

Microelectrode Studies in Toad Urinary Bladder Epithelium

Effects of Na Concentration Changes in the Mucosal Solution on Equivalent Electromotive Forces

JAVIER NARVARTE and ARTHUR L. FINN

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

ABSTRACT Microelectrode techniques were employed to measure membrane potentials, the electrical resistance of the cell membranes, and the shunt pathway, and to compute the equivalent electromotive forces (EMF) at both cell borders in toad urinary bladder epithelium before and after reductions in mucosal sodium concentration. Basal electrical parameters were not significantly different from those obtained with impalements from the serosal side, indicating that mucosal impalements do not produce significant leaks in the apical membrane. A decrease in mucosal Na concentration caused the cellular resistance to increase and both apical and basolateral EMF to depolarize. When Na was reduced from 112 to 2.4 mM in bladders with spontaneously different base-line values of transepithelial potential difference (V_{ms}), a direct relationship was found between the change in V_{ms} brought about by the Na reduction and the base-line V_{ms} before the change. A direct relationship was also found by plotting the change in EMF at the apical or basolateral border caused by a mucosal Na reduction with the corresponding base-line EMF before the change. These results indicate that resting apical membrane EMF (and, therefore, resting apical membrane potential) is determined by the Na selectivity of the apical membrane, whereas basolateral EMF is at least in part the result of rheogenic Na transport. These results are consistent with data of others that suggested a link between the activity of the basolateral Na pump and apical Na conductance.

The ionic permeability of cell membranes can be estimated from the changes in electrical potential measured after modifications in the ionic concentrations of the bathing solutions (1-4). This approach is more difficult in epithelial tissues than in nonpolar cells because the transmural route in epithelia consists of a cellular pathway, constituted by the two membranes in series, in parallel with a paracellular shunt pathway (5, 6). A minimum equivalent circuit for

the tissue has to include six elements: a resistor and an electromotive force (EMF) at each cell membrane and at the shunt pathway (7).

Based on transepithelial electrical measurements, Koefoed-Johnsen and Ussing (8) proposed that the outside surface of the frog skin was selectively permeable to Na. By a similar approach, Leb et al. (9) and Gatzky and Clarkson (10) concluded that the mucosal border of toad urinary bladder was more permeable to Na than to other monovalent cations, although the serosal surface was more permeable to K. Because the membrane potential at each border is a function of all the elements in the circuit, and because it is likely that several of them (at least ipsilateral membrane and shunt resistances and EMF) would be modified by a change in the bathing solution, the conclusions derived from transepithelial electrical measurements are subject to several errors. The only way to characterize by electrical measurements the effect of a change in ionic composition of the bathing fluid on the ipsilateral EMF is to compute the values of all the elements in the circuit before and after the solution change. When considering recent data that apparently gave different conclusions from those obtained from previous work, a reexamination of the ionic permselectivity characteristics of toad bladder using intracellular microelectrode techniques appears even more justified. Finn (11) reported that the change in transepithelial potential difference (V_{ms}), after Na-K substitutions in the bathing solutions of toad bladders with different rates of transport, is a function of the base-line V_{ms} in control conditions, suggesting that either the membrane permselectivity changes with the level of activity of the Na pump, or that V_{ms} is not the result of ionic diffusion.

The experiments reported here were performed to characterize the permselectivity features of the apical membrane, by measuring the effects of ionic concentration changes in the bathing solutions on the membrane EMF of toad bladder. In addition, we shall present data that indicate that microelectrode impalements from the serosal side yield results identical with mucosal impalements, suggesting that the latter do not produce significant leaks in the apical membrane. Our experiments indicate that (a) the apical membrane is largely sodium selective, but the selectivity is a function of base-line EMF, (b) basolateral EMF must be, at least in part, determined by rheogenic Na extrusion, and (c) apical Na conductance is a function of basolateral pump activity, as suggested by others (12-15).

METHODS

Colombian and Mexican toads (*Bufo marinus*) were obtained from the Charles P. Chase Company or the Pet Farm, Miami, Fla., and kept in a container at room temperature with free access to water. Bladders were removed from pithed toads and mounted as flat sheets, mucosal side upward, between the two halves of a lucite chamber. The bladder was supported by a nylon mesh and the exposed area was 3.41 cm². The serosal chamber was continuously perfused and a small negative pressure was applied to the lower half of the chamber. The mucosal solution was either continuously replaced by gravity superfusion or changed at 10-20-min intervals. The change of solution was brought about by two pipettes (for superfusion and suction,

respectively) placed close to the surface of the bladder. All experiments were performed at room temperature ($22^\circ \pm 1^\circ\text{C}$).

Solutions

Standard Ringer's solution had the following composition (mM): NaCl 110, KCl 2.5, NaHCO_3 2.4, CaCl_2 0.9, pH ~ 8.5 , gassed with room air. Na was replaced with K, choline, or *N*-methyl-D-glucamine (NMDG). NMDG⁺ was prepared by titrating the base with HCl to pH 8. The drugs were dissolved in the Ringer's solution. Ouabain was obtained from Sigma Chemical Co., St. Louis, Mo., and amiloride was a gift from Merck Sharp and Dohme Div., Merck and Co., Inc., West Point, Pa.

Electrical Measurements

POTENTIALS Two silver-silver chloride electrodes were connected to each side of the chamber (mucosal and serosal) by means of polyethylene agar-Ringer bridges. One pair was used for the measurement of the V_{ms} (610B electrometer, Keithley Instruments, Inc., Cleveland, Ohio) with the serosal side grounded, and the other for passing transepithelial DC current pulses (302T Anapulse Stimulator and 305-2 Stimulus Isolator, W-P Instruments, Inc., New Haven, Conn.).

Apical (V_{mc}) and basolateral (V_{cs}) membrane potentials were measured with glass microelectrodes prepared by pulling 1-mm o.d. by 0.6-mm i.d. Pyrex glass (Drummond Scientific Co., Broomall, Pa.) with several fiberglass strands placed within, and filled by injecting 4 M potassium acetate through a syringe and a 28-gauge needle. The electrodes were beveled (16) to a resistance of 10–20 M Ω , and had tip potentials ranging from 2 to 8 mV. The cellular impalements were performed with mechanical manual or motor-driven micromanipulators, under visual control with an inverted, phase-contrast microscope (E. Leitz, Inc., Rockleigh, N.J.) at $\times 200$. The criteria for successful impalements were the same as previously described (17). Potentials were measured with M-4 electrometer probes (W-P Instruments, Inc.) and displayed on a storage oscilloscope (Tektronix, Inc., Beaverton, Oreg.). All potentials measured with nonidentical composition of the solutions were corrected for the liquid junction potentials measured in the same system when a short agar 3 M KCl bridge was substituted for the tissue.

RESISTANCES The total transepithelial resistance (R_t) was calculated from

$$R_t = \frac{\Delta V_{ms}}{i} \cdot S$$

where ΔV_{ms} is the voltage deflection produced by a transepithelial DC pulse of intensity i (7–55 μA), and S is the exposed area of the preparation.

The ratio of the apical (R_a) to the basolateral (R_b) cell membrane resistances was calculated as the ratio of voltage deflections produced by a transepithelial pulse with the microelectrode in a cell. The apical membrane voltage deflection (ΔV_{mc}) was calculated by subtracting the basolateral membrane deflection (ΔV_{cs}) from the transepithelial voltage deflection (ΔV_{ms}).

$$\frac{\Delta V_{ms} - \Delta V_{cs}}{\Delta V_{cs}} = \frac{\Delta V_{mc}}{\Delta V_{cs}} = \frac{R_a}{R_b} = a. \quad (1)$$

The resistances of the cell membranes and the shunt were calculated by using method B of Reuss and Finn (17–19). According to the equivalent circuit shown in

Fig. 1:

$$R_t = \frac{(R_a + R_b)R_s}{R_a + R_b + R_s} \quad (2)$$

If amiloride is added to the mucosal solution, the electrical resistance of the transcellular pathway, R_c ($R_c = R_a + R_b$), will increase. If we assume that there is no change in R_b or R_s in this circumstance, an expression for R_s can be obtained from the application of Eqs. 1 and 2 before and after adding amiloride:

$$R_s = \frac{R_t R_t' (a' - a)}{R_t (a' + 1) - R_t' (a + 1)}, \quad (3)$$

where the primes represent the determinations after addition of amiloride. According to Eq 3, R_s can be calculated from the measurement of R_t and a before and after

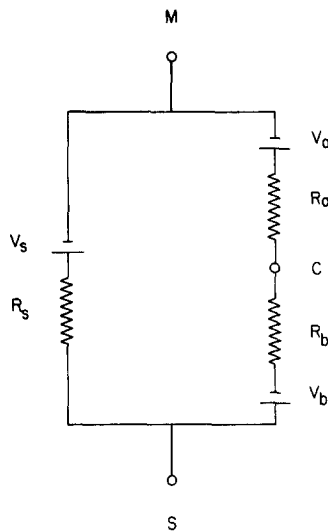


FIGURE 1. Electrical equivalent circuit of the toad urinary bladder epithelium. Each element is represented by its equivalent electromotive force in series with the respective equivalent resistance— M , C , S : mucosal, cellular, and serosal compartments, respectively; V_a , R_a : equivalent electromotive force and resistance of the apical membrane; V_b , R_b : equivalent electromotive force and resistance of the basolateral membrane; V_s , R_s : equivalent electromotive force and resistance of the shunt pathway.

adding amiloride. Once the value of R_s is calculated, the values of R_a and R_b are calculated from Eqs. 1 and 2.

CALCULATION OF THE EQUIVALENT ELECTROMOTIVE FORCES GENERATED AT THE CELL MEMBRANES AND THE SHUNT As shown in Fig. 1, the cell membranes and the shunt pathway can each be represented by a battery in series with a resistor. Under control conditions (Ringer's solution bathing both sides of the tissue), V_s was assumed to be zero because bulk solution concentrations were the same in both media. The EMF

can be calculated from:

$$V_a = V_{mc} + V_{ms} \frac{R_a}{R_s} \quad (4)$$

$$V_b = V_{cs} + V_{ms} \frac{R_b}{R_s} \quad (5)$$

with the polarities defined as V_{cs} , V_b : serosal solution – cell; V_{mc} , V_a : cell – mucosal solution; V_{ms} , V_s : serosal solution – mucosal solution. With this convention all the potentials and EMF have a positive sign if the polarities are those found in control conditions in this tissue.

If the composition of one of the bathing solutions is changed, V_s is no longer equal to zero, and the following equations hold:

$$V_a = \frac{V_{mc}(R_a + R_b + R_s) - R_a(V_s - V_b)}{R_b + R_s} \quad (6)$$

$$V_b = \frac{V_{cs}(R_a + R_b + R_s) - R_b(V_s - V_a)}{R_a + R_s} \quad (7)$$

The values of R_t , a , V_{mc} , and V_{cs} were measured (in at least five different cells in each condition) before and after adding 10^{-5} M amiloride to the standard Ringer solution in the mucosal chamber; the medium was then replaced with an experimental solution and the sequence repeated. The first impalement was usually performed within 1 min after the change in solution, and the time needed to accumulate a set of at least five impalements varied from 8 to 30 min. From these measurements all the resistances could be computed from method B, and V_a and V_b were calculated from Eqs. 4 and 5 in control conditions, and Eqs. 6 and 7 after a solution change. The value of V_s was calculated from the Goldman-Hodgkin-Katz constant field, zero-current equation (1), using bulk solution concentrations and the following shunt permeability ratios from Finn and Bright (20):

$$\frac{P_{Na}}{P_K} = 0.70 \quad \text{and} \quad \frac{P_{Cl}}{P_K} = 0.54.$$

Permeabilities to choline and NMDG were assumed to be equal to that of Na. It can be shown that this assumption introduces no more than a 3-mV error in the computation of EMF.

Only bladders with V_{ms} over 40 mV were accepted, except when studying spontaneously low V_{ms} bladders (see Results). Because of the long time required to perform all the measurements, most bladders did not return to control conditions. Inasmuch as changes in EMF and potentials were found to be related to the base-line potentials of the bladders (see Results), the absence of a complete recovery made it impossible to compare the effect of different Na concentrations in the same tissue.

Results are expressed as means \pm SEM. Differences between means were analyzed using Student's *t* test.

RESULTS

General

The electrical parameters of bladders in control conditions are similar to those previously reported (13; Table I).¹ These results confirm previous observations related to the potential profile ("stair-step-like") in toad urinary bladder (17–19, 24–27) with Ringer's solution bathing both sides of the tissue.² The same potential profile was found in a group of bladders with spontaneous V_{ms} values < 40 mV (Table I). This potential profile is different from that reported for rabbit (12) and *Necturus* (28) urinary bladders with low spontaneous V_{ms} .

Effects of Reductions in Mucosal Na Concentration on Cellular and Paracellular Resistances

Table II shows the effect of K-for-Na replacements on cellular and paracellular resistances. As the Na concentration decreased, there is a concentration-

TABLE I
ELECTRICAL CHARACTERISTICS OF BLADDERS WITH SPONTANEOUS V_{ms} OVER AND BELOW 40 mV, IN CONTROL CONDITIONS WITH RINGER'S SOLUTION ON BOTH SIDES

	V_{ca}	V_{mc}	R_a	R_b	R_s	V_a	V_b	n
$V_{ms} > 40$ mV	32.3 ± 1.0	29.0 ± 1.1	4.0 ± 0.2	2.9 ± 0.2	20.7 ± 1.5	43.1 ± 1.4	43.3 ± 1.3	64
$V_{ms} < 40$ mV	18.5 ± 0.8	15.1 ± 0.8	11.3 ± 2.8	7.2 ± 1.8	15.5 ± 1.3	37.5 ± 3.8	34.7 ± 2.5	15
P	< 0.01	< 0.01	< 0.01	< 0.01	> 0.10	> 0.10	< 0.01	

For abbreviations, see the text. Resistances in $K\Omega cm^2 \cdot V$ in mV.

dependent increase in R_t which is explained by an increase in R_a and R_b . Although the mean values of R_s decreased in each of the three groups, those changes were not significant. When NMDG or choline was used to replace sodium, concentration-dependent increases of R_t and R_a were found. The changes in R_b were not significant, and the mean changes of R_a were smaller

¹ The mean value of ($V_a + V_b$) in control conditions was 87 ± 3 mV, with a range of 60–150 mV. The equivalent circuit of Fig. 1 can be simplified by representing the cellular limb by an overall cell EMF and an equivalent cell resistance in series. Such an EMF equals $V_a + V_b$, and if Na is the only ion actively transported across the tissue, it represents the EMF of this overall transport process, or E_{Na} . Using other techniques, a somewhat higher value of 105 ± 2.9 mV has been reported (21). This difference may be the result of the presence of active transport of Cl^- (22) and H^+ (23), which would reduce $V_a + V_b$ (because they are derived from the measured membrane resistances and voltages and therefore include contributions of ions other than sodium) to a value less than E_{Na} .

² Associated with the mechanical noise invariably seen as the electrode strikes the tissue surface, there is often a negative deflection just before the usual positive potential is recorded. This negative potential appears as a spike of 5–30 mV in amplitude, and of a duration that varies with the speed of the impalement. With care, the electrode may be held steady enough to record prolonged (up to several seconds) negative deflections; under such conditions, there is no change in potential upon passage of a transepithelial DC pulse, indicating that the microelectrode has not yet passed a significant resistive barrier. Thus the negative potentials are likely to be of extracellular or cell surface origin.

than the changes measured with K as a substitute. As a result of the presence in those series of some bladders with extremely high values of R_s (see Discussion), it was not possible to obtain good measurements of the changes in R_s in the choline and 12 mM Na NMDG series.

Effects of Reduction in Mucosal Na Concentration on Potentials and EMF

As has been previously shown (9-11), a decrease of the mucosal Na concentration elicits a decrease in V_{ms} . By studying a group of bladders with different values of control V_{ms} , as well as some of the bladders in which the base-line

TABLE II
EFFECTS OF MUCOSAL K FOR Na SUBSTITUTIONS ON THE RESISTANCES OF THE CELL MEMBRANES AND THE SHUNT

Mucosal solution	R_t	R_a	R_b	R_s	n
Ringer's	5.40 ± 0.94	4.7 ± 1.0	3.4 ± 0.7	20.3 ± 4.4	7
61 mM Na	5.93 ± 0.98	6.3 ± 1.2	4.5 ± 1.0	18.7 ± 5.8	
Difference	-0.53 ± 0.08	-1.6 ± 0.7	-1.1 ± 0.4	1.6 ± 4.9	
<i>P</i>	<0.01	>0.10	>0.05	>0.20	
Ringer's	5.35 ± 1.13	4.6 ± 0.6	3.6 ± 0.6	16.6 ± 5.3	6
12 mM Na	6.48 ± 1.08	10.6 ± 1.1	6.1 ± 0.6	11.5 ± 2.9	
Difference	-1.13 ± 0.05	-6.0 ± 0.9	-2.5 ± 0.5	5.1 ± 2.5	
<i>P</i>	<0.001	<0.01	<0.01	>0.10	
Ringer's	5.34 ± 0.50	6.1 ± 1.2	5.5 ± 1.0	21.6 ± 7.8	7
2.4 mM Na	7.90 ± 0.71	14.2 ± 2.5	11.5 ± 1.7	12.2 ± 1.5	
Difference	-2.56 ± 0.64	-8.1 ± 2.8	-6.0 ± 1.6	9.4 ± 7.4	
<i>P</i>	<0.01	<0.05	<0.02	>0.50	

Resistances in $K\Omega\text{cm}^2$. Difference: resistance in Ringer's - resistance in low Na solution.

V_{ms} fell after several hours in experimental conditions (mucosal Na concentration changes), the same relationship reported by Finn (11), using graded inhibition of active transport, was obtained. Fig. 2 shows the direct relationship between the base-line V_{ms} just before the solution change, and the change in V_{ms} brought about by reducing the mucosal Na to 2.4 mM, using K as a substitute. A group of three bladders to which ouabain (10^{-3} M) was added is included in the figure, but the relationship is not changed significantly with the inclusion of these bladders. A similar relationship was found in another group of bladders in which choline was used as the substitute instead of K ($r = 0.953$, $P < 0.001$). Given this relationship between the spontaneous base-line values of V_{ms} and the magnitude of the reduction in V_{ms} after lowering the mucosal Na concentration to a single value, the slope of the relationship between V_{ms} and mucosal Na concentration will depend on the base-line values of V_{ms} . Thus, a bladder with a low base-line V_{ms} will yield smaller changes in V_{ms} for a given change in sodium concentration than a bladder with a high base-line V_{ms} . In 12 bladders with a control V_{ms} of 40-60 (51 ± 1) mV, the slope obtained from all the experimental points was 19 ± 5 mV/10-fold change in $[Na]_m$. The slope for eight bladders with V_{ms} over 60 mV (71

± 4 mV) was 36 ± 1 mV, which differs significantly from 19 ($P < 0.05$). These effects of changes in mucosal sodium on V_{ms} are strikingly similar to those previously reported (11) when V_{ms} was varied by inhibiting transport with ouabain, amiloride, or cold, namely, that the change in V_{ms} was related to the base-line V_{ms} . To determine the mechanisms responsible for this finding, we

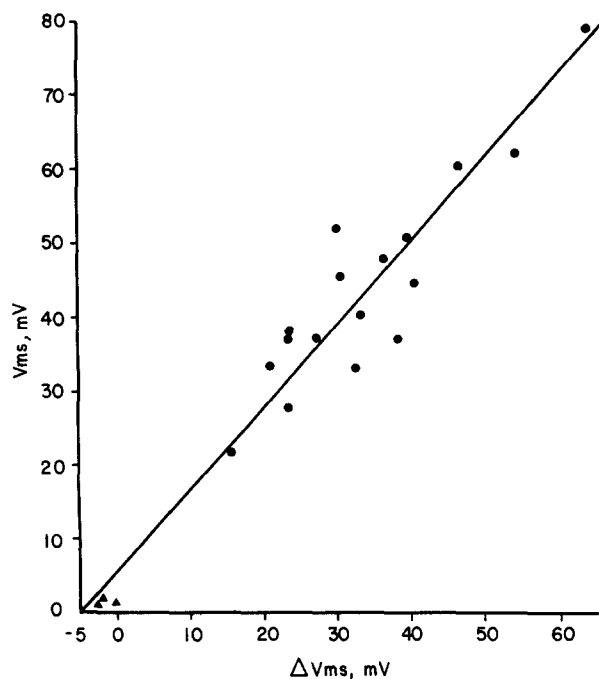


FIGURE 2. Relationship between V_{ms} and ΔV_{ms} . V_{ms} in control conditions (ordinate) is plotted as a function of the change in V_{ms} (abscissa) brought about by a decrease of mucosal Na concentration from 112 to 2.4 mM (K replacement) in bladders incubated without (●) or with 10^{-3} M ouabain (▲). $r = 0.959$, $P < 0.001$, $n = 20$.

studied the effect of changes in mucosal Na on membrane potentials and EMF as illustrated in Table III. The change in V_{ms} is explained by a change in both V_{mc} and V_{cs} as shown in Fig. 3. As Table III shows, lowering the mucosal Na concentration causes graded changes in V_a and V_b . The relationship between V_a or V_b and mucosal Na concentration is shown in Fig. 4. The least squares fit of the mean values gives a slope of 20 ± 1 for V_a and 18 ± 1 for V_b . When choline or NMDG were used as Na substitutes, the effects on potentials and EMF were similar to those found using K as a substitute (Fig. 5). Once again, lowering the mucosal Na concentration caused depolarization of V_{mc} , V_{cs} , V_a , and V_b .

Effects of Reductions of Mucosal Na Concentration on Bladders Treated with ouabain

To study the EMF changes with mucosal Na in relation to different control states of the tissue, ouabain (10^{-3} M) was added to the serosal solution to obtain the lowest base-line values of EMF. Table IV shows the effect of

TABLE III
EFFECTS OF MUCOSAL K FOR Na SUBSTITUTIONS ON TRANSEPITHELIAL AND MEMBRANE POTENTIALS AND EQUIVALENT ELECTROMOTIVE FORCES

Mucosal solution	V_{ms}	V_{mc}	V_{cs}	V_a	V_b	n
Ringer's	55.4 ± 4.5	25.2 ± 2.8	30.2 ± 2.1	39.4 ± 1.7	40.9 ± 2.3	7
61 mM Na	42.8 ± 3.9	19.4 ± 2.1	23.4 ± 1.9	37.0 ± 3.2	35.7 ± 2.1	
Difference	12.6 ± 1.7	5.8 ± 1.4	6.8 ± 0.9	2.4 ± 3.2	5.2 ± 2.1	
P	<0.001	<0.01	<0.001	>0.20	<0.05	
Ringer's	63.0 ± 6.5	31.4 ± 4.0	31.6 ± 2.9	53.5 ± 4.5	49.4 ± 4.9	6
12 mM Na	28.3 ± 4.6	13.0 ± 3.3	15.3 ± 1.4	37.9 ± 7.0	30.1 ± 3.4	
Difference	34.7 ± 2.5	18.4 ± 1.9	16.3 ± 1.8	15.6 ± 6.5	19.3 ± 3.3	
P	<0.001	<0.001	<0.001	>0.10	<0.01	
Ringer's	49.7 ± 5.8	23.6 ± 3.1	26.1 ± 2.9	45.6 ± 4.6	44.8 ± 4.2	7
2.4 mM Na	12.7 ± 2.7	4.8 ± 1.7	7.9 ± 1.0	12.9 ± 5.6	14.2 ± 4.5	
Difference	37.0 ± 4.1	18.8 ± 2.2	18.2 ± 3.4	32.7 ± 8.3	30.6 ± 7.5	
P	<0.001	<0.001	<0.01	<0.01	<0.01	

Difference: value in Ringer's solution - value in low Na solution.

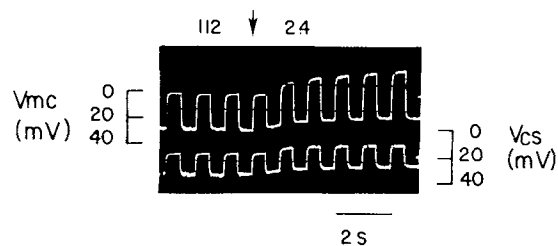


FIGURE 3. Effect of a change in Na concentration in the mucosal solution on cell potentials. The figure starts with the microelectrode in the cell, recording apical (V_{mc}) and basolateral (V_{cs}) membrane potentials. At the arrow, Na concentration was changed to 2.4 mM. The deflections in both recordings (ΔV_{mc} and ΔV_{cs}) were produced by transepithelial DC pulses of $7.9 \mu\text{A}/\text{cm}^2$. Note the rapid depolarization of both V_{mc} and V_{cs} and the increase in R_a/R_b ($\Delta V_{mc}/\Delta V_{cs}$).

10^{-3} M ouabain on the electrical parameters of eight bladders: there are significant increases of R_t ($1.5 \pm 0.4 \text{ K}\Omega\text{cm}^2$, $P < 0.01$), R_a and R_b , and decreases of V_{ms} , membrane potentials, and EMF. When the sodium concentration was lowered to 2.4 mM (using either K or choline as the Na substitute), no significant changes were found either in cellular or in paracellular resistances, as shown in Table V. The changes in EMF were nonsignificant, and no consistent changes were found in membrane potentials, as can be seen in

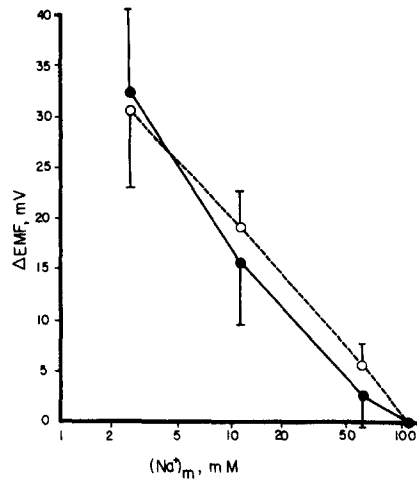


FIGURE 4. Relationship between ΔEMF and mucosal Na concentration. Changes in apical (ΔV_a , ●) and basolateral (ΔV_b , ○) EMF are plotted as functions of mucosal Na concentration. $\Delta\text{EMF} = \text{EMF}$ in 112 mM Na - EMF in low Na, isosmolar K replacement. Each point represents the mean \pm SEM of at least six experiments.

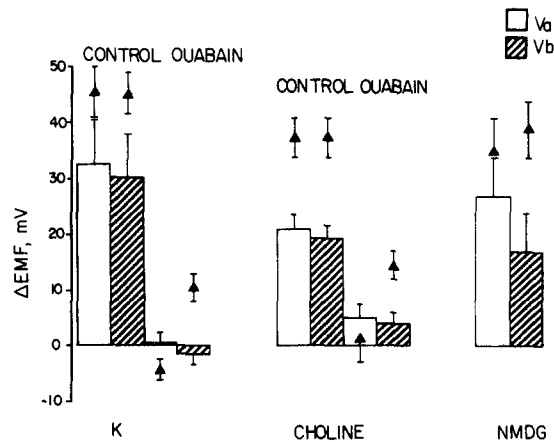


FIGURE 5. Effect of different Na substitutes and ouabain on ΔEMF . The changes in V_a and V_b , when mucosal Na concentration was lowered from 112 to 2.4 mM, are shown for three Na substitutes, K, choline, and NMDG. The triangles represent the base-line value of EMF (before the change in Na concentration). The changes are presented for the three substitutes in bladders incubated in Ringer's solution (control) and for K and choline when the change in mucosal Na concentration was performed in tissues incubated for 1 h with 10^{-3} M serosal ouabain. There are no significant differences in ΔEMF between the three substitutes. When Na concentration was reduced in tissues exposed to ouabain, ΔEMF was not significantly different from zero.

Table V and Fig. 5. Fig. 6 illustrates the relationship between the change in EMF after reduction of mucosal Na to 2.4 mM (K or choline replacement) and the base-line values of EMF before the change, using bladders with different spontaneous values of EMF as well as the tissues treated with ouabain. As shown above for V_{ms} , there is a direct relationship between the base-line value of EMF and the change in EMF brought about by a decrease in mucosal Na concentration ($r = 0.793$, $P < 0.001$).

TABLE IV
EFFECTS OF 10^{-3} M OUABAIN ON RESISTANCES, POTENTIALS, AND ELECTROMOTIVE FORCES

Serosal solution	R_a	R_b	R_s	V_{mc}	V_{cs}	V_a	V_b	n
Ringer's	4.1 ± 0.5	2.7 ± 0.3	20.7 ± 4.9	26.5 ± 3.9	30.4 ± 3.2	43.7 ± 7.7	40.1 ± 4.0	8
Ringer's + 10^{-3} M Ouabain	16.7 ± 4.0	7.7 ± 2.2	9.1 ± 1.0	-7.6 ± 1.6	10.7 ± 1.4	-3.0 ± 2.0	12.7 ± 1.4	
Difference	-12.6 ± 3.4	-5.0 ± 2.1	11.6 ± 4.9	34.1 ± 2.8	19.7 ± 2.9	46.7 ± 7.4	27.4 ± 4.7	
P	<0.01	<0.05	<0.05	<0.001	<0.001	<0.001	<0.001	

The measurements in the presence of ouabain were performed in the steady-state period of the drug action, i.e., at least 60 min after adding ouabain to the serosal solution.

TABLE V
EFFECTS OF Na SUBSTITUTIONS ON RESISTANCES, POTENTIALS, AND EQUIVALENT ELECTROMOTIVE FORCES OF BLADDERS IN THE PRESENCE OF 10^{-3} M OUABAIN

Mucosal solution	R_a	R_b	R_s	V_{mc}	V_{cs}	V_a	V_b	n
Ringer's	15.2 ± 6.2	6.6 ± 2.7	7.3 ± 1.2	-7.0 ± 1.1	8.2 ± 1.0	-4.8 ± 1.7	9.1 ± 1.0	3
2.4 mM Na (K)	17.6 ± 4.7	8.0 ± 2.1	5.2 ± 1.5	-5.5 ± 1.1	8.2 ± 1.8	-5.2 ± 2.5	10.4 ± 2.5	
Difference	-2.4 ± 1.5	-1.4 ± 0.8	2.1 ± 0.9	-1.5 ± 0.1	0.0 ± 0.8	0.4 ± 1.8	-1.3 ± 1.8	
P	>0.20	>0.20	>0.10	<0.01		>0.80	>0.50	
Ringer's	13.0 ± 4.4	5.2 ± 1.1	11.0 ± 1.6	-6.1 ± 4.4	12.0 ± 3.4	0.7 ± 4.7	14.8 ± 2.6	3
2.4 mM Na (choline)	14.7 ± 3.9	6.2 ± 2.2	9.6 ± 1.1	-7.2 ± 5.1	9.2 ± 3.8	-4.3 ± 7.0	10.9 ± 2.5	
Difference	-1.7 ± 2.2	-1.1 ± 1.4	1.4 ± 0.7	1.1 ± 1.3	2.8 ± 0.6	5.0 ± 2.5	3.9 ± 1.0	
P	>0.20	>0.20	>0.10	>0.50	<0.05	>0.10	>0.05	

See footnote to Table IV.

DISCUSSION

Impalement Artifacts

We have examined the effects of different sodium concentrations in the solutions bathing toad urinary bladders on the measured transepithelial electrical parameters and cellular potentials, and on the computed values of cellular and shunt resistances and equivalent electromotive forces. The reliability of the computed values of resistances and EMF rests on the assumptions used to compute them and on the validity of the measured membrane potentials. Intracellular potential recording in toad bladder has been recently questioned (26-31). However, we have evidence that impalement artifacts

have not played a significant role in our results. (a) The fact that there is a finite delay between the response of V_{mc} and V_{cs} after a sudden change in the composition of the mucosal solution (and no such delay when a DC current pulse is passed; 32, 33) makes untenable the argument that the change in V_{cs} is the result of the flow of current through an impalement shunt (29). (b) Lewis et al. (31) derived equations from an equivalent electric circuit that incorporates the effect of a microelectrode impalement shunt to predict the

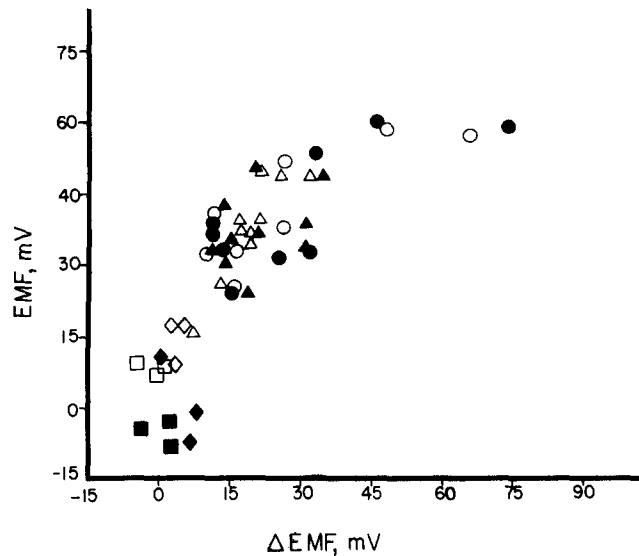


FIGURE 6. Relationship between EMF and Δ EMF. EMF in control conditions (ordinate) is plotted as a function of the change in EMF (abscissa) brought about by a change of mucosal Na concentration from 112 to 2.4 mM (K or choline replacement) in bladders incubated with or without ouabain 10^{-8} M. ●, V_a , K replacement; ○, V_b , K replacement; ▲, V_a , choline replacement; △, V_b , choline replacement; ■, V_a , K, ouabain; □, V_b , K, ouabain; ◆, V_a , choline, ouabain; ◇, V_b , choline, ouabain.

membrane potentials as a function of short circuit current. These authors suggested that impalement damage of either the apical or apical and basolateral membranes could cause such a delay. However, this is true only for short circuit current values much larger than those found in toad or rabbit urinary bladders in control conditions (12, 31). In addition, no delay is predicted when amiloride is washed out of the mucosal solution (31). The experimental observation, however, is that the delay is independent of the basal short circuit current and that it is still present when amiloride is washed out of the mucosal solution (32, 33).³ Thus, the arguments of Lewis et al. are invalid. (c) In addition, when amiloride was added to the mucosal solution of preparations

³ Bisson, M. A., and A. L. Finn. Unpublished observations.

kept short-circuited, the membrane potential became more negative, i.e., V_{mc} went from -8.5 ± 1.7 mV to -14.3 ± 2.2 mV ($P < 0.05$) in four cells where the microelectrode remained intracellular during the change in solution. Similar results were obtained by impaling several cells before and after the addition of amiloride, where V_{mc} went from -7.7 ± 0.8 mV to -12.3 ± 0.9 mV (83 cells, two bladders, $P < 0.001$). These results indicate that the microelectrode was therefore monitoring the transport pathway in this and, by implication, in the studies performed under open circuit conditions, because such a potential change could not have occurred were the electrode monitoring an effective shunt pathway (damaged cell; 29) when transepithelial potential was held at zero. (d) Another group of investigators (28, 30) has raised the possibility of artifacts as the main reason for the differences between the results obtained in *Necturus* and toad urinary bladders. Thus, in *Necturus* urinary bladders with high V_{ms} (>90 mV), the cell potential difference was found to be positive to the mucosal and negative to the serosal medium (stair-step profile), whereas in bladders with low V_{ms} , the cell was negative to both media (trough or well-type profile; 28). On the other hand, in toad urinary bladder with normal Ringer on both sides, we have always found a stair-step profile irrespective of the values of V_{ms} (see Table I). Furthermore, Higgins et al. (28) found that the mucosal membrane had a resistance about 13 times that of the serosal membrane in *Necturus* urinary bladders when the microelectrode was advanced from the serosal side (28). As reported in the Appendix, serosal impalements in toad urinary bladders gave results identical to those previously reported with mucosal impalements. Unless the microelectrode always damages both membranes in both types of impalements, these results strongly suggest that the differences between toad and *Necturus* urinary bladder are real and cannot be explained by microelectrode-induced shunts at the apical cell membrane. (e) Amiloride or sodium-free medium in the mucosal solution results in a two- to threefold increase in R_a/R_b (34), suggesting that any leak must be small.

Assumptions

The method used to compute cellular and shunt resistances relies on several assumptions, some of them discussed previously (17). The assumption that amiloride affects only Na movements at the apical membrane is well supported by observations in different tissues (26, 35–40).

It was observed that the relative increase in R_t after amiloride (R'_t/R_t) varied from bladder to bladder, with a range of 1.3 to 3.8, and that bladders with a high R'_t/R_t ratio usually have very high shunt resistances. The association of high R'_t (transepithelial resistance after amiloride) and high R_s corresponds to a critical region in the relationship between R_s and a' (Eq. 3), where small changes in the measured a' lead to extremely large changes in the computed R_s , yielding uncertainties in this value. However, even extremely large positive values of R_s lead at most to an underestimation of R_a and R_b of $\sim 10\%$. Because of the lack of accuracy in the computation of values of R_s over $50 \text{ K}\Omega \text{ cm}^2$, only values less than this were included when computing the

mean values of R_s before and after a solution change.

When studying the effect of external ionic composition on membrane EMF, to avoid the influence that changes in intracellular composition could have on the measured EMF values (3, 4, 41), it is very important to make the measurements as soon as possible after the alteration in the external solution. Unfortunately, it takes several minutes to make all the measurements needed to compute the EMF. However, the values of the membrane potentials, R_a/R_b and R_t , do not differ significantly if measured immediately after the solution change or during the steady-state period (see Results and Fig. 5). It is therefore reasonable to assume that R_a , R_b , and R_s also change abruptly and thereafter remain relatively constant.

Apical Membrane Effects

EMF represent the lumped contributions of all the diffusion potentials and rheogenic pumps in the membrane. Because the intracellular ionic composition differs from that in the mucosal solution, an equivalent circuit for the apical membrane has to include two batteries in parallel (each with a resistor in series), one for the Na pathway and another for ions other than Na (e.g., K, Cl; 42). In this case, V_a is not a function only of the ionic battery being tested by the change in the mucosal concentration of the respective ion, but is also a function of the resistor in series and the parallel resistances and batteries. Thus, if all movement is diffusive at the apical membrane, the value of the slope we expect for the relationship of V_a to $\log [\text{Na}]_m$ will be always <58 mV/10-fold change in Na concentration. It is impossible, at the present time, to predict the slope, especially if there are ionic concentration-dependent resistance changes.

Reductions in mucosal sodium concentration caused changes in the EMF in the direction expected for an apical Na diffusion potential (see Fig. 4). However, the magnitude of this change was much less than that expected if Na is the only permeating species. We define the Na-dependent partial potential ratio (3, 4) as

$$T_{\text{Na}} = \frac{\Delta V_a}{\frac{RT}{zF} \ln \frac{[\text{Na}]_1}{[\text{Na}]_2}}$$

where $\Delta V_a = V_{a1} - V_{a2}$ (V_{a1} and V_{a2} are the apical EMF before and after the change in solution), $[\text{Na}]_1$ and $[\text{Na}]_2$ are the mucosal sodium concentrations before and after the change, and R , T , z , and F have their usual meanings. The denominator represents the change in EMF that would be measured if the membrane were perfectly permselective for Na. Considering the changes from 112 mM Na to each one of the different mucosal concentrations (with the same substitute), or when the value of T_{Na} for a single change (going from 112 mM Na to either 12 or 2.4 mM Na) was compared between the three different substitutes, the computation of the mean value of T_{Na} for each one of the different series of mucosal Na substitutions (i.e., K replacement at 61, 12, and 2.4 mM Na concentrations, NMDG replacement at 12 and 2.4 mM

Na concentrations, and choline replacement at 2.4 mM Na concentration) yielded no significant differences. When pooling the values from all the substitutes at the different Na concentrations studied, T_{Na} ranged from 0.20 ± 0.03 to 0.35 ± 0.09 with a mean value of 0.25 ± 0.05 .

There are several possible explanations for the low T_{Na} in a membrane that has been considered to behave as a Na electrode. The first is to assume that there are important contributions to the total membrane conductance given by other ions (K^+ , Cl^- , H^+ , or HCO_3^-). Current evidence suggests that the contribution of these ions to apical conductance is small (9, 43-47). However, using chloride substitutions in the mucosal solution, we have recently shown a chloride conductance in the apical membrane, which accounts for a part of the amiloride-insensitive conductance (48).

Another possibility is that important cellular Na concentration changes took place shortly after the change. This would be true only if the sodium pool were very small, as has been suggested recently (36, 49). In that circumstance, after a reduction of mucosal Na, the ratio of mucosal to cell sodium would be larger than the ratio obtained with the same mucosal concentration but without any change in cell Na concentration. Assuming that the Na EMF is given by the Nernst equation, this would result in an EMF that is larger than if cell Na were constant, and a change from control that is smaller. In addition, a low cell Na concentration may also have an effect on apical membrane conductance, as has been suggested for other tissues (12-15).

Another possibility to consider is that either the Na substitutes employed are partially penetrating cations or that they can modify the membrane conductance to Na and/or other ions. In this regard, it has been reported that choline may enhance Na transport in frog skin (50).

An increase in Na permeability at low $[Na]_m$ could also be invoked to explain small changes in EMF with changes in mucosal Na, because there is evidence in frog bladder and frog skin (51, 52) that a reduction in mucosal Na concentration enhances apical Na permeability.

In summary, a low T_{Na} can be explained by (a) contributions of other ions to the apical conductance, (b) fast changes in Na pool with changes in $[Na]_m$, (c) dependence of g_{Na}^a on $[Na]_m$ and/or $[Na]_e$, and (d) effects of the substitutes on apical conductance.

Ouabain Effects

Ouabain caused an increase in cellular resistance and a depolarization of V_a and V_b . The effect on V_b is not surprising because by inhibiting the basolateral Na-K pump (a) the potassium gradient across that membrane decreases (53, 54) and (b) the contribution of any electrogenic component of the pump to V_b is eliminated. The effect on V_a may be explained by the secondary changes in intracellular ionic composition induced by the drug. The increase in cellular Na concentration will decrease the Na gradient across the apical membrane, and (as suggested for other tissues [12-15]) may be the cause of the decrease in apical Na conductance. If V_a is at least in part the result of a Na diffusion potential, these changes can readily explain a depolarization.

Basolateral Membrane Effects

A repeated observation in our experiments is the change of both V_{mc} and V_{cs} , and also of both V_a and V_b in the same direction after a change only in the mucosal solution. Because both membrane potentials change in a depolarizing direction, the change in V_{cs} cannot be the result of current flow across the shunt secondary to the depolarization of V_a (32). In addition, as can be seen in Fig. 3, V_{cs} changes less than a second after the solution change, and then becomes fairly constant, which makes unlikely the possibility of late changes in cellular ionic composition as the explanation for the change in V_{cs} . Because membrane potential changes are completed in seconds, and assuming that the resistance changes take place within the same time period, it is reasonable to assume that the computed EMF will have identical values immediately after the changes, as after several minutes of exposure to the different solutions (when all the actual resistance measurements can be made). If the Na pool were very small (36, 49), a decrease in mucosal Na concentration could cause a decrease in that pool shortly after the change, and this might reduce the activity of the basolateral Na pump.⁴ If this pump were rheogenic, the decrease in Na extrusion would explain the depolarization of V_b . Alternatively, if the Na pump were neutral, a depolarization of V_b could be explained by a decrease of the chemical gradient of K across the basolateral membrane (i.e., by an increase of [K] in the unstirred layer). Because the change in V_{cs} starts milliseconds after the change in $[Na]_m$ (32, 33), the first mechanism is more likely (11, 33, 56).

Relationship between EMF and Δ EMF

Another important finding was the dependence of the change in V_{ms} , membrane potentials, and EMF on the base-line state of the bladder. That is, high V_{ms} or EMF before the solution change are associated with large changes of the respective parameter induced by modifications in the ionic environment, whereas low base-line values lead to small changes. Theoretically, different "base-line states" of the bladders might be the reflection of several possible physiological variables, such as intracellular concentration of Na and/or other ions, conductance of cellular membranes (a function of the individual ion conductances, and possibly varying also with cellular and/or extracellular ion concentrations), electrical field in the membranes (which may also affect the membrane conductance), rate of active transport (which may affect cellular ionic concentrations, membrane conductances, and membrane potentials), etc. If apical membrane resistance and V_a are inversely related, and R_a increases as transport is inhibited, the relationship between V_a and ΔV_a might be explained. However, we found no relationship between V_a and R_a ($P > 0.10$). This can be interpreted in at least three ways: (a) there is no relationship between EMF and g_{Na}^a , (b) there is a large variability in the apical conductance

⁴ Although the experiments were done under open circuit conditions to evaluate permselectivities, previous reports on the relationship between $[Na]_m$ and Na transport in short-circuited preparations have established that reductions in $[Na]_m$ to values used in this paper cause a decrease in transepithelial Na transport (55).

to other ions that is independent of EMF, and (c) apical Na conductance varies inversely with conductance to other ions as V_a changes. We can distinguish among these alternatives by computing the amiloride-sensitive apical conductance $g_{Na}^a = g^a - g_x^a$, where g^a is total apical conductance before amiloride, and g_x^a the conductance with amiloride present. Fig. 7 shows a direct relationship between the change in V_a brought about by lowering $[Na]_m$ to 2.4 mM and the ratio g_{Na}^a/g_x^a computed before the change in $[Na]_m$ ($r = 0.703$, $P < 0.001$). The ratio represents the relative permselectivity of the

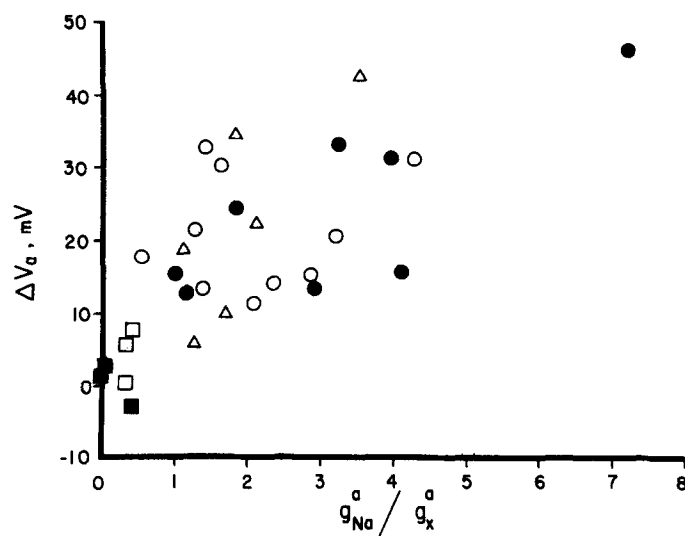


FIGURE 7. Relationship between ΔV_a and the ratio of apical Na conductance (g_{Na}^a) to apical conductance to other ions (g_x^a). The change in V_a (ordinate) brought about by changing the mucosal Na concentration from 112 to 2.4 mM (K, choline, or NMDG replacement) in bladders incubated with or without ouabain 10^{-3} M, is plotted as a function of the ratio g_{Na}^a/g_x^a (abscissa) before the change in $[Na]_m$. g_{Na}^a was computed as $g^a - g_x^a$, where g^a and g_x^a are the apical membrane conductance before and after the addition of amiloride (10^{-5} M) to the mucosal solution, respectively. ●, K replacement; ○, choline replacement; △, NMDG replacement; ■, K, ouabain; □, choline, ouabain.

apical barrier to sodium. The figure indicates that the change in V_a effected by changing $[Na]_m$ is directly related to the sodium selectivity before the concentration change. We also found a direct relationship between g_{Na}^a/g_x^a and V_a ($r = 0.746$, $P < 0.001$) in Na-Ringer. This indicates that the failure to find a relationship between R_a or g_{Na}^a and V_a may be the result of tissue-to-tissue variation in nonsodium conductance, g_x^a . We can conclude from these data that (a) apical Na selectivity varies from tissue to tissue, (b) the more selective the barrier is to sodium, the higher the base-line potential difference and EMF, and (c) the more selective the barrier, the greater will be the change in EMF as mucosal sodium is varied. That apical EMF is directly related to Na selectivity is consistent with observations in other tissues (12-15) that

suggest a direct relationship between the activity of the basolateral Na pump and g_{Na}^a (mediated possibly by changes in intracellular Na concentration). That is, the spontaneous and ouabain-induced variations in base-line V_a might reflect changes in apical Na selectivity secondary to different degrees of activity of the Na pump. Note that g_x^a still includes a component of Na conductance, because the residual short-circuit current after amiloride was significantly different from zero ($0.30 \pm 0.09 \mu\text{A}/\text{cm}^2$, $P < 0.01$). Thus, the true values of g_{Na}^a/g_x^a are larger than the ones shown in Fig. 7.

The relationship between changes in V_b and base-line values of V_b may well represent control of the basolateral sodium pump mechanism by sodium in the transport pool. It has been shown that net sodium transport is a saturating function of mucosal sodium concentration (55), and this may represent the effects of cell Na on the pump mechanism. Furthermore, Finn (57) showed that the rate coefficient for sodium extrusion from the cell to serosal medium was an inverse function of pool size, indicating that the pump saturates as substrate (pool Na content or concentration) increases. The relationship between V_a and V_b is highly linear in a range of EMF from 10 to 70 mV, and the slope of the line is not significantly different from 1.⁵ Because V_a is directly related to the sodium selectivity of the apical membrane, this relationship may be taken to indicate, once again, a control of V_a by the sodium pump. As pump rate (and V_b) decreases, apical sodium conductance (and V_a) decreases because cell sodium has increased. If this is true, then bladders with low V_b will be characterized by high cell Na, low g_{Na}^a , and low V_a . A reduction in $[\text{Na}]_m$ will then have a smaller effect on V_b because those bladders will be in the saturating portion of the curve relating Na pool and pump activity.

In summary, we have shown that reductions in Na concentration in the mucosal medium depolarize EMF at both apical and basolateral membranes, and that the change in EMF is a function of the base-line EMF.

These results are taken to indicate that V_a is determined by apical membrane Na selectivity; the latter is in turn controlled by basolateral Na pump activity, perhaps mediated through changes in cell Na. V_b , on the other hand, is at least in part determined by rheogenic Na extrusion at the basolateral membrane.

APPENDIX

Serosal Impalements in Toad Urinary Bladder Epithelium

It has been recently proposed (28) that microelectrode impalements from the serosal side give more reliable results in *Necturus* urinary bladder when compared with impalements from the apical side of the tissue. The rationale of this approach is that more favorable ratios of electrode-leak resistance to membrane resistance can be obtained at the serosal membrane because the mucosal membrane was found to have a resistance 13 times higher than the serosal one when measured with the use of serosal impalements (28, 30). These data were taken to indicate that the differences between the electrical parameters of *Necturus* and toad urinary bladders were the result of the presence of impalement artifacts in the toad bladder experiments, where the micro-

⁵ Narvarte, J., and A. L. Finn. Unpublished observations.

electrodes were advanced from the mucosal side. We report in this Appendix the results of serosal impalements in toad urinary bladder. Bladders were mounted serosal side upward with no support other than the Ringer's solution under the mucosal side. We could not get good preparations when any other support, such as nylon mesh, was used under the mucosal side. The chamber was placed on the stage of an inverted microscope, and transepithelial potential and resistance were monitored continuously. Two stainless steel microneedles, attached to holders and placed in micromanipulators, were used to dissect away the subepithelial connective tissue under direct vision, at $\times 35$. In this way, small areas with several epithelial cells could be exposed with no change in transepithelial electrical parameters. The serosal solution was continuously replaced by gravity superfusion (except during the impalements) and the mucosal solution was changed at 10- to 20-min intervals. Polarity convention and the methods used to measure potential and resistances were the same as described in Methods. We found it impossible to obtain successful impalements without removing the connective tissue. On the other hand, impalements through the stripped area gave results in every way identical to those previously reported with impalements from the mucosal side in control conditions (17). Thus, 47 impalements in seven bladders resulted in a value of

TABLE AI
ELECTRICAL PARAMETERS OF BLADDERS IN CONTROL CONDITIONS
MEASURED WITH MICROELECTRODES ADVANCED FROM THE SEROSAL
SIDE

V_{ms}	V_{cs}	V_{mc}	R_t	R_a	R_b	R_s
mV	mV	mV	$K\Omega cm^2$	$K\Omega cm^2$	$K\Omega cm^2$	$K\Omega cm^2$
40.1 ± 1.7	20.0 ± 1.4	20.1 ± 0.4	3.2 ± 0.4	3.1 ± 0.6	1.7 ± 0.3	10.7 ± 2.0

R_a/R_b of 1.68 ± 0.11 , and 28 impalements in five bladders exposed to 10^{-4} M amiloride in the mucosal medium gave a value of R_a/R_b of 4.97 ± 0.52 . Cellular and shunt resistances were measured in three bladders; those results are illustrated in Table AI. It is evident that the potential profile, the ratio of apical to basolateral membrane potential, and the mean values of cellular and shunt resistances are not significantly different from those described previously (17). It is therefore highly likely that mucosal impalements in toad urinary bladder do not produce significant leaks in the apical membrane.

The authors are grateful to Dr. C. William Davis for his help with the experiments reported in the Appendix.

This work was supported by grant AM17854 from the National Institute of Arthritis, Metabolism, and Digestive Diseases.

Received for publication 21 September 1979.

REFERENCES

1. HODGKIN, A. L., and B. KATZ. 1949. The effect of sodium on the electrical activity of the giant axon of the squid. *J. Physiol. (Lond.)* **103**:37-77.
2. HODGKIN, A. L., and P. HOROWICZ. 1959. The influence of potassium and chloride ions on the membrane potential of single muscle fibres. *J. Physiol. (Lond.)* **148**:127-160.
3. STRICKHOLM, A., and B. G. WALLIN. 1967. Relative ion permeabilities in the crayfish giant axon determined from rapid external ion changes. *J. Gen. Physiol.* **50**:1929-1953.

4. REUSS, L., and A. L. FINN. 1975. Electrical properties of the cellular transepithelial pathway in *Necturus gallbladder*. II. Ionic permeability of the apical membrane. *J. Membr. Biol.* **25**: 141–161.
5. FRÖMTER, E., and J. M. DIAMOND. 1972. Route of passive ion permeation in epithelia. *Nat. New Biol.* **235**:9–13.
6. USSING, H. H., D. ERLIJ, and U. LASSEN. 1974. Transport pathways in biological membranes. *Annu. Rev. Physiol.* **36**:17–49.
7. SCHULTZ, S. G. 1972. Electrical potential differences and electromotive forces in epithelial tissues. *J. Gen. Physiol.* **59**:794–798.
8. KOEFOED-JOHNSEN, V., and H. H. USSING. 1958. The nature of the frog skin potential. *Acta Physiol. Scand.* **42**:298–308.
9. LEB, D. E., T. HOSHIKO, and D. LINDLEY. 1965. Effects of alkali metal cations on the potential across toad and bullfrog urinary bladder. *J. Gen. Physiol.* **48**:527–540.
10. GATZY, J. T., and T. W. CLARKSON. 1965. The effect of mucosal and serosal solution cations on bioelectric properties of the isolated toad bladder. *J. Gen. Physiol.* **48**:647–671.
11. FINN, A. L. 1974. Transepithelial potential difference in toad urinary bladder is not due to ionic diffusion. *Nature (Lond.)*. **250**:495–496.
12. LEWIS, S. A., D. C. EATON, and J. M. DIAMOND. 1976. The mechanism of Na transport by rabbit urinary bladder. *J. Membr. Biol.* **28**:41–70.
13. CUTHBERT, A. W., and W. K. SHUM. 1977. Does intracellular sodium modify membrane permeability to sodium ions? *Nature (Lond.)*. **266**:468–469.
14. TURNHEIM, K., R. A. FRIZZELL, and S. G. SCHULTZ. 1977. Effects of anions on amiloride-sensitive, active sodium transport across rabbit colon, in vitro. Evidence for “trans-inhibition” of the Na entry mechanism. *J. Membr. Biol.* **37**:63–84.
15. TURNHEIM, K., R. A. FRIZZELL, and S. G. SCHULTZ. 1978. Interaction between cell sodium and the amiloride-sensitive sodium entry step in rabbit colon. *J. Membr. Biol.* **39**:233–256.
16. BROWN, K. T., and D. G. FLAMING. 1974. Beveling of fine micropipette electrodes by a rapid precision method. *Science (Wash. D. C.)*. **185**:693–695.
17. REUSS, L., and A. L. FINN. 1974. Passive electrical properties of toad urinary bladder epithelium. Intercellular electrical coupling and transepithelial cellular and shunt conductances. *J. Gen. Physiol.* **64**:1–25.
18. REUSS, L., and A. L. FINN. 1975. Effects of changes in the composition of the mucosal solution on the electrical properties of the toad urinary bladder epithelium. *J. Membr. Biol.* **20**:191–204.
19. FINN, A. L., and L. REUSS. 1975. Effects of changes in the composition of the serosal solution on the electrical properties of the toad urinary bladder epithelium. *J. Physiol. (Lond.)*. **250**:541–558.
20. FINN, A. L., and J. BRIGHT. 1978. The paracellular pathway in toad urinary bladder: permselectivity and kinetics of opening. *J. Membr. Biol.* **44**:67–83.
21. YONATH, J., and M. M. CIVAN. 1971. Determination of the driving force of the Na⁺ pump in toad bladder by means of vasopressin. *J. Membr. Biol.* **5**:366–385.
22. FINN, A. L., J. S. HANDLER, and J. ORLOFF. 1967. Active chloride transport in the isolated toad bladder. *Am. J. Physiol.* **213**:179–184.
23. LUDENS, J. H., and D. D. FANESTIL. 1972. Acidification of urine by the isolated urinary bladder of the toad. *Am. J. Physiol.* **223**:1338–1344.
24. FRAZIER, H. S. 1962. The electrical potential profile of the isolated toad bladder. *J. Gen. Physiol.* **45**:515–528.

25. RAMSEY, A. G., D. L. GALLAGHER, R. L. SHOEMAKER, and G. SACHS. 1976. Barium inhibition of sodium ion transport in toad bladder. *Biochim. Biophys. Acta.* **436**:617–627.
26. SUDOU, K., and T. HOSHI. 1977. Mode of action of amiloride in toad urinary bladder. An electrophysiological study of the drug action on sodium permeability of the mucosal border. *J. Membr. Biol.* **32**:115–132.
27. KIMURA, G., S. URAKABE, S. YUASA, S. MIKI, Y. TAKAMITSU, Y. ORITA, and H. ABE. 1977. Potassium activity and plasma membrane potentials in epithelial cells of toad bladder. *Am. J. Physiol.* **232**:F196–F200.
28. HIGGINS, J. T., JR., G. GEBLER, and E. FRÖMTER. 1977. Electrical properties of amphibian urinary bladder epithelia. II. The cell potential profile in *Necturus maculosus*. *Pflugers Arch. Eur. J. Physiol.* **371**:87–97.
29. LINDEMANN, B. 1975. Impalement artifacts in microelectrode recordings of epithelial membrane potentials. *Biophys. J.* **15**:1161–1164.
30. FRÖMTER, E., and B. GEBLER. 1977. Electrical properties of amphibian urinary bladder epithelia. III. The cell membrane resistances and the effect of amiloride. *Pflugers Arch. Eur. J. Physiol.* **371**:99–108.
31. LEWIS, S. A., N. K. WILLS, and D. C. EATON. 1978. Basolateral membrane potential of a tight epithelium: ionic diffusion and electrogenic pumps. *J. Membr. Biol.* **41**:117–148.
32. REUSS, L., and A. L. FINN. 1975. Dependence of serosal membrane potential on mucosal membrane potential in toad urinary bladder. *Biophys. J.* **15**:71–75.
33. FINN, A. L., and L. REUSS. 1978. Electrical interaction between apical and basolateral cell membranes in toad urinary bladder epithelium: evidence for rheogenic Na extrusion. *In Membrane Transport Processes*. J. F. Hoffman, editor. Raven Press, New York. **1**:229–241.
34. REUSS, L., J. T. GATZY, and A. L. FINN. 1978. Dual effects of amphotericin B on ion permeation in toad urinary bladder epithelium. *Am. J. Physiol.* **235**:F507–F514.
35. BIBER, T. U. L. 1971. Effects of changes in transepithelial transport on the uptake of sodium across the outer surface of frog skin. *J. Gen. Physiol.* **58**:131–144.
36. MACKNIGHT, A. D. C., M. CIVAN, and A. LEAF. 1975. The sodium transport pool in toad bladder epithelial cells. *J. Membr. Biol.* **20**:365–386.
37. HONG, C. D., and A. ESSIG. 1976. Effects of 2-deoxy-D-glucose, amiloride vasopressin and ouabain on active conductance and E_{Na} in the toad bladder. *J. Membr. Biol.* **28**:121–142.
38. BENTLEY, P. J. 1968. Amiloride: a potent inhibitor of sodium transport across the toad bladder. *J. Physiol. (Lond.)*. **195**:317–330.
39. EHRLICH, E. N., and J. CRABBE. 1968. The mechanism of action of amipramizide. *Pflugers Arch. Eur. J. Physiol.* **302**:79–96.
40. FRAZIER, L. W., and J. C. VANATTA. 1971. Characteristics of H^+ and NH_4^+ excretion by the urinary bladder of the toad. *Biochim. Biophys. Acta.* **241**:20–29.
41. REUSS, L., and A. L. FINN. 1975. Electrical properties of the cellular transepithelial pathway in *Necturus gallbladder*. I. Circuit analysis and steady-state effects of mucosal solution ionic substitutions. *J. Membr. Biol.* **25**:115–139.
42. SCHULTZ, S. G., R. A. FRIZZELL, and H. N. NELLANS. 1977. An equivalent electrical circuit model for “sodium-transporting” epithelia in the steady state. *J. Theor. Biol.* **65**:215–229.
43. ROBINSON, B. A., and A. D. C. MACKNIGHT. 1976. Relationships between serosal medium potassium concentration and sodium transport in toad urinary bladder. III. Exchangeability of epithelial cellular potassium. *J. Membr. Biol.* **26**:269–286.
44. MACKNIGHT, A. D. C. 1977. Contribution of mucosal chloride to chloride in toad bladder

- epithelial cells. *J. Membr. Biol.* **36**:55-63.
45. FINN, A. L., and H. NELLANS. 1972. The kinetics and distribution of potassium in the toad bladder. *J. Membr. Biol.* **8**:189-203.
 46. LUDENS, J. H., and D. D. FANESTIL. 1972. Acidification of urine by the isolated urinary bladder of the toad. *Am. J. Physiol.* **223**:1338-1344.
 47. CHEN, J. S., and M. WALSER. 1977. Bicarbonate ions in active Na transport across toad bladder. *Am. J. Physiol.* **232**:F210-F214.
 48. NARVARTE, J., and A. L. FINN. 1979. Effects of mucosal Cl substitutions on the electrical parameters of toad urinary bladder: microelectrode studies. *Biophys. J.* **25**:31a.
 49. MACKNIGHT, A. D. C., and A. LEAF. 1978. The sodium transport pool. *Am. J. Physiol.* **234**:F1-F9.
 50. MACEY, R. I., and D. C. KOBlick. 1963. Effects of choline and other quarternary ammonium compounds on Na movements in frog skin. *Am. J. Physiol.* **205**:1063-1066.
 51. LINDEMANN, B., and U. GEBHARD. 1973. Delayed changes of Na-permeability in response to steps of (Na)_o at the outer surface of frog skin and frog bladder. In *Transport Mechanisms in Epithelia*. H. H. Ussing and N. A. Thorn, editors. Munksgaard, International Booksellers and Publishers, Ltd., Denmark. 115-127.
 52. FUCHS, W., E. H. LARSEN, and B. LINDEMANN. 1977. Current voltage curve of sodium channels and concentration dependence of sodium permeability in frog skin. *J. Physiol. (Lond.)*. **267**:137-166.
 53. RICK, R., A. DORGE, A. D. C. MACKNIGHT, A. LEAF, and K. THURAU. 1978. Electron microprobe analysis of the different epithelial cells of toad urinary bladder. Electrolyte concentrations at different functional states of transepithelial sodium transport. *J. Membr. Biol.* **39**:257-271.
 54. DELONG, J., and M. M. CIVAN. 1978. Dissociation of cellular K⁺ accumulation from net Na⁺ transport by toad urinary bladder. *J. Membr. Biol.* **42**:19-43.
 55. LEAF, A. 1965. Transepithelial transport and its hormonal control in toad bladder. *Ergeb. Physiol. Biol. Chem. Exp. Pharmacol.* **56**:216-263.
 56. SCHULTZ, S. G. 1978. Is a coupled Na-K exchange "pump" involved in active transepithelial Na transport? A status report. In *Membrane Transport Processes*. J. F. Hoffman, editor. Raven Press, New York. 1:213-227.
 57. FINN, A. L. 1971. The kinetics of sodium transport in the toad bladder. II. Dual effects of vasopressin. *J. Gen. Physiol.* **57**:349-362.