES OF THE EPITHELIUM 30 MIN AFTER RETURNING TO A Na^+-CONTAINING BATHING SOLUTION

ΔE_{Cl} is the intracellular potential measured with the Cl^- selective microelectrode. The numbers in parentheses indicate the number of impalements.

Experiment	PD (mV)	R ($k\Omega \cdot cm^2$)	$\psi_{TS,C}$ (mV)	ΔE_{Cl^-} (mV)	a_{Cl^-} (mM)	$a_{Cl^-,0}/a_{Cl^-,e}$
1	11.5	2.0	51.0 ± 0.9 (7)	23.6 ± 0.5 (6)	27	2.7
2	14.0	–	52.0 ± 0.3 (3)	20.2 ± 0.2 (3)	22	2.3
3	30.0	3.8	33.0 ± 0.4 (4)	9.2 ± 0.6 (3)	32	1.5
4	16.4	3.0	40.2 ± 1.9 (5)	16.4 ± 0.5 (5)	32	2.0
5	15.0	2.1	48.3 ± 0.3 (4)	18.7 ± 0.6 (6)	25	2.3
6	26.0	2.5	30.8 ± 0.7 (6)	12.0 ± 0.4 (4)	39	1.7
7	24.5	3.0	31.7 ± 0.5 (5)	9.3 ± 0.3 (6)	33	1.5
8	25.5	3.0	35.6 ± 0.9 (5)	15.3 ± 0.3 (5)	38	1.9
9	28.0	2.9	35.2 ± 0.8 (5)	10.1 ± 0.4 (4)	31	1.6
10	14.0	1.8	43.8 ± 0.6 (6)	16.4 ± 0.3 (5)	28	2.0
11	24.0	2.5	42.2 ± 0.4 (5)	13.3 ± 0.5 (5)	27	1.8
Mean	20.8	2.7	40.4	15.0	30	1.9
S.E.	2.0	0.2	2.3	1.4	2	0.1

TABLE VI

INTRACELLULAR Na^+ AND K^+ ACTIVITIES OF THE EPITHELIUM IN NORMAL BATHING SOLUTION

ΔE_{Na} and ΔE_K are the intracellular potentials measured with the Na^+ and K^+ selctive microelectrode, respectively. The numbers in parentheses indicate the number of impalements.

Experiment	PD (mV)	R ($k\Omega \cdot cm^2$)	$\psi_{TS,C}$ (mV)	ΔE_{K^+} (mV)	ΔE_{Na^+} (mV)	a_{K^+} (mM)	a_{Na^+} (mM)
12	27.0	2.5	38.7 ± 0.6 (6)	34.2 ± 0.3 (5)	54.8 ± 0.9 (6)	95	28
13	16.0	3.0	45.0 ± 2.0 (7)	25.3 ± 1.0 (6)	62.2 ± 1.8 (3)	87	29
14	23.0	4.0	41.2 ± 0.6 (3)	30.4 ± 0.2 (4)	–	91	–
15	16.0	2.8	40.8 ± 0.7 (7)	20.1 ± 0.9 (5)	67.7 ± 1.1 (7)	61	19
16	37.0	2.9	18.4 ± 0.4 (5)	46.0 ± 0.2 (5)	50.2 ± 1.1 (6)	69	15
17	38.5	2.9	20.6 ± 0.1 (10)	42.7 ± 0.7 (5)	45.0 ± 0.6 (5)	67	23
18	28.0	2.8	30.9 ± 0.6 (12)	30.9 ± 0.4 (5)	62.2 ± 1.1 (9)	62	13
19	25.0	3.5	26.6 ± 0.5 (14)	34.8 ± 0.3 (4)	39.8 ± 1.2 (6)	62	43
20	36.0	3.5	19.7 ± 0.4 (12)	39.7 ± 0.5 (6)	47.3 ± 1.2 (6)	60	20
21	29.5	4.1	26.2 ± 0.9 (6)	35.6 ± 0.2 (4)	41.8 ± 1.2 (6)	62	42
22	40.5	2.8	24.3 ± 0.4 (7)	42.1 ± 0.7 (4)	51.0 ± 0.8 (5)	76	15
23	33.5	3.2	28.7 ± 0.5 (5)	40.3 ± 0.5 (3)	49.2 ± 0.6 (3)	84	23
24	26.0	2.6	40.9 ± 0.8 (4)	31.3 ± 0.5 (4)	64.8 ± 0.8 (4)	94	12
Mean	28.9	3.1	30.9	34.9	53.0	75	24
S.E.	2.2	0.1	2.6	2.0	2.7	4	3

rect way of determining a_{Na^+} is the rather large standard error in a_{Na^+} for a single tissue. Due to accumulation of errors in $\psi_{TS,C}$, the potential recordings of the Na^+ and K^+ selective microelectrodes and in their conversion to ion activities, the relative standard error of a_{Na^+} may amount to 20% for a single tissue.

Comparison of the measured a_{K^+} in the cells and the measured potential across the aqueous side membranes leads to the conclusion that the K^+ activity in the cell is higher than expected on the basis of passive distribution across that membrane. The average electrochemical potential difference for K^+ across the aqueous side membrane averages 14 mV, forcing K^+ out of the cell into the aqueous solution. The electrochemical potential difference for Na^+ across the tear side membrane averaged 84 mV driving Na^+ into the cell.

In preliminary experiments it was found that the cell activities of Na^+ and K^+ can be altered during incubation with ouabain or amphotericin B. In both cases the Na^+ as well as the K^+ activity changes towards the respective values of the bathing solution but this process takes several hours. Experiments with a Na^+-free solution at the aqueous side (substitution by triethanolamine or choline) indicated that the liquid ion exchange solutions have a very high sensitivity for these large cations. From these experiments it could also be concluded that triethanolamine and choline were able to enter the cells.

Discussion

The results lead to several interesting conclusions. In the first place, it was found that the method for obtaining ion activities in rabbit corneal epithelium may give rise to large artifacts. When 3 M KCl filled microelectrodes were used with a tip opening as large as that of the ion selective microelectrodes, pre-tip potentials of up to 25 mV could be detected. Similar findings were obtained in frog skin [18] and frog cornea [17]. The pre-tip potentials are probably due to fixed negative charges in the cell cytoplasm and in the connective tissue. These pre-tip potentials were reduced to zero by lowering the KCl concentration of the filling solution of the microelectrodes to 150 mM. The reliability of the 150 mM KCl filled microelectrodes is supported by the finding that in corneas treated with amphotericin B and ouabain a zero intracellular potential was measured while

the 3 M KCl filled electrodes gave a potential of −18 mV.

The observed inverse relationship between $\psi_{TS,C}$ and PD was previously found in rabbit corneal epithelium [22] and in frog skin [24]. Linear relationships were also found between the PD and I_{sc} [22,24]. Therefore the tear side membrane of amphibian corneal epithelium is considered to be a rate limiting step in the transport of Na^+ [1] and Cl^- [25]. This holds also for rabbit corneal epithelium [2,13]. The lower values of R_{TS} may be due either to an increased Na^+ or to an increased Cl^- permeability of the TS membrane. An increase in Na^+ permeability would result in absorption, while an increase in Cl^- permeability would result in secretion. In particular secretion is regulated in vivo [15] as suggested by the sensitivity towards adrenaline.

A Cl^- activity of 28 mM was measured in the basal cells of rabbit corneal epithelium. This value is in close agreement with the value of 29 mM reported for frog cornea [12]. By measuring the effect of suddenly increasing the Cl^- permeability of the TS membrane on $\psi_{TS,C}$ at various Cl^- concentrations in the tear side bathing solution, Klyce and Wong [13] found a Cl^- concentration of 41.5 mM in the squamous and wing cells of rabbit corneal epithelium which correspond to $a_{Cl^-} = 34$ mM. The difference might be explained by the fact that the intracellular potential of the squamous and wing cells is several mV lower than that of the basal cells ([22] and Festen, unpublished data).

The K^+ activity and Na^+ activity of the basal cells of corneal epithelium were measured to be 75 and 24 mM, respectively. The Na^+ activity is much lower than can be expected for passive distribution across the TS membrane. This difference must be due to active Na^+ extrusion by the ($Na^+ + K^+$)-ATPase. The finding that K^+ activity is slightly larger than expected on the basis of a passive distribution across the AS membrane points at a process that accumulates K^+ in the cell. Another possibility is that the K^+ activity between the cells is somewhat higher than in the aqueous bathing solution.

The K^+ activity reported here is much lower than the value of 124 mM reported previously for rabbit corneal epithelium [26]. However, in those experiments the KCl filled microelectrodes may have given rise to tip potential artifacts as described above. The observed value of K^+ activity is in the same range as the values reported for *Necturus* gallbladder [27], for *Necturus* proximal tubule [28] and for the epithelial cells of bullfrog small intestine [29]. The Na^+ activity reported here is also in the same range as the values reported for gallbladder [27] the *Necturus* proximal tubule [28] and for bullfrog small intestine [29].

Knowing the cellular activities of Na^+, K^+ and Cl^-, the diffusion potentials for these ions across the TS and AS membranes of the epithelium can be calculated with the Nernst equation, e.g. in the case of Cl^-,

$$E_{Cl^-,TS} = (RT/F)\ln(a_{Cl^-,b}/a_{Cl^-,C})$$

$E_{Cl^-,TS}$, $E_{Na^+,TS}$ and $E_{K^+,AS}$ amounted to −31, +42 and −76 mV, respectively. The TS membrane potential amounted to −36 mV and the AS membrane potential to −63 mV (all values cell interior negative; $n = 24$). These calculations indicate that the tear side membrane potential is far from the Na^+ equilibrium potential. It is also larger than the Cl^- equilibrium potential. The aqueous side membrane potential is somewhat lower than expected on the basis of a K^+ equilibrium potential. It was calculated that Cl^- activity was a factor 1.9-times larger than predicted for passive distribution across the tear side membrane. The ratio $a_{Cl^-,0}/a_{Cl^-,e}$ is reversibly lowered to 1.4, after substitution of Na^+ with choline or triethanolamine in the aqueous bathing solution. The relatively slow decrease of Cl^- activity in the epithelial cells is probably due to the large stromal layer, serving as a compartment which is not easily depleted when the bathing solution is made Na^+-free. The findings suggest that the presence of Na^+ in the aqueous bathing solution is an important parameter in accumulation of Cl^- in rabbit corneal epithelium. Dependence of Cl^- transport on serosal Na^+ was reported before in frog corneal epithelium [30]. The results can be summarized in a model of the transport mechanism in rabbit corneal epithelium shown in Fig. 4. In the aqueous side membrane a ($Na^+ + K^+$)-ATPase, a neutral NaCl cotransport mechanism and a K^+ permeability are located, while the tear side membrane is permeable for Na^+ and Cl^- as reported earlier [15]. Sodium translocated across the aque-

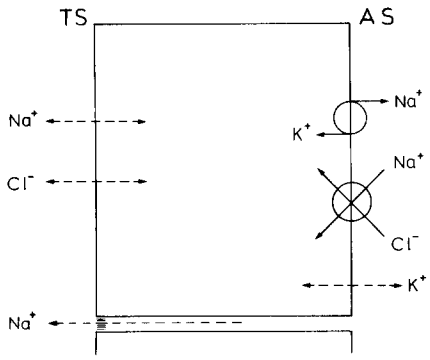

Fig. 4. Model of the transport mechanisms of rabbit corneal epithelium. (For explanation see text).

ous side membrane by $(Na^+ + K^+)$-ATPase is replaced by Na^+ from the tear side bathing solution. Na^+ entry is permitted by a Na^+ permeability in the tear side membrane. Since entry of Na^+ across the tear side membrane is rate-limiting cellular Na^+ activity will decrease relative to Na^+ activity in the tear side bathing solution. The Na^+ gradient across the aqueous side membrane can energize NaCl co-transport and realize Cl^- entry across the aqueous side membrane. Cl^- ions leak out across the tear side membrane via a Cl^- permeability due to the favourable electrochemical gradient. This Cl^- flux must be accompanied by a flux of Na^+ across the junctional route in order to obey electroneutrality. Additional support for the hypothesis that Cl^- transport from aqueous side to tear side is dependent on the established gradients rather than on an active Cl^- transport mechanism is provided by the fact that inhibition of Cl^- transport is not accompanied by an alteration in O_2 consumption in amphibian corneal epithelium [31]. Accumulation of Cl^- into the cellular compartment can take place, when the exit of Cl^- across the tear side membrane or the accompanying flux of Na^+ across the shunt pathway (cf. Fig. 4) is rate-limiting.

A serious problem of the epithelial transport model in Fig. 4 is the accumulation of K^+ into the cells. In the case of net Na^+ transport, K^+ must recirculate across the aqueous side membrane. K^+ uptake is sustained by the $(Na^+ + K^+)$-ATPase. The passive efflux of K^+ across the aqueous side membrane is hampered by the absence of a Cl^- permeability of that membrane [15]. However, there may be electroneutral efflux of K^+ out of the cell. A neutral KCl cotransport mechanism has been described in erythrocytes [32] and ascites cells [33]. This mechanism is inhibited by furosemide.

In amphibian cornea, DIDS, a stilbene derivative, inhibits Cl^- transport but not Na^+ transport [34]. DIDS is a good inhibitor of anion exchange in red blood cells [35]. The qualitative similarity between amphibian and rabbit corneal epithelium transport processes, suggests that an anion exchange mechanism like a Cl/HCO_3^- exchange would operate in rabbit corneal epithelium. The aqueous side sodium effect on Cl^- transport could be explained by the presence of Cl^-/HCO_3^- exchange in parallel to a Na^+/H^+ exchange mechanism. A combination of these exchange mechanisms has been demonstrated in membrane vesicles of isolated brush borders of rat smal intestine [36,37] and in rabbit gallbladder epithelium [38]. Further experiments are necessary to investigate whether HCO_3^- and/or the pH are more directly involved in the Cl^- uphill transport mechanism across the aqueous membrane.

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