J. Membrane Biol. 73, 275–282 (1983)

The Journal of
Membrane Biology

Cellular and Paracellular Pathway Resistances in the "Tight" Cl^--Secreting Epithelium of Rabbit Cornea

William S. Marshall* and Stephen D. Klyce
Lions Eye Research Laboratories, Louisiana State University Eye Center, New Orleans, Louisiana 70112

Summary. The high transverse resistance of the isolated rabbit cornea (6–12 $k\Omega \cdot cm^2$) is associated with the corneal epithelium, a Cl^--secreting tissue which is modulated by β-adrenergic and serotonergic receptors. Three methods were employed to determine the resistances for the apical membrane, basolateral membrane, and paracellular conductive pathways in the epithelium. In the first method, the specific resistance of the apical membrane was selectively and reversibly changed. Epinephrine was used to increase apical Cl^- conductance and Ag^+ was used to increase apical cation permeability. The second method utilized a direct measure of the spontaneous cellular ionic current. The third method obtained estimates of shunt resistance using transepithelial electrophysiological responses to changes in apical membrane resistance. The results of the first method were largely independent of the agent used. In addition, the three methods were in general agreement, and the ranges of mean values for apical membrane, basolateral membrane, and shunt resistances were 23–33, 3–4, and 12–16 $k\Omega \cdot cm^2$, respectively, for the normal cornea. The apical membrane was the major, physiologically-modulated barrier to ion permeation. The shunt resistance of the corneal epithelium was comparable to that found previously for other "tight" epithelia. Experiments using Ag^+ in tissues that were bathed in Cl^- and HCO_3-free solutions indicated that under resting conditions the apical membrane is anion-selective.

Key Words epithelial transport · corneal epithelium · paracellular pathway · epinephrine · silver ion · rabbit cornea

Introduction

The importance of intercellular "tight" junctions in characterizing the transport and permeability properties of epithelia is well-recognized (e.g. Frömter & Diamond, 1972; Diamond, 1978). In some "tight" epithelia, i.e., those with high resistance apical intercellular junctions (the morphological equivalent being the zonula occludentes), several methods have been employed to determine the resistance of the shunt pathway. A common approach has been to model the epithelium as a simple Thévenin equivalent circuit which represents the barrier properties of the apical membrane, basolateral barrier, and shunt. The resistance values for the three barriers may be determined by specific perturbation of one of the two membrane analogues. Two agents, amiloride and nystatin, have been used previously in this method. Amiloride decreases apical membrane sodium permeability in some epithelia [e.g., toad urinary bladder (Reuss & Finn, 1974, 1975; Narvarte & Finn, 1980*a*, *b*)]. In contrast, nystatin increases apical membrane cation permeability [e.g., in rabbit urinary bladder (Lewis, Eaton, Clausen & Diamond, 1977)]. Tight Na^+-transporting epithelia, such as the urinary bladder of toad and rabbit, have shunt resistances that are higher than that of the cellular transport pathway. Hence the paracellular pathway is thought to constitute the major epithelial barrier to solute diffusion in tight epithelia (Diamond, 1978). Whereas this could be a general characteristic of tight epithelia that transport Na^+, it will be shown here that the barrier resistances of the normal corneal epithelium, which primarily transports Cl^-, may follow a different pattern.

While the above methods are aptly suited to studies using urinary bladder preparations, they are inappropriate for use with the corneal epithelium, because the cornea does not tolerate well the methanol vehicle for nystatin and the tissue apparently lacks amiloride-sensitive Na^+ channels at the apical membrane surface (S.D. Klyce and W.S. Marshall, *unpublished*). Instead, epinephrine and Ag^+ treatments are used here to perturb apical membrane conductance, as it has been shown that the former specifically increases apical membrane Cl^- conductance (Klyce & Wong, 1977) and that the latter enhances apical membrane cation conductance (Klyce & Marshall, 1982). The effects of

* *Present address:* Department of Biology, St. Francis Xavier University, Antigonish, Nova Scotia, Canada B2G 1CO

Ag^+ are largely reversed by rinsing or by the addition of reduced glutathione (Klyce, 1975; Klyce & Marshall, 1982); the reversal of the epinephrine effect requires no additional treatment if small pulses of the substance are used. We also wished to compare anion *vs.* cation resistances and to obtain ionic selectivities for the apical membrane. These experiments involved a comparison of component resistances measured in a complete bathing solution with those measured in a low-Cl^-, low-HCO_3^- medium.

To confirm the results from the above perturbation method, an independent procedure, developed previously by Klyce (1972), is used for measurement of barrier resistances. A third "noninvasive" method (Klyce, 1971) is used to substantiate the previous two techniques which require cell impalements. The results from the various methods are evaluated and the component resistances for the cornea are compared to those found previously for cation-transporting tight epithelia. Parts of this work have been presented previously (Marshall & Klyce, 1981 *a*).

Materials and Methods

New Zealand White rabbits (3.5–4.5 kg) were killed by an overdose of sodium pentobarbital. Eyes were enucleated, and the corneas dissected and mounted in Lucite chambers (exposed corneal surface = 1.0 cm^2), as described previously (Klyce & Wong, 1977). Corneas were bathed in normal Ringer's solution or a low-Cl^-, low-HCO_3^- solution.

Bathing Solutions

The normal Ringer's solution used in the corneal experiments comprised (in mM): NaCl, 99.7; KCl, 3.7; Na_2SO_4, 6.9; $NaHCO_3$, 20.0; K_2HPO_4, 0.6; Na/HEPES (pH 7.4), 25.0; calcium gluconate, 1.4; $MgSO_4$, 0.6; and glucose, 26.0 (all from Sigma Chemical Co.). To make low Cl^-/HCO_3^- solution, these ions were replaced with SO_4^{2-}, with sufficient sucrose added to maintain the normal osmolarity of 305 mOsm·l^{-1}. The former solution was aerated with 95% O_2/5% CO_2, while the latter was gassed with air. Both solutions had a stable pH of 7.4 ± 0.2 at 37 °C. The tissues in the chambers were maintained at 35 °C; flow-through perfusion of the hemichambers was gravity-fed to minimize vibration in the system.

Microelectrode Experiments

The *in vitro* cornea chamber and associated instrumentation used here have been described (Klyce & Wong, 1977). Microelectrodes were filled with 1.0 M potassium-citrate and had tip resistances of 30–60 MΩ, measured in Ringer's solution. The microelectrodes were manipulated using a sliding micromanipulator (Zeiss Jena) and a digitally-operated hydraulic microdrive (David Kopf Instruments); the microdrive was in turn controlled manually or with a control circuit (Marshall & Klyce, 1981 b) which was devised to advance the microelectrode continuously through the bathing medium of the tears (apical) side of the cornea and to halt the microelectrode when a superficial cell was impaled. To obtain complete voltage/resistance profiles of the epithelium, the microelectrode was advanced in small (2–5 μm) steps, thereby impaling sequentially the underlying cell layers. The measured parameters included transcorneal potential (V_{ts}), apical barrier potential (V_{tc}), basal barrier potential (V_{cs}), where the voltages of the normal "well"-type potential profile of the epithelium (Klyce, 1972) are presented as absolute values in the calculations (*vide infra*). Transepithelial (R_{ts}), apical barrier (R_{tc}), and basal barrier (R_{cs}) resistances (lumped parameters), and the voltage divider ratio were calculated from the voltage responses of the epithelium to 2.0–5.0 μA·cm^{-2} square current pulses (I_e) of 1.0 sec duration applied at intervals of 4–5 sec

Determination of Component Resistances

General Assumptions. For the purposes of estimating component resistances by any of the three methods employed here, it is assumed that the corneal epithelium is a functional syncytium of similar cells except at the juncture of the basal cells (those adjacent to the basal lamina) and the deep wing cells (those immediately anterior to the basal cells); these two cell layers are incompletely coupled (Klyce, 1972). The resistance at this location is small (400–600 Ω·cm^2) compared to total corneal resistance (6–12 kΩ·cm^2). Membrane and shunt potentials are assumed to comprise an electrogenic component, an ohmic component, and an ion diffusion component (Klyce, 1973), where the latter can be described by the "constant field equation" (Goldman, 1943). Also, the basal barrier resistance is a lumped parameter, which reflects the equivalent resistance of all conductive pathways between the microelectrode and the stromal-side bath, to include the stroma and endothelium. The contribution of the later two components is small (50–100 Ω·cm^2; Klyce, 1972).

As in other studies of this type, it is necessary to minimize the possible contribution of peripheral "edge damage" in the preparation, as this will be reflected as a part of the shunt measurement. For this reason, we used as large a surface area as was practical (1.0 cm^2), a gentle mounting procedure for the *in vitro* preparation (Klyce, Neufeld & Zadunaisky, 1973), and a protective coating of stopcock grease (Corning High Vacuum) where the epithelium contacted the hemichamber surface. Also, only those epithelia which developed at least 20 mV transcorneal potential and 6.0 kΩ·cm^2 transcorneal resistance within the first hour of incubation in normal Ringer's solution were considered acceptable for the experiments. For the microelectrode techniques, there is the possibility of current leakage around the shank of the electrode if the impaled membrane does not seal properly. To minimize this effect, which would tend to reduce the estimated value of apical membrane resistance, only those stable impalements that conformed to previously established electrical criteria (Klyce, 1972) were accepted. The latter assumption is tested here, in that both "noninvasive" and microelectrode techniques should yield similar estimates of cellular and shunt resistances, if the microelectrodes are properly sealed.

In those experiments to determine the component resistances where apical membrane resistance was modified, it is assumed that intracellular ion composition is in steady-state during the treatment (*cf.* Klyce & Wong, 1977). For this reason, only small doses of epinephrine and Ag^+ were used throughout the experimental series. Otherwise, changes in intracellular ion concentration, which may occur during marked stimulation of ion transport by the tissue, could alter the apparent resistances of the apical membrane and basal barrier, hence leading to inaccurate estimates of shunt resistance.

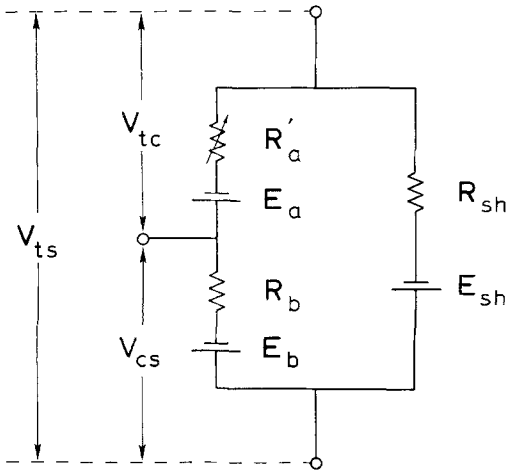

Fig. 1. The Thévenin equivalent circuit depicted here includes conductive pathways in the epithelium where subscripts *t* and *s* refer to the tear side and stromal side bathing solutions (respectively), subscript *c* refers to the intracellular compartment, and subscripts *a*, *b* and *sh* reflect apical membrane, basolateral barrier, and shunt pathways, respectively. R'_a is depicted as a variable resistor because epinephrine and Ag^+ specifically modify ion transport at this location. This circuit was used for component resistance measurement by *methods 1* and *2*

Method 1. For this approach (*cf.* Reuss & Finn, 1974), the corneal epithelium was modeled using a simple Thévenin equivalent circuit (Fig. 1), with the paracellular pathway resistance in parallel to the serially-arranged apical membrane and basal barrier resistances. To obtain data for the calculation of the component resistances, individual cells were impaled and the epithelium was treated with epinephrine or $AgNO_3$ and reduced glutathione. Most intracellular recordings were from basal cells. During each 15–45 min impalement, the apical surface of the cornea could be treated repeatedly with epinephrine (final concentration $=2\times10^{-10}$ M). Alternatively, the epithelium was exposed (once) to Ag^+ (final concentration $=2\times10^{-8}$ M), followed by reduced glutathione (2×10^{-6} M final concentration). Epinephrine stimulates only Cl^- transport in the rabbit cornea (Klyce et al., 1973). If this agent is added in small pulses (as above), it specifically increases the conductance of the apical membrane to Cl^- without apparently altering intracellular ion composition nor activating electrogenic transport processes (Klyce & Wong, 1977). Ag^+ stimulates sodium uptake in the corneal epithelium by increasing cation conductance of the apical barrier (Klyce & Marshall, 1982). The increased current is largely the result of increased Na^+ uptake, although there may be a small (<1%) K^+-dependent component.

The resistances of the apical membrane (R_a), basolateral barrier (R_b), and paracellular pathway (R_{sh}) were calculated according to Reuss and Finn (1974),

$$R_b=\frac{(\alpha-\alpha')\,R_{ts}\cdot R'_{ts}}{[(\alpha+1)(\alpha'+1)(R_{ts}-R'_{ts})]} \tag{1}$$

where $\alpha=\Delta V_{tc}/\Delta V_{cs}$, the voltage divider ratio, V_{ts} is the transcorneal potential and $R_{ts}=\Delta V_{ts}/I_e$, the transcorneal resistance. The superscript ′ refers to the new value of a variable at the peak of a response to an agent. Also,

$$R_a=\alpha\cdot R_b \tag{2}$$

$$R'_a=\alpha'\cdot R_b \tag{3}$$

and

$$R_{sh}=1/[1/R_{ts}-(1/R'_a+R_b)] \tag{4}$$

where R_a is the apical membrane resistance and R_{sh} is the resistance of shunt.

The contribution of anions and cations to the cellular resistance analogues (Fig. 1) was also estimated using this perturbation technique. With the depletion of major permeant anions (Cl^- and HCO_3^-) by total SO_4^{2-} substitution, the corneal epithelium continues to transport Na^+ actively, at a low rate, from tears (apical) to stromal (basal) side (Klyce et al., 1973). Under these conditions, the residual apical and basolateral membrane conductances may be taken to reflect that of cations, assuming that cellular conductance to SO_4^{2-} is negligible. As epinephrine-sensitive Cl^- transport is absent in sulfate Ringer's, Ag^+ (at 4×10^{-9} M final concentration) was used as the perturbing agent. The estimated resistance of the tissue to anions (R^-) was calculated from the formula for two resistors in parallel, *viz.*

$$R^-=(R^+\cdot R^{+,-})/(R^+-R^{+,-}) \tag{5}$$

where $R^{+,-}$ is the resistance measured in normal Ringer's and R^+ is that measured in low-Cl^-, low-HCO_3^- solution. In this way, the cellular resistor analogues in the Thévenin equivalent circuit (Fig. 1) may be partitioned into parallel resistors for anions and cations.

Method 2. The normal voltage profile of the corneal epithelium includes a small (2–10 mV) drop in the resting potential between the wing cell layers and the basal cell layer (Klyce, 1972). This voltage drop (V_{wb}) is apparently an ohmic result of the open-circuit loop current: spontaneous fluctuations in V_{wb} vary linearly with the measured resistance at this location (R_{wb}) such that extrapolation of the linear regression of R_{wb} *vs.* V_{wb} predicts V_{wb} is nil at $R_{wb}=0.0$ (*cf.* Klyce, 1972). We may therefore estimate the loop current (I_l) as V_{wb}/R_{wb}. In consideration of the resistance between the wing and basal cell layers, the Thévenin equivalent circuit used in this derivation partitions the basal barrier resistance into R_{wb} and the rest of the contributing basal resistance (including that of the basolateral membrane). The calculated loop current, and other appropriate information from the votage/resistance profile of the epithelium, are used to calculate the component resistances of the epithelium including R_a, R_{sh} and R_b, according to the following equations (Klyce, 1972):

$$V_{wb}=V_{cs}-V_{tw}-V_{ts} \tag{6}$$

$$E_a=[V_{tc}\cdot R_{ts}+(V_{ts}-V_{cs})]/[R_{ts}-R_{tc}] \tag{7}$$

$$R_a=[(V_{cs}-E_a)(V_{tc}-E_a)\,R_{ts}]/[(V_{cs}-E_a-V_{ts})V_{ts}] \tag{8}$$

$$R_b=R_a[(R_{ts}-R_{tc})/R_{tc}] \tag{9}$$

and

$$R_{sh}=[R_{ts}(R_a+R_b)]/[R_a+R_b-R_{ts}] \tag{10}$$

where V_{tw} is the intracellular potential in the wing cell layer referenced to the tears side, V_{cs} is the potential in the basal cell layer with reference to the stromal side, and E_a is the estimated ion diffusion component of the apical membrane voltage.

Method 3. An estimate of paracellular pathway resistance may also be obtained using a "noninvasive" technique wherein the Thévenin equivalent circuit is simplified (Fig. 2) (Klyce, 1971; Yonath & Civan, 1971). In this circuit analogue, a possible emf in the paracellular pathway is ignored, the serial cellular pathway resistances are lumped into a single element (R_{ser}),

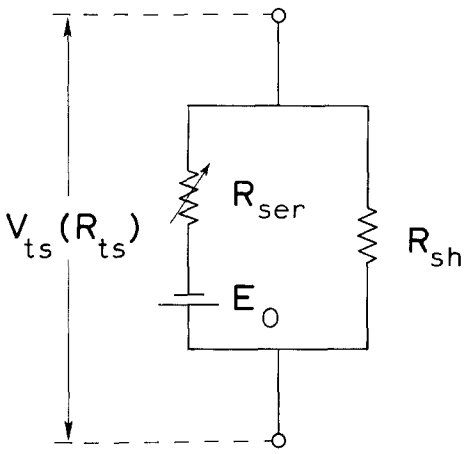

Fig. 2. This simplified Thèvenin equivalent circuit includes the shunt (R_{sh}) and R_{ser}, the lumped resistance of the cellular pathway, and a single electromotive driving force (E_o). This circuit was used for shunt measurements by *method 3*

and a single potential driving force for ion flows is included as E_o. In differential form, the effect of a specific change in R_{ser}, such as that produced by epinephrine or Ag^+, on the circuit (Fig. 2) can be written:

$$(dV_{ts}/dR_{ts})_{R_{sh}} = (\partial V_{ts}/\partial R_{ser})(\partial R_{ser}/\partial R_{ts}) \quad (11)$$

when R_{sh} is constant. Simple analysis of the circuit reveals:

$$V_{ts} = E_o R_{sh}/(R_{ser} + R_{sh}) \quad (12)$$

and

$$R_{ts} = R_{ser}\, R_{sh}/(R_{ser} + R_{sh}). \quad (13)$$

The integral form of Eq. (11) can be written

$$(V_{ts})_{R_{sh}} = -(E_o/R_{sh})R_{ts} + E_o. \quad (14)$$

Converting to conductances and rearranging, Eq. (14) becomes

$$G_{ts} = G_{ts} V_{ts}/E_o + G_{sh} \quad (15)$$

which can be used to determine E_o and G_{sh} as the slope and intercept, respectively, from the linear relationship between G_{ts} and $G_{ts}V_{ts}$, the latter expression being equivalent to the "quasi-instantaneous"[1] short-circuit current across the tissue.

Data Analysis

Statistical treatment of the data from *method 1* used no more than two individual determinations of the component resistances from each cell impalement and no more than four determinations from each corneal preparation to avoid over-weighting of any particular impalement or cornea in the experimental series. Similarly, the results of *method 2* also were limited to four individual voltage/resistance profiles for any one corneal preparation. Results of *method 3* comprised one linear regression of G_{ts} *vs.* $G_{ts}V_{ts}$ per corneal preparation. Data are expressed as the mean ± 1 SE.

[1] The "quasi-instantaneous" short-circuit current is defined as $G_{ts}V_{ts}$ where V_{ts} is the resting epithelial potential and G_{ts} is measured as $\Delta I_e/\Delta V_{ts}$ for a 1 sec transcorneal current pulse. Steady-state short-circuit current is substantially smaller than the above, as a result of polarization effects (*cf.* Kidder & Rehm, 1970).

Results

Method 1

Epinephrine Experiments. The shunt resistance calculated from the epinephrine experiments was significantly smaller than that of the apical membrane before and after epinephrine addition (Table 1), indicating that the major barrier to solute diffusion across the epithelium was the apical membrane, at least in these sub-maximally stimulated corneas. Basal barrier resistance was relatively lower than apical membrane or shunt resistances. It should be pointed out that the epinephrine dose used here was near the threshold of the response, and that higher doses, near the maximal, would reduce R_a more markedly, and could result in apical membrane resistances smaller than that of the shunt.

Ag^+ Experiments in Normal Ringer's Solution. The addition of Ag^+ to the tear-side bathing solution rapidly reduced the resistance measured across the apical barrier and primarily depolarized V_{tc}; the voltage across the basal barrier was essentially unchanged for the first 10–20 min after the addition of Ag^+, as observed previously (Klyce & Marshall, 1982). Prolonged exposure of the tissue to Ag^+ eventually results in depolarization of the basal barrier, suggesting (as above) changes in cellular ion composition. In this case, the depolarization likely reflects depletion of intracellular potassium and accumulation of intracellular sodium. In this later stage of the Ag^+ effect, there is also evidence to suggest a nonspecific increase in solute permeability of the paracellular pathway (Klyce & Marshall, 1982). Because we were primarily concerned with a determination of the normal component resistances of the epithelium, the Ag^+ effect was reversed after 10 min, i.e. before there was significant depolarization of the basal barrier or alteration of shunt permeability.

The calculated resistances derived from the experiments using Ag^+ compared well with those from the epinephrine experiments (Table 1), and the apical membrane again appeared to be the major barrier to solute permeation. The reduction in apical membrane resistance after the Ag^+ treatment was more marked than that observed with epinephrine, but this is probably only a dose-related difference.

The addition of reduced glutathione to Ag^+-treated corneas largely reversed the Ag^+ effect. The calculation of the component resistances based on the effect of reduced glutathione suggests a total restoration of the normally-low apical membrane permeability (Table 1).

Table 1. Component resistances of the corneal epithelium: Method 1

Ringer's sol'n	Treatment (conc. M)	n	Resistances ($k\Omega \cdot cm^2$)				
			R_{ts}	R_a	R'_a	R_b	R_{sh}
Normal	epinephrine (2×10^{-10} M)	87	7.9±0.2	32.6± 2.0	18.3±1.2	3.1±0.2	11.5±0.4
Normal	Ag^+ (2×10^{-8} M)	6	8.9±0.4	27.2± 2.7	13.2±1.5	2.8±0.6	13.2±1.2
Normal	Ag^+, GSH (2×10^{-6} M)	6	6.7±0.2	13.7± 2.8	31.3±6.9	4.0±1.2	13.2±1.9
Low Cl^- Low HCO_3^-	Ag^+ (4×10^{-9} M)	11	15.6±1.2[b]	90.2±10.6[c]	65.8±6.1[c]	4.3±0.6	19.3±1.7[a]

Abbreviations: n, number of impalements; R, resistance; R_{ts}, transcorneal; R_a, of apical membrane, initial; R'_a, of apical membrane at peak of effect; R_b, of basal barrier; R_{sh}, of shunt; Ag^+, $AgNO_3$; GSH, reduced glutathione. Shown are t-test comparisons of Ag^+ effect in normal and low Cl^- Ringer's solutions: [a] $P<0.05$; [b] $P<0.01$; [c] $P<0.001$.

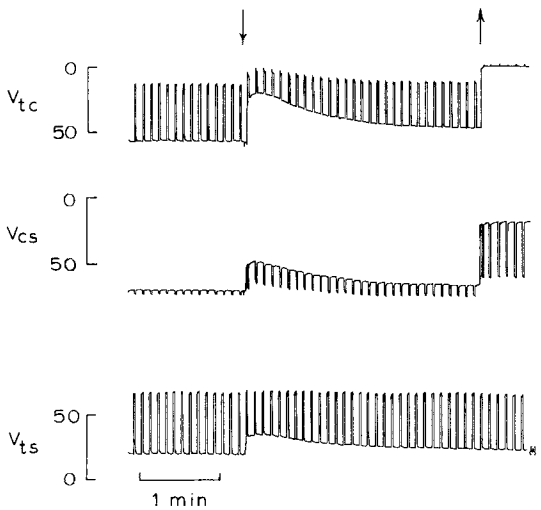

Fig. 3. Example of the effect of a relatively large dose (2×10^{-8} M, final concentration) of Ag^+ (first arrow) on the resting potential measured across the apical membrane, basal barrier, and whole cornea (V_{tc}, V_{cs}, and V_{ts}, respectively) with the preparation bathed in low-Cl^-, low-HCO_3^- Ringer's solution. The marked depolarization after the addition of Ag^+ suggests changes in intracellular ion composition (*cf.* text). After reversal of the Ag^+ response by continous superfusion of the tear-side bath, the microelectrode was withdrawn (second arrow) to the tear-side bathing solution. Voltage deflections are in response to 5.0 μA transcorneal current pulses. (Redrawn from original)

Hence, the estimated values for shunt and membrane resistances are strengthened, inasmuch as perturbations of apical membrane anion *or* cation permeability yielded comparable results.

Ag^+ Experiments in Low-Cl^-, Low-HCO_3^- Solution. The resistances of the paracellular pathway, apical membrane, and of the whole cornea were significantly elevated in low-Cl^-, low-HCO_3^- Ringer's solution, compared to the respective values determined using Ag^+ in normal Ringer's solution (Table 1). The large increase in apical membrane resistance in the modified Ringer's suggests that this barrier is anion selective. Using Eq. (5) and the mean data from Table 1, the apical membrane resistance to anions (Cl^- and HCO_3^-) was estimated to be 38.5 $k\Omega \cdot cm^2$, and that to cations (Na^+ and K^+) was 90.2 $k\Omega \cdot cm^2$. Hence the apical membrane appears to be anion-selective. Whereas the resistance of the basal barrier was not significantly affected by the low-Cl^-/low-HCO_3^- bathing solution, there was a trend to higher resistance in the modified bathing solution. Little can be concluded at this time with regard to the selectivity of the paracellular pathway, inasmuch as the relative permeabilities of the shunt to Cl^- and to SO_4^{2-} are not known.

When high doses of Ag^+ were used in low-Cl^-, low-HCO_3^- Ringer's, there was a rapid, marked depolarization of both V_{tc} and V_{cs} (Fig. 3). This effect was in sharp contrast to that of Ag^+ in normal Ringer's (*cf.* Fig. 10 in Klyce & Marshall, 1982), where V_{tc} depolarized more slowly and the change in V_{cs} was much smaller. To account for this difference, we calculated the expected depolarization of V_{cs} in the ion-substituted Ringer's, assuming that the change in V_{cs} was an IR drop resulting from the change in I_l after Ag^+ treatment. The expected change in V_{cs} (13.4 mV) was less than the observed value (24 mV), suggesting that part of the depolarization of V_{cs} resulted from a change in the ion diffusion component, i.e., that intracellular ion distribution was altered by Ag^+. This likely reflects K^+ depletion and/or Na^+ accumulation

Table 2. Electrical parameters from voltage/resistance profiles of the corneal epithelium ($n=121$)

	Potentials (mV)					Resistances (kΩ·cm²)			α
	V_{ts}	V_{tc}	V_{wb}	V_{cs}	E_a	R_{tc}	R_{wb}	R_{cs}	
Mean	21.4	50.9	6.0	78.2	13.1	6.2	0.6	0.7	8.9
SEM	0.8	1.0	0.4	0.6	3.2	0.1	0.05	0.04	0.7

Abbreviations: n, number of impalements. Subscripts: t, tear side; s, stromal side; c, intracellular; w, wing cell layer; b, basal cell layer. E_a, battery emf of apical membrane; α, voltage divider ratio ($\Delta V_{tc}/\Delta V_{cs}$).

Table 3. Component resistances of the corneal epithelium: Method 2

n	Resistances (kΩ·cm²)			
	R_{ts}	R_a	R_b	R_{sh}
121	7.5±0.2	23.4±1.8	3.8±0.2	15.4±1.0

Abbreviations: n, number of impalements; R, resistance; R_{ts}, transcorneal; R_a, of apical membrane; R_b, of basal barrier; R_{sh}, of shunt.

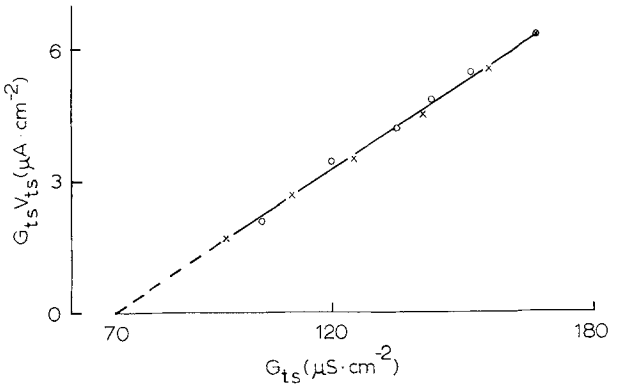

Fig. 4. Example of the effect of a large dose of Ag^+ (4×10^{-6} M final concentration) on the "instantaneous" short-circuit current ($G_{ts}V_{ts}$) and corneal conductance (G_{ts}), with the tissue bathed in normal Ringer's solution. Shown is the least squares line of best fit through points during the development (×) of the Ag^+ response and its reversal (o) by reduced glutathione (2×10^{-5} M, final concentration)

intracellularly, considering the marked effects of Ag^+ on cation transport (Klyce & Marshall, 1982). Of interest is that low-Cl^-, low-HCO_3^- Ringer's apparently impairs the ability of the cell to maintain intracellular ion composition when exposed to Ag^+. This argument underscores the importance of using only small perturbations of membrane permeability in the determination of component resistances.

Method 2

Voltage/Resistance Profiles. Electrical parameters of the corneal epithelium from voltage/resistance profiles (Table 2) are in general agreement with those reported previously (Klyce, 1972), with the exception of the larger transcorneal resistances in the present work. The higher resistances are apparently a result of improved mounting procedures used here, as per Klyce and Wong (1977). In accord with Klyce (1972), the major resistance was seen as the microelectrode entered the outer cell layer of the epithelium; about 70% of total transcorneal resistance was associated with this cell membrane. Whereas the 4–5 outer cell layers (squamous and wing cells) were essentially isopotential, there was a small voltage drop (V_{wb}) which was observed when the microelectrode penetrated to the basal cell layer. V_{wb} was associated with a resistance (R_{wb}) of about 600 Ω·cm² (Table 2); these parameters were used to calculate the open-circuit loop current (V_{wb}/R_{wb}).

Component Resistances. The calculated resistances for resting corneal epithelia using *method 2* (Table 3) were comparable to those of *method 1* (Table 1). Parenthetically, the apical membrane resistance calculated here should be compared not to the corresponding estimate prior to the addition of epinephrine or Ag^+, but to that *after* the addition of reduced glutathione. A "noninvasive" technique, one not requiring intracellular recordings, was used to confirm the estimates of R_{sh} by the previous two methods.

Method 3

The plot of G_{ts} *vs.* $G_{ts}V_{ts}$ for a cornea treated with Ag^+ (Fig. 4) depicts a linear relationship, as would be expected from a specific increase in the conductance of the cellular pathway. It would also suggest that Ag^+ treatment, at least during the first 30 min of the effect, did not significantly change the conductance of the shunt, in accord with our previous findings. The effect of Ag^+ was entirely reversible, in that the line traced by the onset of the Ag^+ response was superimposable on the line from the subsequent reversal of the effect, i.e., after addition of reduced glutathione (Fig. 4). The shunt conductance from this example is equivalent to a resistance of 14.3 kΩ·cm². Table 4 summarizes data from plots similar to Fig. 3 for both epinephrine- and Ag^+-treated corneas.

The shunt conductances estimated using the present method (Table 4) were comparable to the measured shunt conductances from the other two

Table 4. Shunt resistance and effective emf from plots of G_{ts} *vs.* G_{ts} V_{ts}: Method 3

Treatment (dose)	*n*	R_{sh} (kΩ·cm²)	E_o (mV)
Epinephrine (10^{-10}–10^{-9} M)	13	16.4±2.2	48±2
Ag^+ (10^{-8}–10^{-6} M)	6	11.9±1.4	70±2

Abbreviations: n, number of corneas; G_{sh}, shunt conductance; R_{sh}, shunt resistance; E_o, effective emf of the cellular pathway.

methods (*compare* with Tables 1 and 3). The estimate of E_o for the epinephrine-treated corneas agrees with previous estimates of the electrochemical potential gradient for Cl^- across the apical membrane (Klyce & Wong, 1977), and that estimated by Nagel and Reinach (1980) for bullfrog corneal epithelium (about 45 mV). If, instead, the E_o estimates originate from variation in sodium transport, as with the Ag^+-treated corneas, E_o approximates the basal barrier emf, about 70 mV (Table 4), consistent with previous estimates of this parameter (Klyce, 1971). Although the slope ($1/E_o$) for the Ag^+ treatment is significantly different from that of the epinephrine treatment ($P<0.01$), the intercepts (G_{sh}) show more variation and are not significantly different. The greater variation of the intercept is to be expected, as this is an extrinsic property (i.e., extrapolation) of the data.

Discussion

The present results using three different experimental approaches indicate that each apparently can yield a relatively accurate estimate of shunt resistance. The agreement of the combined results of the first two techniques, involving intracellular recordings (*methods 1* and *2*), with the results using the noninvasive technique (*method 3*), suggests that good membrane seals were maintained around the barrel of the microelectrode in the intracellular measurements. In *method 1*, the results were apparently independent of the agent which was used to perturb membrane conductance, suggesting that an agent which alters transmembrane anion or cation conductance may be used to determine shunt and cellular resistances, given that the agent has been adequately tested for specificity of action on the conductance of one resistive barrier. The present work also demonstrates the utility of Ag^+ as a tool for investigation of ion transport processes by epithelia. In general, Ag^+ may be useful to study ion transport in tissues that normally have low cation permeability, or in amiloride-treated cation-transporting epithelia. The shunt resistance determined here (Tables 1, 3 and 4) approximates that estimated from the (diffusional) backfluxes of Na^+ and Cl^- at open circuit (15–17 kΩ·cm², Klyce, 1975). The slightly higher value for R_{sh} from the flux estimate is expected if the shunt is permeable to ionic species in addition to Na^+ and Cl^-. Because there is apparently no excess conductive flux of ions other than that through the shunt, it is likely that much of the passive transepithelial flux of Na^+ and Cl^- is paracellular.

Shunt and basolateral membrane resistances measured for the corneal epithelium (Tables 1, 3 and 4) are comparable to those of toad urinary bladder [10–20 kΩ·cm² and 3–5 kΩ·cm², respectively (Reuss & Finn, 1974, 1975; Narvarte & Finn, 1980*a, b*)]. Hence it would seem that anion- and cation-transporting tight epithelia both have high-resistance shunts and lower resistance at the basolateral membranes. Relatively more permeable epithelia, such as rabbit descending colon, also show a similar relationship between shunt resistance and that of the basolateral membrane [0.73 and 0.10 kΩ·cm² for the shunt and basolateral barriers, respectively (Wills, Lewis & Eaton, 1979)]. The contributing factors that could account for the high basal membrane conductance of epithelial cells include the large surface area of the basolateral membrane and possibly high potassium permeability at this location.

Apical membrane resistances for the normal corneal epithelium (Tables 1 and 3) are as much as ten times greater than those observed for toad urinary bladder [3–5 kΩ·cm² (Reuss & Finn, 1974, 1975; Narvarte & Finn, 1980*a, b*)]. This difference may be a result of the apparent lack of amiloride-sensitive Na^+ channels in the apical membranes of corneal epithelial cells, as this tissue is unresponsive to amiloride (S.D. Klyce & W.S. Marshall, *unpublished*). If instead we compare apical membrane resistance of the normal corneal epithelium (Tables 1 and 3) with that of the toad urinary bladder *after* amiloride, i.e., in the absence of Na^+-conductive channels in the apical membrane [about 17 kΩ·cm² (Narvarte & Finn, 1980*b*)], then there is better agreement between the apical membrane resistances of the two epithelia. In epithelia with spontaneously low sodium transport rates, such as the rabbit urinary bladder, apical membrane resistances are very high [>100 kΩ·μF (Lewis, Eaton & Diamond, 1976)], and, as seen here for the corneal epithelium, are greater than the shunt resistance.

Lewis et al. (1976) have shown that the transport rate (as I_{sc}) of the rabbit urinary bladder varies directly with apical membrane conductance.

In addition, the physiological modulation of apical membrane permeability is the means by which some hormones and neurotransmitters control epithelial transport rates. Examples include the increase in apical membrane conductance to Cl^- in the corneal epithelium by epinephrine (Klyce & Wong, 1977; Nagel & Reinach, 1980) and by serotonin (Marshall & Klyce, 1982; Klyce et al., 1982), and the opening of apical membrane cation conductance in amphibian skin by aldosterone (e.g., Crabbé, 1980) and vasopressin (e.g., Nagel, 1978).

The able assistance of K.P. Mann and M.B. Rutter is gratefully acknowledged. Major support for the work was from the National Eye Institute research grant EY03311. Calculations were implemented on the LSU/Lions Eye Research Computer Facility, which is supported by grants from the Louisiana Lions Eye Foundation and which operates under the UNIX operating system, courtesy of Bell Laboratories.

References

Crabbé, J. 1980. Decreased sensitivity to amiloride of amphibian epithelia treated with aldosterone. *Pfluegers Arch.* **383**:151–158

Diamond, J.M. 1978. Channels in epithelial cell membranes and junctions. *Fed. Proc.* **37**:2639–2644

Frömter, E., Diamond, J. 1972. Route of passive ion permeation in epithelia. *Nature New Biol.* **235**:9–13

Goldman, D.E. 1943. Potential, impedance, and rectification in membranes. *J. Gen. Physiol.* **27**:37–60

Kidder, G.W., Rehm, W.S. 1970. A model for the long time constant transient voltage response to current in epithelial tissues. *Biophys. J.* **10**:215–236

Klyce, S.D. 1971. Electrophysiology of the corneal epithelium. Ph.D. Thesis. Department of Physiology, Yale University, New Haven

Klyce, S.D. 1972. Electrical profiles in the corneal epithelium. *J. Physiol. (London)* **226**:407–429

Klyce, S.D. 1973. Relationship of epithelial membrane potentials to corneal potential. *Exp. Eye Res.* **15**:567–575

Klyce, S.D. 1975. Transport of Na, Cl, and water by the rabbit corneal epithelium at resting potential. *Am. J. Physiol.* **228**:1446–1452

Klyce, S.D., Neufeld, A.H., Zadunaisky, J.A. 1973. The activation of chloride transport by epinephrine and Db cyclic-AMP in the cornea of the rabbit. *Invest. Ophthalmol.* **12**:127–139

Klyce, S.D., Palkama, K.A., Härkönen, M., Marshall, W.S., Huhtaniitty, S., Mann, K.P., Neufeld, A.H. 1982. Neural serotonin stimulates chloride transport in the rabbit corneal epithelium. *Invest. Ophthalmol. Vis. Sci.* **23**:181–192

Klyce, S.D., Wong, R.K.S. 1977. Site and mode of adrenaline action on chloride transport across the rabbit corneal epithelium. *J. Physiol. (London)* **266**:777–799

Klyce, S.D., Marshall, W.S. 1982. Effects of Ag^+ on ion transport by the corneal epithelium of the rabbit. *J. Membrane Biol.* **66**:133–144

Lewis, S.A., Diamond, J.M. 1976. Na^+ transport by rabbit urinary bladder, a tight epithelium. *J. Membrane Biol.* **28**:1–40

Lewis, S.A., Eaton, D.C., Clausen, C., Diamond, J.M. 1977. Nystatin as a probe for investigating the electrical properties of a tight epithelium. *J. Gen. Physiol.* **70**:427–440

Lewis, S.A., Eaton, D.C., Diamond, J.M. 1976. The mechanism of Na^+ transport by rabbit urinary bladder. *J. Membrane Biol.* **28**:41–70

Marshall, W.S., Klyce, S.D. 1981*a*. Membrane resistances in rabbit corneal epithelium. *Fed. Proc.* **40**:370 (*abstr.*)

Marshall, W.S., Klyce, S.D. 1981*b*. Cell finder speeds impalements with microelectrodes. *Pfluegers Arch.* **391**: 258–259

Marshall, W.S., Klyce, S.D. 1982. Transport effects of serotonin in rabbit corneal epithelium. *Fed. Proc.* **41**:1263 (*abstr.*)

Nagel, W. 1978. Effects of antidiuretic hormone upon electrical potential and resistance of apical and basolateral membranes of frog skin. *J. Membrane Biol.* **42**:99–122

Nagel, W., Reinach, P. 1980. Mechanism of stimulation by epinephrine of active transepithelial Cl transport in isolated frog cornea. *J. Membrane Biol.* **56**:73–79

Narvarte, J., Finn, A.L. 1980*a*. Microelectrode studies in toad urinary bladder epithelium. Effects of Na concentration changes in the mucosal solution on equivalent electromotive forces. *J. Gen. Physiol.* **75**:323–344

Narvarte, J., Finn, A.L. 1980*b*. Anion-sensitive sodium conductance in the apical membrane of toad urinary bladder. *J. Gen. Physiol.* **76**:69–81

Reuss, L., Finn, A.L. 1974. Passive electrical properties of toad urinary bladder: Intercellular electrical coupling and transepithelial and shunt conductances. *J. Gen. Physiol.* **64**:1–25

Reuss, L., Finn, A.L. 1975. Effects of changes in the composition of the mucosal solution in the electrical properties of the toad urinary bladder epithelium. *J. Membrane Biol.* **20**:191–204

Wills, N.K., Lewis, S.A., Eaton, D.C. 1979. Active and passive properties of rabbit descending colon: A microelectrode and nystatin study. *J. Membrane Biol.* **45**:81–108

Yonath, J., Civan, M.M. 1971. Determination of the driving force of the Na^+ pump in the toad bladder by means of vasopressin. *J. Membrane Biol.* **5**:366–385

Received 7 September 1982; revised 2 December 1982