Characterization of the Basolateral Membrane Conductance of *Necturus* Urinary Bladder

JEFFERY R. DEMAREST and ARTHUR L. FINN

From the Departments of Medicine and Physiology, University of North Carolina School of Medicine, Chapel Hill, North Carolina 27514

ABSTRACT *Necturus* urinary bladders stripped of serosal muscle and connective tissue were impaled through their basolateral membranes with microelectrodes in experiments that permitted rapid changes in the ion composition of the serosal solution. The transepithelial electrical properties exhibited a marked seasonal variation that could be attributed to variations in the conductance of the shunt pathway, apical membrane selectivity, and basolateral Na^+ transport. In contrast, the passive electrical properties of the basolateral membrane remained constant throughout the year. The apparent transference numbers (T_i) of the basolateral membrane for K^+ and Cl^- were determined from the effect on the basolateral membrane equivalent electromotive force of a sudden increase in the serosal K^+ concentration from 2.5 to 50 mM/liter or a decrease in the Cl^- concentration from 101 to 10 mM/liter. T_K and T_{Cl} were 0.71 ± 0.05 and 0.04 ± 0.01, respectively. The basolateral K^+ conductance could be blocked by Ba^{2+} (0.5 mM), Cs^+ (10 mM), or Rb^+ (10 mM), but was unaffected by 3,4-diaminopyridine (100 μM), decamethonium (100 μM), or tetraethylammonium (10 mM). We conclude that a highly selective K^+ conductance dominates the electrical properties of the basolateral membrane and that this conductance is different from those found in nerve and muscle membranes.

INTRODUCTION

With a few exceptions, the conductance of the basolateral membranes of epithelial cells is dominated by K^+ channels that appear to be similar to those found in the membranes of nerve and muscle cells (Van Driessche and Zeiske, 1985). The Cl^- conductance of the basolateral membranes is more variable, ranging from a substantial portion of the total conductance to a small or nonexistent fraction (e.g., Lewis et al., 1978; Reuss, 1979).

Recently, Schultz et al. (1984*a*, *b*) analyzed the current-voltage relationship of the basolateral membrane of *Necturus* urinary bladder. From the low value of the basolateral electromotive force, E_b, that they obtained, they concluded that the Cl^- conductance of this membrane is large and may even exceed the

Address reprint requests to Dr. Arthur L. Finn, Depts. of Medicine and Physiology, University of North Carolina at Chapel Hill, Old Clinic Bldg. 226H, Chapel Hill, NC 27514. Dr. Demarest's present address is Physiology-Anatomy Dept., University of California, Berkeley, CA 94720.

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membrane's K^+ conductance. These authors pointed out that if Cl^- were assumed to be passively distributed across the basolateral membrane, their value for the E_b in short-circuited bladders would be consistent with a P_{Cl}/P_K of 0.8. However, intracellular Cl^- has been found to be above equilibrium with respect to the serosal bathing solution in a wide variety of epithelial cells (Nagel et al., 1981; Oberleithner et al., 1983; Reuss and Weinman, 1979; White, 1977), though not all (Lewis et al., 1978). We have made a direct test of the hypothesis advanced by Schultz et al. (1984*a*) by performing ion substitution experiments.

Because of diffusion delays caused by the subepithelial connective and muscle tissue, most electrophysiological investigations in flat-sheet epithelia of the effects of changes in the ion composition of the basolateral bathing solution have been limited to steady state measurements (Lindley and Hoshiko, 1964; Gatzy and Clarkson, 1965; Leb et al., 1965; Ramsay et al., 1976; Nagel, 1979). The interpretation of the results of these studies is complicated by the possible influence of secondary events, such as changes in cell volume and alterations of the intracellular ion composition. By using mechanical dissection of a small, exposed basolateral area of epithelial cells and rapid superfusion, we have reduced the time required for the completion of an intracellular electrical response to a change in the concentration of a single ion to <5 s. Using these techniques, we have directly evaluated the contribution of K^+ and Cl^- gradients to the E_b of *Necturus* urinary bladder. Our results indicate that the basolateral membrane of this tissue is predominantly conductive to K^+ and has only a small Cl^- conductance that accounts for no more than 4% of the total membrane conductance. These conductance properties of the basolateral membrane of the epithelium remain relatively constant throughout the year, while most of its other electrical properties exhibit marked seasonal variation. In addition, we have examined the effects of some of the better-known K^+ channel blockers on the basolateral K^+ conductance. A preliminary report of this study has been presented elsewhere (Demarest and Finn, 1984).

METHODS

Male *Necturus maculosus* (Nasco Biological Supply, Ft. Atkinson, WI) were kept in dechlorinated tap water in a temperature-controlled room at 12–14°C. Urinary bladders were removed from animals that had been killed by decapitation and pithed. The isolated bladders were placed in *Necturus* Ringer solution (see below), cut open to form a flat sheet, and mounted horizontally, serosal side up, in an open-topped Lucite chamber similar to that described previously (Reuss and Finn, 1974) but having a smaller exposed area (either 0.07 or 0.46 cm^2). The serosal muscle and connective tissue were removed from the basolateral surface of the epithelial cell layer using microdissecting scissors and a fine hook made from an insect pin, while the tissue was viewed at 60× through a dissecting microscope. When the larger area was used, the bladder was supported mechanically by a nylon mesh with 125-μm openings in which a 2–3-mm hole had been formed by the tip of a hot dissecting probe. The mesh was placed on the top (serosal) side of the tissue, which was held against the mesh by light hydrostatic pressure (3–4 cm H_2O) applied to the mucosal side of the tissue. No supporting mesh was used with the 0.07-cm^2-area chamber. Only this (the smaller) chamber was used in the experiments involving rapid serosal solution changes, since it was easier to remove the muscle and connective tissue from the entire exposed area of the epithelium. The closed bottom half of the

chamber facing the mucosal surface of the bladder had a volume of ~270 μl and was perfused at a rate of 3–4 ml/min by gravity feed from an overflowing reservoir, the level of which was set to maintain the desired hydrostatic pressure. The open upper compartment of the chamber was superfused by a gravity feed at a rate of 4–5 ml/min. This serosal bath was maintained at a depth of 3–4 mm, corresponding to a volume of ~200 μl by the appropriate positioning of a beveled aspiration tube fashioned from a Pasteur pipette that was placed opposite the perfusion inflow. The inflow was directed onto the exposed basolateral surface of the epithelium by short lengths of polyethylene tubing (i.d. = 0.047 in., Intermedic PE 190, Clay Adams, Parsippany, NJ). Switching between control and experimental solutions was controlled by miniature solenoid valves (The Lee Company, Westbrook, CT) that were mounted adjacent to the chamber on the stage of the inverted microscope (Diavert, E. Leitz, Inc., Rockleigh, NJ) used to view the epithelial cells at 320× with bright-field illumination.

Solutions

Necturus Ringer solution had the following composition (mM/liter): 95 NaCl, 10 $NaHCO_3$, 2.0 $CaCl_2$, 1.0 $MgCl_2$, 1.19 K_2HPO_4, 0.11 KH_2PO_4, 5 glucose. The total osmotic concentration was 210 mosmol/kg and the pH was 7.9 when equilibrated with 99% O_2, 1% CO_2. In solutions with higher-than-normal $[K^+]$, KCl was substituted for NaCl to obtain the concentrations indicated in the text. RbCl and CsCl were substituted for NaCl as indicated in the text. Na methylsulfate (electrical grade, ICN K&K Laboratories, Plainview, NY) was substituted for NaCl to make a 10 mM Cl^- solution. Amiloride (a gift from Merck, Sharp & Dohme Research Laboratories, West Point, PA) was dissolved in *Necturus* Ringer at a final concentration of 10^{-4} M. Decamethonium (DECA) and 3,4-diaminopyridine (3,4-DAP) (Sigma Chemical Co., St. Louis, MO) were dissolved in the experimental solutions at a final concentration of 100 μM/liter. Tetraethylammonium (TEA) Cl (Sigma Chemical Co.) was substituted for an equal amount of NaCl to give a final concentration of 10 mM/liter. The experiments were performed at room temperature (21 ± 1°C).

Electrical Measurements

The transepithelial potential (V_{ms}) was measured with reference to the grounded serosal bathing solution, using a high-impedance (>10^{14} Ω) electrometer connected to the solutions bathing the epithelium by 1-mm-diam sintered Ag/AgCl pellets (In Vivo Metrics, Healdsberg, CA) or, in the experiments with 10 mM Cl^- Ringer, by agar/Ringer bridges. The correction for the liquid junction potential between the bridge and the 10 mM/liter Cl^- Ringer was 5.4 ± 0.1 mV and was determined as previously described (Demarest, 1984). The transepithelial resistance (R_t) was calculated from the change in V_{ms} resulting from square current pulses (amplitude, 5 or 10 μA/cm^2; duration, 500 ms; frequency, 20 or 30/min) generated by a stimulus isolation unit and a stimulator (models 305-2 and 302T, W-P Instruments, Inc., New Haven, CT). Current was passed across the epithelium through platinum wires formed into rings positioned in the chamber baths on either side of the epithelium. With the exception of a few experiments as noted in the Results, measurements were made under open-circuit conditions and the short-circuit currents (I_{sc}) were calculated as V_{ms}/R_t.

The basolateral membrane potential (V_{cs}) was measured by impaling the epithelial cells through their basolateral membranes with microelectrodes pulled from 1.0-mm-o.d. borosilicate glass capillaries containing an internal glass fiber (Kwik-Fil, W-P Instruments, Inc.) and filled with 500 mM KCl. Microelectrodes had resistances of 100–170 MΩ and tip potentials of 5–7 mV with the tips immersed in *Necturus* Ringer. Similar electrodes

filled with 3 M KCl had resistances of 40–70 MΩ under the same conditions. Impalements were made using a motor-driven micromanipulator (model MM33 M, Stoelting Co., Chicago, IL). V_{cs} and the apical membrane potential ($V_{mc} = V_{ms} - V_{cs}$) were displayed on a storage oscilloscope (R5103N, Tektronix, Inc., Beaverton, OR), sampled every 60 ms by a computer (MED-80, Nicolet Instrument Corp., Madison, WI), and stored on floppy disks. The criteria for successful impalements were as previously described (Reuss and Finn, 1974).

The electrical measurements were analyzed according to the equivalent circuit model of the epithelium shown in Fig. 1. R_t was determined by the change in transepithelial voltage resulting from a transepithelial current pulse, and the ratio of the apical to basolateral membrane resistances, R_a/R_b, was given by the ratio of the voltage deflections in V_{mc} and V_{cs} produced by the same current pulses (Reuss and Finn, 1974). For the calculations, we used the average values of the voltage deflections between 120 and 300 ms after the start of the pulses. The shunt resistance, R_s, was estimated as the steady state

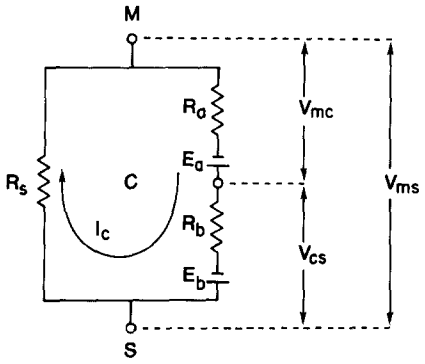

FIGURE 1. Equivalent circuit model of *Necturus* urinary bladder epithelium. The mucosal, cellular, and serosal compartments are designated by the letters M, C, and S. The apical and basolateral cell membranes are represented by electromotive forces E_a and E_b in series with resistances R_a and R_b. The resistance R_s represents the shunt pathway; the emf across the resistance was assumed to be zero (see text for details). The horizontal dashed lines connect the transepithelial (V_{ms}), apical (V_{mc}), and basolateral (V_{cs}) membrane potentials to the points in the circuit from which they were measured. The curved arrow labeled "I_c" represents the current that circulates within the epithelium under open-circuit conditions.

R_t after exposure of the mucosal surface of the bladder to 10^{-4} M amiloride (Ussing and Windhager, 1964). According to the equivalent circuit given in Fig. 1,

$$R_t = [(R_a/R_b)R_s]/(R_a + R_b + R_s). \quad (1)$$

Thus, from the measured values of R_t and R_a/R_b and the estimated value of R_s, the resistances of the apical and basolateral membranes can be calculated:

$$R_a = [(R_a/R_b)R_sR_t]/[(R_a/R_b + 1)\,(R_s - R_t)]; \quad (2)$$

$$R_b = (R_sR_t)/[(R_a/R_b + 1)\,(R_s - R_t)]. \quad (3)$$

Estimating R_s by the above procedure assumes that the transcellular conductance is completely blocked by 10^{-4} M amiloride and that R_s is itself unaffected by amiloride. These assumptions seem justified for the *Necturus* urinary bladder since amiloride (*a*)

virtually abolishes Na transport, i.e., I_{sc}, (*b*) increases R_a/R_b by 20-fold, and (*c*) has no significant effect on the serosal-to-mucosal flux of Na^+ (see Results).

Under open-circuit conditions, an internal current, I_i ($= V_{ms}/R_s$), circulates continuously within the epithelial circuit. Using the calculated values of the resistances in the circuit, the values of the equivalent electromotive forces (emf's) across the apical, E_a, and basolateral, E_b, membranes can be calculated from the measured membrane potentials by correcting for the influence of the internal current:

$$E_a = V_{mc} + I_i R_a \tag{4}$$

and

$$E_b = V_{cs} + I_i R_b. \tag{5}$$

Since measured slope resistances rather than chord resistances were used in the calculation of the membrane resistances, errors were potentially introduced in the calculated values of the membrane emf's (Finkelstein and Mauro, 1963). However, over the range of voltages in this study (±40 mV from open circuit), R_t, R_s, and R_b have been found to be ohmic; therefore, the chord and slope resistances are equal (Fromter et al., 1981; Thomas et al., 1983). Although this is not strictly true for R_a, the resulting errors over this restricted range of voltages under open circuit appear to be small (see Results).

The emf of the shunt pathway was assumed to be zero when the solutions on the two sides of the epithelium were identical; experiments employing nonsymmetrical solutions are discussed in the Results.

The change in E_b resulting from a sudden change in the concentration of an ion in the bathing solution has been compared with that predicted for the new transmembrane chemical gradient, and yields an apparent transference number for the ion, T_i (Strickholm and Wallin, 1967; Brown et al., 1970):

$$T_i = \Delta E_b / [(RT/zF)\ln(C_1/C_2)], \tag{6}$$

where ΔE_b is the change in E_b resulting from a change in the serosal control concentration of the ion, C_1, to a new concentration, C_2.

Flux Measurements

Transepithelial serosal-to-mucosal $^{22}Na^+$ fluxes were measured by a technique described previously (Finn and Bright, 1978), but using a chamber with a smaller exposed tissue area, 0.8 cm^2.

Statistics

All values are given as means ± standard error. Comparisons between means were made using the *t* test for paired or unpaired data, as appropriate.

RESULTS

Seasonal Dependence of the Shunt Resistance

Bladders were used over a 2.5-yr period from January 1982 to May 1984. The mean V_{ms} and R_t for 81 bladders on which successful cell impalements were made were −62.7 mV (range, −11 to −127 mV) and 2.72 kΩ·cm^2 (range, 1.08–8.57). However, there were seasonal trends in some of the electrical properties of the bladders. During the winter and early spring (November to March), the tissues had higher resistances and more negative transepithelial potentials. V_{ms} values

more negative than about −67 mV were associated with "stair-step" profiles of intracellular potential, i.e., $V_{mc} \leq 0$. These bladders have been designated as "winter" in Table I. More depolarized values of V_{ms} were characteristic of the late spring to early fall (April to October) and were associated with "well"-type potential profiles, i.e., $V_{mc} > 0$. These bladders have been designated as "summer" in Table I. It should be noted that we report here results only from bladders whose cells were successfully impaled, and no attempt was made to select tissues on any other basis. However, it was evident that bladders from so-called summer animals had, in general, lower transepithelial resistances than the winter preparations, and in addition were much harder to impale. In fact, we were able to impale only ~10% of those preparations; thus, the mean R_t and V_{ms} given in Table I are clearly higher than the true mean of the entire summer population.

The variation of V_{cs} was considerably smaller than that of V_{mc}; there was no difference between the mean values of V_{cs} for bladders with well profiles and those with stair-step profiles (Table I). Bladders with a low V_{ms} had lower R_t and

TABLE I

Seasonal Variation of Electrical Properties

(A)	V_{ms}	V_{mc}	V_{cs}	R_a/R_b	R_t	R_s
		mV			*kΩ·cm²*	*kΩ·cm²*
Winter (39)	−87.4±1.7	−21.8±1.4	−65.6±1.4	4.11±0.26	3.25±0.19	6.49±0.44
Summer (42)	−39.7±1.3	28.0±1.1	−67.7±0.7	4.50±0.22	2.22±0.15	3.57±0.17

(B)	R_a	R_b	E_a	E_b
	kΩ·cm²	*kΩ·cm²*	*mV*	*mV*
Winter	5.24	1.27	−92	−83
Summer	5.19	1.01	−24	−78

The measured values in part *A* are means ± SE (*n*). The values in part *B* were calculated from the means in *A*.

R_s values than the high-V_{ms} bladders (Table I). During the summer, R_s decreased relative to the control value of R_t. In a number of bladders, R_t did not increase at all after amiloride, even though R_a/R_b increased to its high post-amiloride level (see below and Fig. 2). The method used in this study to calculate the cell membrane resistances cannot be applied when R_t approaches R_s (that is, when amiloride has little or no effect on R_t), since R_a and R_b do not have finite limits in this case (see Eqs. 2 and 3). Therefore, only bladders for which R_t/R_s was <0.75 have been used to calculate the cell membrane resistances. Whereas all winter bladders met this criterion, 31% of summer bladders did not; therefore, the latter have been excluded from the calculations of cell membrane resistances. As indicated in Table I, these resistances remain relatively constant throughout the year. Although there is considerable scatter in the data, there is a significant positive correlation ($r = 0.444$, $P < 0.001$) between R_s and V_{mc} for the individual bladders of Table I (Fig. 2), as would be predicted from a consideration of the internal current flow in the equivalent circuit of Fig. 1 (Schultz, 1972). This relationship suggests that *Necturus* urinary bladders have a seasonally dependent

shunt conductance. However, this relationship can account for only part of the variation of V_{mc}, and there is a large difference between the E_a values of winter and summer bladders (Table I).

Effects of Amiloride

Exposure of the mucosal surface of the bladder to amiloride (10^{-4} M) (Fig. 3) reduced V_{ms} to a low but nonzero value; reversal of polarity was never observed.[1] The effect of amiloride on R_t was variable, as discussed above, ranging from a fourfold increase (R_t values as high as 19.5 k$\Omega \cdot cm^2$) to no effect at all. However, amiloride always reduced V_{ms} to a low value and always resulted in an ~20-fold

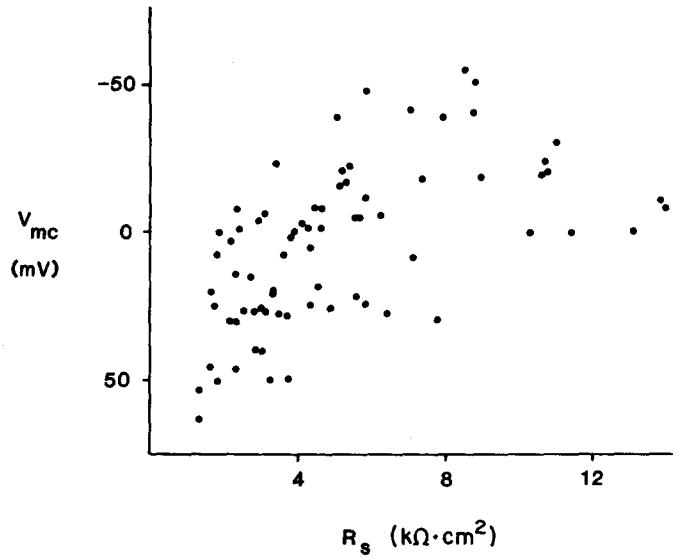

FIGURE 2. The relationship between the apical membrane potential, V_{mc}, and the shunt resistance, R_s. Positive values of V_{mc}, i.e., "summer" bladders with well-type potential profiles, were associated with lower values of R_s; negative values of V_{mc}, i.e., "winter" bladders with stair-step profiles, were associated with higher values of R_s. The correlation coefficient between V_{mc} and R_s for the 81 bladders shown in the figure was 0.444 ($P < 0.001$).

increase of R_a/R_b. For example, in three bladders in which R_t (1.77 ± 0.16 k$\Omega \cdot cm^2$) was unaffected by amiloride (R_t after amiloride was 1.79 ± 0.16 k$\Omega \cdot cm^2$). R_a/R_b increased from 3.84 ± 0.60 to 116.1 ± 38.2. The effects of amiloride on a representative group of winter bladders are illustrated in Fig. 3. After amiloride addition, the membrane potentials of summer and winter bladders were not significantly different from one another. All of the effects of the drug were completely reversible. Note that there was no effect on V_{cs}.

A few experiments were conducted on short-circuited bladders. The intracel-

[1] This is consistent with the absence of net H^+ secretion, in agreement with the rare occurrence of mitochondria-rich cells in this preparation (Karnaky et al., 1984).

lular potential of these bladders (means of 20 impalements performed on four bladders) was −43.5 ± 2.7 mV before and −67.6 ± 3.0 mV after amiloride. The latter was not significantly different from the values of V_{cs} measured under open-circuit conditions both before and after amiloride (Fig. 3). The mean I_{sc} for these bladders was 19 ± 2 $\mu A \cdot cm^{-2}$ under control conditions and declined to 2 ± 1 after amiloride.

The cell membrane resistances calculated using Eqs. 2 and 3 from the means given in Fig. 3 are 6.35 $k\Omega \cdot cm^2$ for the apical membrane and 1.61 for the

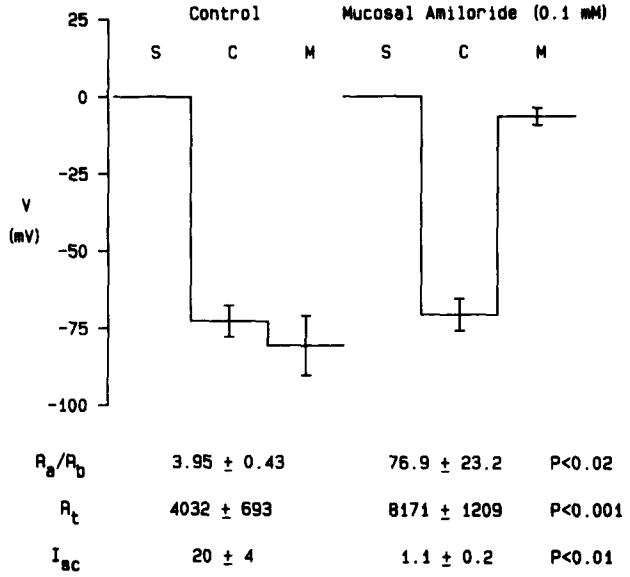

FIGURE 3. Steady state potential profile across a representative group of nine winter bladders with Ringer solution bathing both sides in the absence (control) and presence of 0.1 mM mucosal amiloride. The ratio of the membrane resistances, R_a/R_b, transepithelial resistance, R_t ($\Omega \cdot cm^2$), and the calculated short-circuit current, I_{sc} ($= V_{ms}/R_t$) ($\mu A \cdot cm^{-2}$) under the two conditions are given in the lower part of the figure.

basolateral. Since it has been shown (Davis and Finn, 1982) that amiloride not only increases R_a but indirectly results in an increase in R_b, only a minimum estimate of the value of R_a in the steady state after amiloride can be inferred from the increase in R_a/R_b, assuming that R_b remains constant. Thus, R_a was increased to at least 123.8 $k\Omega \cdot cm^2$, or ~20 times its control value, by amiloride. These calculations of the membrane resistances also include the assumption that R_s was unaffected by amiloride. This seems justified since the drug had no effect on the serosal-to-mucosal flux of Na^+ measured in a separate set of experiments on short-circuited bladders (Fig. 4).

Effects of Changes in Serosal K^+ Concentration

Fig. 5 shows a record of the basolateral membrane potential as the serosal K^+ concentration was increased, by substitution for Na^+, from a control value of 2.5

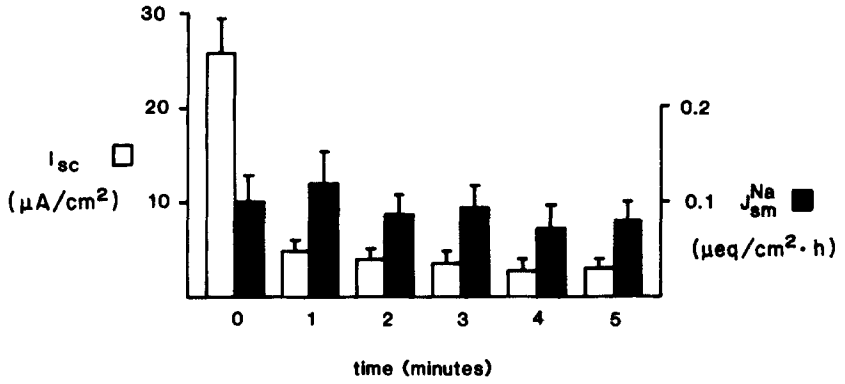

FIGURE 4. The effects of amiloride (0.1 mM) on the short-circuit current, I_{sc} (open bars), and serosal-to-mucosal Na^+ flux, J_{sm} (filled bars). Time zero represents the control values before amiloride (0.1 mM). Flux was measured at 1-min intervals for 5 min after amiloride addition.

to 100 mM/liter. The depolarizations of V_{cs} in response to each step increase in serosal K^+ were rapid, achieving a steady state in ~5 s; they were accompanied by hyperpolarizations of V_{mc}, which could be attributed entirely to changes in the circulating current (that is, E_a was unaffected; see below and Table III). The repeated downward deflections in the membrane potential record were the result

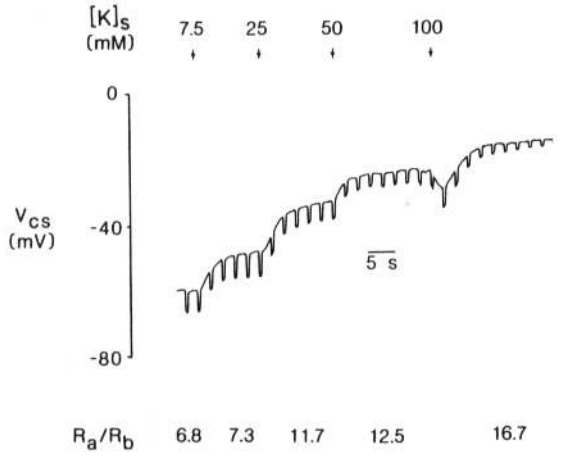

FIGURE 5. A record of the basolateral membrane potential, V_{cs}, of a cell as the serosal K^+ concentration was increased in a stepwise manner, by substitution for Na^+, from a control value of 2.5 to 100 mM/liter. The repeated downward deflections in the record were the result of transepithelial current pulses passed across the epithelium every 2 s. The quasi-steady state values of R_a/R_b for each K^+ concentration shown at the top of the figure are given across the bottom of the figure. The transient hyperpolarization of V_{cs} after the switch to 100 mM/liter K^+ was due to a dead space in the superfusion system, which caused a small amount of control 2.5 mM/liter K^+ Ringer to be flushed into the chamber ahead of the 100 mM/liter K^+ solution.

of transepithelial current pulses passed across the bladder every 2 s. The ratio of the membrane resistances increased in a stepwise manner from a control value of 6.8 with 2.5 mM/liter serosal K^+ to 16.7 with 100 mM/liter serosal K^+, which indicates a large reduction in the relative resistance of the basolateral membrane. The changes resulting from increased serosal K^+ were completely reversible, but it was seldom possible to follow the return to control conditions in the same cell (since the impalements were usually lost 30–60 s after the increase of serosal K to 100 mM because of the contraction of the few remaining muscle fibers). When separate cells were used for each of the step changes in serosal K^+, bracketed by control conditions, the results were identical to those measured in single cells; all changes were monotonic and potentials reached a steady state within 5–10 s.

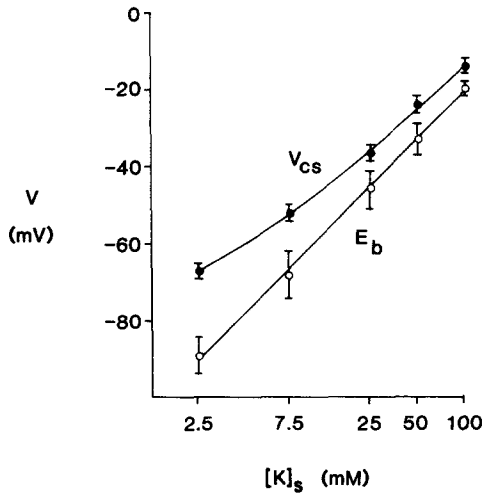

FIGURE 6. Relationships between the basolateral membrane potential, V_{cs} (filled circles; $n = 7$), and the emf, E_b (open circles; $n = 4$), and the serosal K^+ concentration plotted on a logarithmic scale. The slope of the relationship between E_b and serosal K^+ concentration was 43.4 ± 2.9 mV ($r = 0.962$, $P < 0.001$). See text for details.

The results are summarized in Fig. 6, where V_{cs} and E_b are plotted as a functions of the serosal K^+ concentration. The (semilogarithmic) relationship between V_{cs} and the serosal K^+ concentration is nearly linear above 25 mM/liter, with a slope of 38.3 ± 2.4 mV ($n = 7$, $P < 0.001$); below 25 mM/liter, the slope decreases and the relationship departs from linearity. In contrast, E_b is linearly related to the serosal K^+ concentration over the entire range of concentrations and has a slope of 43.4 ± 2.9 mV ($n = 4$, $r = 0.962$, $P < 0.001$). With a serosal K^+ concentration of 100 mM/liter, V_{cs} was −14.0 ± 1.7 mV; thus, almost complete substitution of serosal K^+ for Na^+ did not completely depolarize V_{cs}, which indicates that factors in addition to the K^+ gradient (such as an electrogenic component of the Na-K pump and/or a Donnan potential due to nondiffusable intracellular anions) contribute to the basolateral membrane potential.

TABLE II

Effects of Increasing Serosal K^+ on the Transepithelial and Cellular Electrical Properties

$[K]_s$	V_{ms}	V_{mc}	V_{cs}	R_a/R_b	R_t
mM/liter			*mV*		*kΩ·cm²*
2.5	−78.4±2.7	−14.5±2.7	−63.9±1.5	3.27±0.60	3.70±0.51
50	−60.0±3.0	−28.8±3.1	−31.2±2.5	6.36±0.55	2.91±0.26
Δ	18.4±2.8	−14.3±2.5	32.7±2.5	3.08±0.56	−0.79±0.32
P	<0.001	<0.001	<0.001	<0.001	<0.05

Measurements were made by continuously recording in single cells as the serosal solution was changed. K^+ was increased by substitution for an equal amount of Na^+. Values are means ± SE of single cells for each of 13 bladders. Δ is the difference between 2.5 and 50 mM K^+.

Apparent Basolateral Transference Numbers for K^+ and Cl^-

The effects of suddenly increasing serosal K^+ to 50 mM/liter are summarized in Table II. In addition to the effects mentioned above, the rise in serosal K^+ depolarized V_{ms}, reduced R_t, and resulted in a slight but significant inhibition of the calculated I_{sc} from 27 ± 2 to 23 ± 2 μA/cm². In Table III, the effects on the membrane resistances and emf's are shown. The apical membrane resistance and emf were unaffected, whereas there was a >50% reduction in R_b and a 53-mV depolarization of E_b. The apparent transference number for K^+, T_K, calculated from the data in Table II using Eq. 6, was 0.71 ± 0.05, which is not significantly different from the T_K of 0.74 calculated from the slope of the relationship between E_b and the serosal K^+ concentration shown in Fig. 6.

The values calculated for Table III rest on two assumptions, which were tested in a separate set of experiments. First, R_s was assumed to be unaffected by 50 mM/liter serosal K^+; this assumption is justified since in five bladders R_s was 7.22 ± 1.12 kΩ·cm² with 2.5 mM/liter serosal K^+ and 7.23 ± 1.12 kΩ·cm² after serosal K^+ was increased to 50 mM/liter. Since R_s is unaffected by increased serosal K^+, an alternative method for the calculation of R_s, based on a comparison of the K^+-induced changes in V_{cs} and V_{ms}, can be applied (Spenney et al., 1974;

TABLE III

Effects of Increased Serosal K^+ on Cell Membrane Resistances and Electromotive Forces

$[K]_s$	R_a	R_b	E_a	E_b
mM/liter	*kΩ·cm²*	*kΩ·cm²*	*mV*	*mV*
2.5	6.52±1.18	2.28±0.33	89±9	−96±6
50	5.50±0.83	1.00±0.20	86±14	−43±4
Δ	−1.02±1.22	−1.29±0.24	−3±14	53±5
P	NS	<0.001	NS	<0.001

Values were calculated from the data summarized in Table II as described in the text. The value of R_s was 5.97 ± 1.60 kΩ·cm². NS, not significant. The values of E_a and E_b are reported with respect to the mucosal and serosal bathing solutions, respectively. Δ is the difference between 2.5 and 50 mM K^+.

TABLE IV

Effects of Reducing Serosal Cl^- on the Transepithelial and Cellular Electrical Properties

$[Cl]_s$	V_{ms}	V_{mc}	V_{cs}	R_a/R_b	R_t
mM/ liter		*mV*			*kΩ·cm²*
101	−49.4±7.3	18.5±7.4	−67.9±4.9	6.51±1.33	2.23±0.39
10	−43.7±6.1	19.8±7.2	−63.5±5.3	5.36±1.09	2.34±0.41
Δ	5.7±1.3	1.3±0.6	4.4±0.7	−1.14±0.26	0.11±0.02
P	<0.05	NS	<0.005	<0.02	<0.005

Experiments were performed as described in the legend to Table II. Cl^- was reduced by replacing it with an equal amount of methylsulfate. Values are means ± SE of single cells for each of five bladders. NS, not significant. Δ is the difference between 110 and 10 mM Cl.

Lewis and Wills, 1982). Using the mean values for the K^+-induced changes in V_{cs} and V_{ms} shown in Table III, R_s is 7.35 kΩ·cm², which is not significantly different from the value of R_s estimated as the R_t after mucosal amiloride, which was 5.97 ± 1.60 kΩ·cm². The second assumption was that the shunt emf remained at zero after the increase in serosal K^+. This was not strictly true, since the V_{ms} after amiloride, 4.0 ± 1.2 mV, was reduced to 1.2 ± 0.8 mV by 50 mM/liter serosal K^+. However, since V_{ms} was not zero after amiloride, a portion of the depolarization was probably due to a cellular effect. In any case, the change was too small to affect significantly the values calculated for Table III.

The results of a similar set of experiments designed to evaluate the apparent transference number of the basolateral membrane for Cl^- are shown in Table IV. Reducing serosal Cl^- from the control concentration of 101 mM/liter to 10 mM/liter, by replacing Cl^- with methylsulfate, resulted in small but significant depolarizations of V_{ms} and V_{cs}, whereas V_{mc} was not changed. There was a significant increase in R_t. Table V shows that low serosal Cl^- increased both membrane resistances. E_a was unchanged and E_b was slightly but significantly depolarized. The apparent T_{Cl} of the basolateral membrane was 0.04 ± 0.01. Thus, the basolateral membrane is predominantly K^+ selective, although there is a small Cl^- conductance.

TABLE V

Effects of Decreasing Serosal Cl^- on the Cell Membrane Resistances and Electromotive Forces

$[Cl]_s$	R_a	R_b	E_a	E_b
mM/liter	*kΩ·cm²*	*kΩ·cm²*	*mV*	*mV*
101	5.45±1.23	0.97±0.24	46±6	−83±6
10	5.99±1.47	1.26±0.27	45±6	−80±6
Δ	0.54±0.16	0.29±0.05	1±3	3±1
P	<0.05	<0.01	NS	<0.01

Values were calculated from the data summarized in Table IV as described in the text. The value of R_s was 3.60 ± 0.60 kΩ·cm². NS, not significant. The values of E_a and E_b are reported with respect to the mucosal and serosal bathing solutions, respectively. Δ is the difference between 110 and 10 mM Cl.

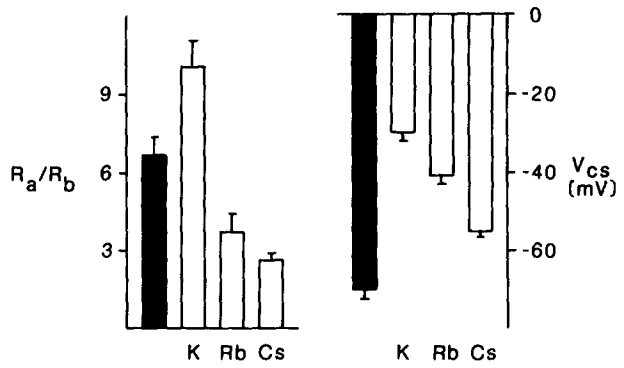

FIGURE 7. The effects of increasing the serosal concentration of K^+, Cs^+, or Rb^+ to 47.5 mM/liter on the ratio of the cell membrane resistances, R_a/R_b, and on the basolateral membrane potential, V_{cs}. The control values are given by the filled bars.

Selectivity of the Basolateral K^+ Channel

The selectivity of the K^+ conductance was assessed by comparing the effects of raising the serosal concentration of K^+, Rb^+, or Cs^+ by 47.5 mM/liter, substituting each ion for an equal amount of Na^+. The effects on V_{cs} and R_a/R_b are shown in Fig. 7. K^+ depolarized V_{cs} by 60% from its control value of 70.5 ± 2.4 mV and approximately doubled R_a/R_b, which can be attributed entirely to a decrease in the resistance of the basolateral membrane, as shown above in Table II. Rb^+ depolarized V_{cs} by 26.7 ± 1.6 mV and Cs^+ depolarized V_{cs} by 16.8 ± 1.3 mV. However, Rb^+ and Cs^+ both significantly decreased R_a/R_b, which indicates increases in the relative resistance of the basolateral membrane. These results suggest that Rb^+ and Cs^+ are less permeant than K^+ and actually block the basolateral K^+ conductance.

Experiments of the type shown in Table II were performed with Cs^+, Rb^+, and Ba^{2+} in the serosal solution in order to evaluate their effects on T_K. Both Cs^+ (10 mM/liter) and Ba^{2+} (0.5 mM/liter) significantly reduced T_K (Table VI). Although Rb^+ (10 mM/liter) also reduced T_K, its effect was not significant. Thus, the basolateral K^+ conductance is highly selective. It is blocked by relatively low concentrations of Ba^{2+} as well as by the closely related ions Cs^+ and Rb^+.

Effects of Nerve and Muscle K^+ Channel Inhibitors

Several of the better-studied K^+ channels of nerve and muscle cells are blocked

TABLE VI

Effect of K^+ Channel Blocking Ions on the Apparent Transference Number of the Basolateral Membrane for K^+ (T_K)

	Control	Ba^{2+}	Cs^+	Rb^+
T_K	0.71±0.05	0.52±0.03	0.50±0.07	0.60±0.07
n	13	6	10	8
P	—	<0.02	<0.05	NS

Values are means ± SE. NS, not significant. The Ba concentration was 0.5 mM/liter; Cs and Rb were used at a concentration of 10 mM/liter.

by small amounts of specific inhibitors. The voltage-sensitive "delayed rectifier" K^+ channel, which is involved in the generation of action potentials in nerve cells, is blocked by 100 μM/liter 3,4-DAP (Yeh et al., 1976). This channel is also blocked by higher concentrations (≥10 μM/liter) of TEA (Armstrong, 1974). However, in the urinary bladder, neither 3,4-DAP at 100 μM/liter nor TEA at 10 mM/liter in the serosal solution had any effect on V_{cs}, R_a/R_b, R_t, or the changes in V_{cs} and R_a/R_b caused by increasing serosal K^+ to 50 mM/liter (Table VII).

The high-conductance, so-called "maxi-K^+ channels" of sarcoplasmic reticulum and the cell membranes of various endocrine cells are blocked by 100 μM/liter DECA (Coronado and Miller, 1980). Serosal DECA at 100 μM/liter had no effect on the basolateral K^+ conductance of the urinary bladder (Table VII).

TABLE VII

Effects of Nerve and Muscle K^+ Channel Inhibitors on the Electrical Properties and Responses to Increased Serosal K^+

	V_{cs}	R_a/R_b	R_t	ΔV_{cs}	$\Delta R_a/R_b$
	mV			*kΩ·cm²*	*mV*
Control	−74.3±3.8	2.86±0.34	2.49±0.15	46.0±1.8	4.38±0.80
DECA	−75.6±4.8	2.86±0.32	2.47±0.18	41.3±1.8	4.41±1.08
Control	−70.6±2.0	5.45±1.74	3.12±0.24	39.4±3.1	7.52±1.88
3,4-DAP	−72.6±1.9	5.16±1.65	3.17±0.26	35.8±2.5	6.52±1.52
Control	−67.3±2.8	2.67±0.21	1.84±0.06	43.4±4.3	2.00±0.76
TEA	−68.5±2.5	2.34±0.26	1.81±0.04	41.5±4.1	2.22±0.85

ΔV_{cs} and $\Delta R_a/R_b$ are the changes in V_{cs} and R_a/R_b resulting from an increase in serosal K^+ to 50 mM/liter. DECA, 100 μM/liter (n = 5); 3,4-DAP, 100 μM/liter (n = 7); TEA, 10 mM/liter (n = 8). None of the effects was significant.

DISCUSSION

Seasonality of Transport Properties

In agreement with a previous report (Karnaky et al., 1984), the transepithelial electrical properties of the urinary bladders of male *Necturus* were found to be seasonally dependent (Table I). Karnaky et al. showed that striking morphological differences in the bladder epithelium were correlated with seasonal differences in the electrical properties. Bladders studied during the winter and early spring exhibited a high (~90 mV) V_{ms} and were composed of a single layer of columnar cells ~70 μm in height, while the epithelia of bladders studied during the summer and autumn had a low V_{ms} and were composed of several layers of squamous cells with a total height of 6–15 μm. Although the present studies were not designed to test this observation, it is possible that this morphologic finding correlates with the comparative difficulty we experienced in impaling the cells from summer bladders (see Results). In the studies of Karnaky et al., this group of bladders exhibited significantly lower short-circuit currents as well. Since no significant seasonal differences in the cell membrane resistances were observed

in the present study, Fig. 2 indicates that at least part of the seasonal variation of the transepithelial electrical properties can be attributed to variations in the paracellular shunt resistance. Similar seasonal variations observed in the frog skin may also be due to seasonal variations in the paracellular shunt (Nagel et al., 1983). While part of the seasonal variation in V_{mc} may therefore be attributable to differences in the internal circulating current, the lower E_a of summer bladders suggests a change in apical membrane selectivity as well. However, the dramatic increase in R_a/R_b after amiloride, which was observed even in bladders in which R_t was unaffected, indicates that the Na^+ conductance still dominates. A nonselective leak conductance in parallel with the dominant Na^+ conductance has been reported by Fromter and Gebler (1977). However, the observed reduction in E_a is also compatible with the opening of a small but finite conductive pathway for K^+ in the apical membrane. An apical K^+ conductance has been observed in frog skin (Zeiske and Van Driessche, 1979; Nagel and Hirschmann, 1980; Nielsen, 1984).

The reversal potential (chord formalism) of the apical, amiloride-sensitive Na^+ channel of 70 mV determined by Fromter et al. (1981) for a *Necturus* urinary bladder with a high V_{ms} (~80 mV) is somewhat lower but is in reasonable agreement with our estimate of E_a, the Thevenin equivalent emf (slope formalism), for similar bladders (winter bladders, Table I). It has recently been pointed out (Thompson, 1986) that, in general, the use of the slope formalism may result in an estimate of E_a that is smaller than the reversal potential, if the current-voltage relationship of the membrane is nonlinear. At least two factors contribute to the perhaps unexpected agreement observed in this case. The relatively low Na^+ permeability of 0.54×10^{-6} cm/s (Fromter et al., 1981) of the apical membrane under the conditions of these experiments, i.e., a mucosal Na^+ concentration >100 mM/liter, results in minimal curvature of the current-voltage relationship (see Fig. 1 of Thompson, 1986). Second, the slope conductance was determined under open-circuit conditions, where V_{mc} is closer to the reversal potential and the slope and chord conductances are more nearly equal. The latter appears to be the case in our experiments since we were unable to detect an increase in R_a, which would be predicted for a significantly nonlinear apical current-voltage relationship, when the apical membrane was hyperpolarized by elevating the serosal K^+ concentration (Table III).

The reduction in the I_{sc}, calculated from the data in Table I, which accompanies the decreased shunt resistance in summer bladders, may reflect a decrease in Na^+ pump activity as well. The Na^+-K^+ ATPase activity in *Necturus* kidney has been reported to decrease during the summer months (Spector et al., 1974). In spite of these seasonal differences, the electrical properties of the basolateral membrane remain relatively constant throughout the year (Table I).

Contrary to a previous report (Higgins et al., 1975), the relationship between V_{ms} and R_t found in this study was direct rather than inverse. It should be noted that the former study included data from both males and females without specifying the time of year at which the experiments were conducted. Urinary bladders from female *Necturus* are strikingly different from those of males, both morphologically and electrophysiologically (LeFevre et al., 1977; Higgins et al.,

1977). The grouping of data derived from these two electrophysiologically distinct populations may have resulted in a spurious inverse correlation.

Transference Numbers of the Basolateral Membrane for K^+ and Cl^-

Several factors contribute to the measured value of the basolateral membrane potential: (*a*) the emf made up of contributions from the gradients of all of the conductive ion species, (*b*) current flow across the membrane resulting from rheogenic transport mechanisms residing in the membrane, and (*c*) current flow caused by the ionic current, which circulates continuously within the epithelium under open-circuit conditions (Fig. 1). In order to evaluate the relative permselectivities of the basolateral membrane, the influence of changes of ion composition on the emf alone must be determined. Using Eqs. 4 and 5, the apparent emf values across the apical and basolateral membrane can be calculated from V_{mc} and V_{cs} by correcting for the influence of the circulating current, but these apparent emf values may still be influenced by the presence of rheogenic transport mechanisms. Eq. 6 is strictly applicable only under zero-current conditions, so that the determination of apparent transference numbers will be affected by the presence of rheogenic transport mechanisms in the basolateral membranes. However, for the brief perturbation employed for the determination of the transference numbers, it is unlikely that the current produced by these mechanisms would be significantly affected by, for example, changes in the intracellular ion concentration. An increase in the current caused by the stimulation of such a mechanism, e.g., stimulation of the Na^+-K^+ ATPase by increasing extracellular K^+, will tend to hyperpolarize V_{cs}, resulting in an attenuation of the response of E_b to increased K^+, and lead to an underestimate of T_K. However, it has been shown (Sjodin, 1983) that the change is small enough that its effect on the potential-concentration relationship of the parallel conductive pathways across the basolateral membrane will be too small to be determined experimentally. Therefore, its influence on the transference numbers calculated using Eq. 6 can be neglected.

The sum of the transference numbers for K^+ and Cl^-, 0.75, was considerably less than 1, which might suggest that the basolateral membrane has a significant permeability for some other ion(s). Na^+ would seem to be an unlikely candidate to account for such a large fraction of the basolateral conductance in a tight epithelium (Koefoed-Johnsen and Ussing, 1958; Lindley and Hoshiko, 1964; Leb et al., 1965), although we did not specifically address this point in these studies. The lack of an effect of serosal verapamil on the electrical properties of the *Necturus* urinary bladder, as shown in the accompanying article (Demarest and Finn, 1987), suggests that Ca conductance does not make a significant contribution. An appreciable bicarbonate conductance also seems unlikely in the absence of net hydrogen ion transport, i.e., in the absence of a reversed short-circuit current after the addition of amiloride. There is, however, an alternative explanation. If the cellular K^+ and/or Cl^- activities are above electrochemical equilibrium, then the sum of the apparent transference numbers determined in this manner will be less than unity (Strickholm and Wallin, 1967). The prelimi-

nary estimate of the intracellular K^+ activity of 95 mM reported by Garcia-Diaz and Armstrong (1980) for *Necturus* urinary bladder suggests that K^+ is above equilibrium. Cl^- is also likely to be distributed above equilibrium in *Necturus* urinary bladder since it has been shown to be above equilibrium in frog skin and a number of other epithelia (White, 1977; Reuss and Weinman, 1979; Nagel et al., 1981; Oberleithner et al., 1983; however, see also Lewis et al., 1978). Furthermore, to the extent that there is a change in intracellular ion activities or an alteration in the activity of an electrogenic transport mechanism during the brief changes in medium concentration (e.g., a fall in cell Cl^- during reductions in medium Cl or the activation of the Na^+-K^+ ATPase caused by increases in the extracellular K^+ concentration), the changes in transmembrane potential will be mitigated and hence decrease the calculated T_i values.

In any case, the transference numbers indicate that G_K is the dominant factor determining the basolateral membrane potential, whereas G_{Cl} makes only a small contribution. There are several observations supporting the conclusion of a low Cl^- conductance. Higgins and Fromter (1974) reported that in Cl^--free solution, increasing serosal K^+ depolarized the V_{cs} of *Necturus* urinary bladder by "more than 38 mV" per decade change in K^+, a value that is not different from the slope of 38.3 ± 2.4 mV determined in this study in the presence of Cl^- (Fig. 6). Second, after serosal K^+ is increased to 50 mM/liter, cell K^+ and Cl^- are both likely to be below electrochemical equilibrium.[2] Therefore, both ions should enter the cell if conductive pathways exist for them and this should result in immediate cell swelling. However, in the frog urinary bladder, even complete replacement of serosal Na^+ with K^+ does not result in any increase in cell volume for 10 min (Davis and Finn, 1982). Basolateral Cl^- permeability of the frog skin is low under control conditions but is increased in osmotically swollen cells (Ussing, 1982). Both of these observations suggest that the basolateral Cl conductance is very low under control, volume-static conditions.

The results of this study do not support the suggestion made recently (Schultz et al., 1984*a*) that the Cl^- conductance of the basolateral membrane of *Necturus* urinary bladder is large and indeed may even exceed the K^+ conductance. This suggestion was prompted by the very low values for E_b that these authors obtained from the current-voltage relationship of the basolateral membrane of bladders studied under short-circuit conditions with low mucosal Na concentrations. The values of V_{cs} and E_b measured under open-circuit conditions, and the intracellular potential under short-circuit conditions obtained in the present study with a mucosal Na^+ concentration of 105 mM/liter, are all higher than would be predicted from the data of Schultz et al. (1984*a*), but are in general agreement with the observations of Fromter and Gebler (1977). The discrepancies in the conductances cannot be ascribed to the difference between our analysis using slope resistance formalism and that of Schultz et al. using chord resistance

[2] V_{cs} was depolarized to −31 mV by increasing serosal K^+ to 50 mM/liter (Table II). Assuming that there was no change in intracellular K^+ during the brief exposure to high serosal K^+, the Nernst equilibrium potential for K^+, E_K, would be −23 mV. E_{Cl} would remain between −40 and −50 mV under these conditions, assuming an intracellular Cl^- of 15–20 mM.

formalism, since both Schultz et al. (1984*a*) and Fromter et al. (1981) have shown that R_b is linear over the range of voltages encountered in our study.[3] It is of interest that in the experiments of Schultz et al. (under short-circuit conditions), both apical and basolateral membrane resistances were extremely low (the values obtained with the highest Na concentrations used were ~1.5 $k\Omega \cdot cm^2$ for the apical membrane and 0.3 $k\Omega \cdot cm^2$ for the basolateral). Since it is known that short-circuiting, at least in frog skin, leads to cell swelling (Voute and Ussing, 1968), and that cell swelling in several systems leads to an increase in the Cl conductance (Grinstein et al., 1982; Ussing, 1982; Davis and Finn, 1985), it is possible that the "extra" conductance of the basolateral membrane seen by Schultz et al. was a result of the conditions of their experiments.

Selectivity and Blockage of the Basolateral K^+ Conductance

Since T_K was evaluated by increasing serosal K^+ 20-fold, the reductions given in Table VI caused by Ba^{2+}, Cs^+, and Rb^+ are likely to be underestimated, because these ions probably compete with K^+ for access to the conductance pathways. Previous studies provide ample evidence for such an interaction. Ba^{2+} block of the basolateral K^+ conductance in the frog skin can be partially overcome by high K^+ concentrations (Nagel, 1979). Tracer flux studies of the basolateral K^+ conductance in the turtle colon have shown an Rb^+/K^+ permeability ratio of 0.1 and positive flux coupling between the two ions, which is consistent with a single-file mechanism for ion permeation (Kirk and Dawson, 1983). Blockage of several of the K^+ channels in nerve and muscle membranes produced by all three ions has been shown to be competitive (Latorre and Miller, 1983). Although the reductions of T_K caused by either 10 mM Cs^+ or Rb^+ were smaller than that caused by 0.5 mM Ba^{2+}, all three ions were equally effective in increasing the basolateral membrane resistance at these concentrations in the presence of 2.5 mM K^+ (Demarest and Finn, 1987). Thus, the basolateral K^+ conductance of *Necturus* urinary bladder appears to be highly selective for K^+, since the closely related Rb ion blocks it.

Several recent patch-clamp studies on pancreatic and lacrimal gland acinar cells reported high single channel conductance (>200 pS) K^+ channels that exhibited virtually no conductance for Rb^+ (Findlay, 1984; Gallacher et al., 1984). These exceptionally selective, high-conductance K^+ channels resembled the maxi-K^+ channels of mammalian sarcoplasmic reticulum (Latorre and Miller, 1983). Thus, it was of interest to evaluate the effects of DECA, a potent blocker

[3] A time dependence to the response to square-wave current pulses in the absence of amiloride was noted in both of these studies. Our experience resembles that of Fromter et al. (1981), who observed an initial capacitative transient lasting 5–89 ms and a variety of longer transients that became increasingly complex at higher current densities. They attributed these long transients to concentration polarization in the unstirred layers adjacent to the cell membranes. Schultz et al. (1984*a*) indicated that R_b and E_b increased in a time-dependent manner after the initiation of a current pulse and chose the time point 20 ms after the onset of the pulses for analysis. They have subsequently (Schultz et al., 1984*b*) pointed out that their measurements may have been influenced by a capacitative transient. The analysis in the current study was carried out over the 120–300-ms interval after the initiation of a current pulse in order to minimize the influence of the capacitative transient. These differences in data analysis may account for some but not all of the differences observed.

of such channels (Coronado and Miller, 1980), on the K^+ conductance of the basolateral membrane of *Necturus* urinary bladder. DECA had no effect on either the control electrical properties or the responses to increased serosal K^+ (Table VII). Thus, epithelial basolateral membranes may possess yet another class of at least pharmacologically distinct, highly selective K^+ channels. In this study, the K^+ conductance of the basolateral membrane certainly represents an ensemble of whatever types of K^+ channel may be functional under normal conditions, e.g., Ca activated, voltage dependent, etc. As we undertook no systematic investigation to examine Ca or voltage effects, further speculation about the nature of these channels seems unwarranted at this time. In this regard, it should be noted that Ba^{2+} blocks all known K^+-selective channels (Wills et al., 1982; Latorre and Miller, 1983).

In conclusion, we have shown by direct measurement, using ion substitution and minimizing unstirred layers, that the basolateral membrane of *Necturus* urinary bladder is predominantly K^+ conductive under open-circuit conditions, and that there is a minimal, but significant, contribution of Cl^- to this conductance. Furthermore, the K^+ channels are highly selective and are blocked by Ba^{2+}, Rb^+, and Cs^+, but not by compounds that block K^+ channels in excitable tissues.

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