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Effects of Changes in the Composition of the Mucosal Solution on the Electrical Properties of the Toad Urinary Bladder Epithelium

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Summary. By the use of microelectrode techniques, the potential profile and the electrical resistances of the cellular and shunt pathways across the toad urinary bladder epithelium were measured under control conditions and after exposing the mucosal side to solutions of low and high NaCl concentrations and osmolalities. The resistance of the shunt pathway increases at low NaCl concentration (even if the osmolality is kept constant), and decreases at high NaCl concentration (by a nonspecific osmotic mechanism). The inverse relationship between mucosal NaCl concentration and shunt resistance suggests a regulatory mechanism of net sodium transport by reduction of the passive blood-to-urine sodium flux at low urinary sodium concentrations. In addition, the transepithelial potential and the potentials at both cell borders fall in both low and high mucosal NaCl, and the magnitude of these changes is such that they cannot be explained by changes in the shunt pathway alone.

The transepithelial electrical resistance of the toad urinary bladder consists of a cellular pathway given by the resistances of the apical (mucosal) and basal-lateral (serosal) cell membranes, in parallel with a paracellular shunt pathway. The use of microelectrode techniques has allowed us to measure these resistances and show that the shunt resistance is about 1.7 times that of the transcellular pathway (Reuss & Finn, 1974).

Increases in the osmolality of the mucosal or external solution produce reductions of the total electrical resistance across frog skin (Ussing & Windhager, 1964) and toad urinary bladder (Urakabe, Handler & Orloff, 1970; DiBona & Civan, 1973; Wade, Revel & DiScala, 1973). In the latter tissue, there is electron-microscopic evidence of concomitant development of localized dilatations in the zonulae occludentes of the junctional complexes, which become permeable to normally nonpermeating substances (Wade & DiScala, 1971; Erlij & Martinez-Palomo, 1973). These observations suggest

that the decrease in tissue resistance is the consequence of an increase in the conductance of the paracellular shunt pathway. However, recent observations have shown that after exposure to a high urea concentration on the mucosal side, the epithelial cells undergo ultrastructural alterations (Erlij & Martinez-Palomo, 1973) and the permeability of the epithelium to nonpolar substances – assumed to go across the cells – increases (Bindslev, Tormey, Pietras & Wright, 1974).

The experiments reported here were designed to investigate the effects of changes in mucosal osmolality and sodium concentration on the electrical resistances of the cell membranes and the shunt pathway. The potential profile across the epithelium was measured under all these conditions with two purposes: first, to determine the effect of a unilateral change in solution on the membrane potential at each cell border, and second, to test the hypothesis advanced by Schultz (1972), i.e., that the "opening" of the paracellular shunt pathway in a tight epithelium would change the potential profile to that seen in leaky epithelia, where the cell is negative to both bathing solutions.

Materials and Methods

Experimental Procedure

Urinary bladders were isolated from Colombian toads *(Bufo marinus)* obtained from The Pet Farm, Miami, Florida. The preparations were set up horizontally in a lucite chamber, serosal side lying on an agar-Ringer's cylinder, as previously described (Reuss & Finn, 1974). The potential profile and the resistances were measured at room temperature with amphibian Ringer's solution bathing both sides of the tissue (composition in mM: NaCl 109, KCl 2.5, $CaCl_2$ 0.9, $NaHCO_3$ 2.4, glucose 5.5, pH about 7.8 when gassed with room air). Then, the mucosal solution was changed and, after the transepithelial potential and resistance had reached a new steady state, the potential profile and resistances were measured again.

The potential difference across the basal-lateral membrane was measured with an intracellular microelectrode, and the transepithelial potential was measured by means of Ag – AgCl macroelectrodes connected to the solutions with agar-Ringer's bridges, in order to minimize the corrections for liquid junction potentials when the mucosal solution was changed. All potentials were measured with respect to the serosal solution taken as ground. A separate pair of macroelectrodes was employed to pass transepithelial d-c pulses. The microelectrodes were prepared from Pyrex glass tubing of 0.6 mm ID and 1 mm OD (Drummond Scientific Company, Broomall, Pennsylvania) by means of a horizontal, two-stage puller (Industrial Science Associates, Ridgewood, New York) and filled with 4 M potassium acetate by the method of Tasaki, Polley and Orrego (1954). The criteria for successful impalement were as previously described (Reuss & Finn, 1974).

The transepithelial resistance was calculated from

$$R_t = (\Delta E/i) \cdot s$$

where ΔE is the transepithelial voltage deflection one second after the initiation of a depolarizing d-c pulse of intensity i (close to the short-circuit current), and s is the macroscopic surface area of the preparation.

The voltage divider ratio was calculated from the voltage deflections across the apical and basal membrane produced by a transepithelial pulse. Usually, the apical membrane voltage deflection (ΔV_a) was calculated by subtracting the basal membrane deflection (ΔV_b) from the transepithelial voltage deflection (ΔV_t):

$$(\Delta V_t - \Delta V_b)/\Delta V_b = \Delta V_a/\Delta V_b = R_a/R_b.$$

The voltage divider ratio was calculated as the mean of at least six impalements in each condition.

Determination of the Resistances

In a series of experiments in which urea was added to the mucosal solution, the cellular and shunt pathway resistances were determined by Method A, as previously described (Reuss & Finn, 1974). Briefly, this method consists of the measurement of the total transepithelial resistance (R_t), the voltage divider ratio across the cellular transepithelial pathway, and the spread of intracellularly applied current into the epithelium. To avoid cellular electrical uncoupling induced by the impalement, the mucosal solution was replaced with mineral oil for the cable analysis measurements (Reuss & Finn, 1974). R_t and R_a/R_b were determined at least 30 min after the addition of urea, and the current spread subsequently (following the mineral oil substitution). The observation that the potential and resistance changes produced by urea are irreversible after 30 min *(see below)* validates the implicit assumption that any cellular alteration induced by urea persists during the exposure to mineral oil. For full discussion of Method A, *see* Eisenberg and Johnson (1970), Shiba (1971), Frömter (1972), and Reuss and Finn (1974).

The remaining experiments were performed by Method B, also previously described (Reuss & Finn, 1974). Briefly, this consists of the measurement of R_t and the voltage divider ratio before and after selectively changing the apical membrane resistance by the addition of amiloride (10^{-5} M, final concentration). This requires the assumption that amiloride does not change the basal-lateral membrane resistance or the shunt resistance. As shown previously, Method B yields values for the resistances identical to those found with Method A.

Results

Effects of Reductions in Mucosal NaCl Concentration on the Cellular and Paracellular Transepithelial Resistances

The effects of mucosal NaCl concentrations of 11 and 60 mM are shown in Table 1. At the lower NaCl concentration, the total transepithelial resistance increases by 20%. This increase is secondary to an increase in the shunt resistance to more than twice its control value. No changes were evident in the resistances of the cell membranes. At the intermediate NaCl concentration of 60 mM, the mean value of the shunt resistance was 40% higher than control. However, given the variation of these values, this change was not significant. Again, the resistance of the cellular pathway

Table 1. Changes in resistances of cellular and shunt pathways after exposure to low NaCl mucosal solutions

	Total transepithelial resistance (Ω cm^2)	Apical membrane resistance (Ω cm^2)	Basal membrane resistance (Ω cm^2)	Shunt resistance (Ω cm^2)
Control	4240 ± 580	4460 ± 1230	5190 ± 2580	13130 ± 2500
60 mM NaCl	5420 ± 790	5030 ± 910	5650 ± 1420	18570 ± 6930
Δ [a]	−1180 ± 630	−570 ± 1290	−460 ± 1610	−5440 ± 6040
P_{diff}	N.S.	N.S.	N.S.	N.S.
Control	4010 ± 350	4500 ± 740	3910 ± 930	10740 ± 1120
11 mM NaCl	5050 ± 500	4010 ± 650	4130 ± 970	25710 ± 3750
Δ [a]	−1040 ± 320	490 ± 640	−220 ± 480	−14970 ± 3710
P_{diff}	<0.01	N.S.	N.S.	<0.005

Resistances were measured in the same bladders under control conditions and after exposure to the low NaCl mucosal solution. The concentrations of the other solutes were kept constant. No solute was added to compensate for the reduction in osmolality. 60 mM NaCl, n = 5; 11 mM NaCl, n = 16.

[a] Δ = control-experimental.

remained unaltered. All these resistance changes were completely reversible even after prolonged exposure to the low NaCl mucosal solutions. To distinguish between the effect of the reduction in osmolality and that of NaCl concentration as the mechanism of the increase in the shunt resistance, a series of experiments was performed in which the resistances were measured at 11 mM mucosal NaCl concentration before and after the addition of sucrose to isosmolality. The results are shown in Fig. 1. No difference was observed for any of the measured parameters. Thus, the possibility that the increase of the shunt resistance at low mucosal NaCl concentration is due to the low osmolality of the mucosal solution can be ruled out.

Effects of Reductions in Mucosal NaCl Concentration on the Potential Profile of the Epithelium

The results of the simultaneous measurements of the apical and basal-lateral membrane potentials at the two low mucosal NaCl concentrations are summarized in Fig. 2. As expected, the transepithelial potential decreases as the external sodium concentration is diminished. These observations are consistent with the hypothesis that the apical membrane is sodium permselective. However, as shown in the Figure, the change in transepithelial potential

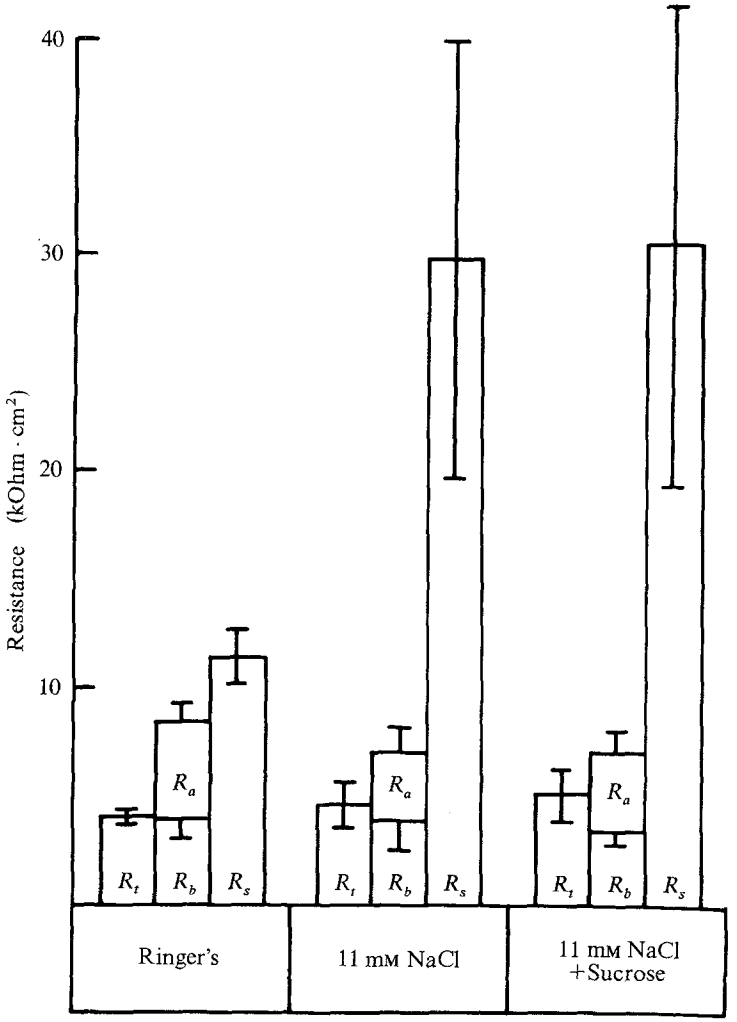

Fig. 1. Effect of low mucosal NaCl concentration on shunt resistance at low and normal total osmolalities. Resistances measured by Method B in the same bladders at 11 mM NaCl mucosal solution, before and after adding sucrose to isosmolality. Means ± SEM; n = 4 experiments; R_t = total transepithelial resistance, R_a = apical membrane resistance, R_b = basal-lateral membrane resistance, R_s = shunt resistance. All resistances are expressed per cm² of macroscopic surface of the bladder

is the consequence of reductions in the potential differences across both the apical and basal cell membranes, and not across the apical membrane alone, as might be expected. The potentials were not significantly changed by the addition of sucrose to bladders exposed to 11 mM NaCl on the mucosal side. These effects, as the resistance changes, were completely reversible.

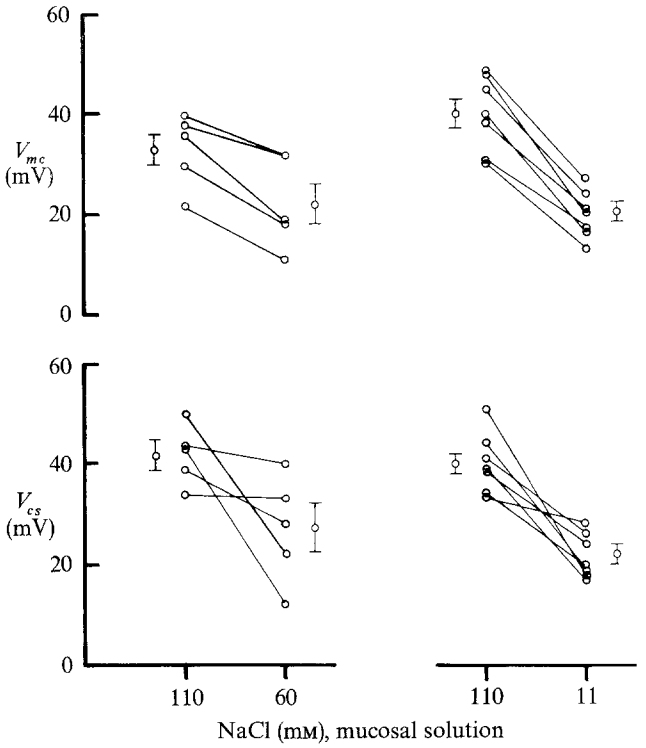

Fig. 2. Effect of low mucosal NaCl concentrations on apical and basal-lateral membrane potentials. For convenience, the values are plotted with the mucosal solutions as ground. Each value is the mean of at least 6 impalements in a given bladder. V_{mc} = apical membrane potential (cell-mucosal solution); V_{cs} = basal-lateral membrane potential (serosal solution-cell). Means ± SEM are given in addition to the individual results

Effects of Increases in Mucosal Solution Osmolality on the Cellular and Paracellular Transepithelial Resistances

The increase in osmolality was produced by the addition of either urea or NaCl. The effects of doubling the osmolality by the addition of urea are shown in Fig. 3. From the experiments performed by Method A the space constant ranged from 280 to 700 μm with a mean value of 390 μm. This is similar to the mean of 460 μm found under control conditions (Reuss & Finn, 1974). As shown in the Figure, the transepithelial resistance drops to 15% of its control value. Although the resistance of the cellular pathway is reduced by almost 50%, the main mechanism of this reduction of transepithelial resistance is a decrease in the resistance of the shunt to less than 10% of its control value. The effect of urea occurs within a few seconds, as shown in Fig. 4, and is reversible – at this concentration – only within

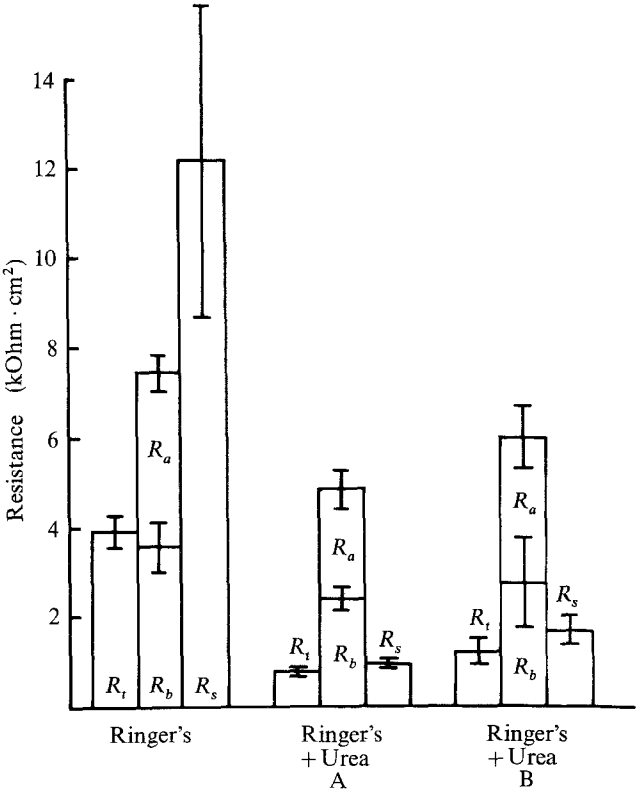

Fig. 3. Effect of the addition of urea (240 mM) to the mucosal solution on the transepithelial resistances. Abbreviations and units as in Fig. 1. The results obtained by Method A (n = 5) and Method B (n = 9) are plotted separately. Statistical comparisons show decrease by both methods, as compared to controls, of $R_t (p < 0.001)$, $R_a + R_b (p < 0.05)$, and $R_s (p < 0.001)$

about 30 min after the exposure. When Method B was employed to measure the resistances, essentially the same results were obtained, i.e., reduction of the total transepithelial resistance mainly by diminution of the shunt resistance with a moderate drop in the resistance of the cells.

In another series of experiments, NaCl was used instead of urea to increase the mucosal osmolality by 50 and 100% as compared to control. The effects on the resistances are summarized in Table 2. Doubling the mucosal osmolality with NaCl produces almost the same effects obtained with urea, i.e., a drop in the total resistance mainly due to reduction of the shunt resistance. The change of the cellular resistance is not statistically significant, but the mean value changes in the same direction as observed with urea. An intermediate increase of NaCl concentration also produces a significant reduction of the shunt resistance.

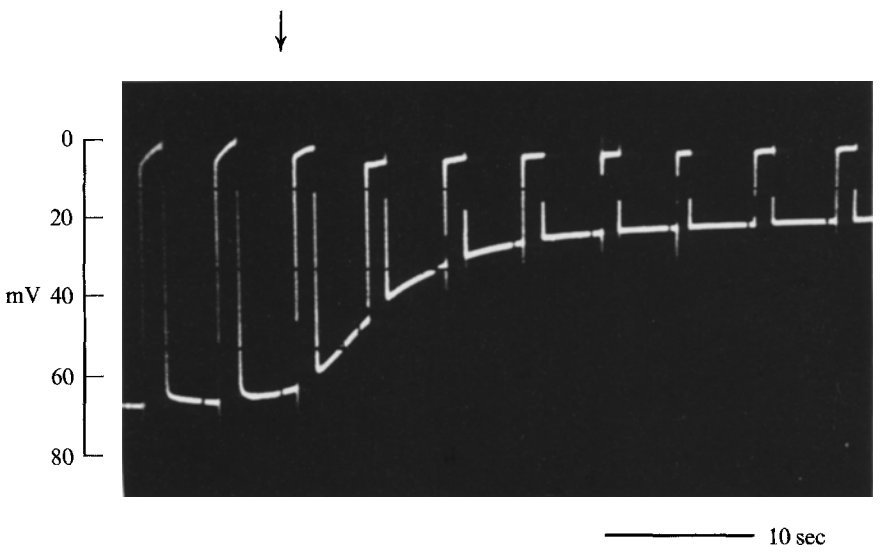

Fig. 4. Time course of the effect of urea (240 mM), added at the arrow to the mucosal solution, on transepithelial potential and resistance. The record shows a single typical experiment in which the transepithelial potential is measured continuously. Identical transepithelial pulses of 30 μA/cm² were passed every 5 sec. The magnitude of the potential change allows calculation of the transepithelial resistance which, as shown, falls within seconds after the change in solution

Table 2. Changes in resistance of cellular and shunt pathways after exposure to high NaCl mucosal solutions

	Total transepithelial resistance (Ω cm²)	Apical membrane resistance (Ω cm²)	Basal membrane resistance (Ω cm²)	Shunt resistance (Ω cm²)
Control	3860±450	4440±1010	4820±2140	12010±2330
170 mM NaCl	2750±320	4270±740	3340±750	7870±2020
△[a]	1110±530	170±680	1490±1460	4130±1590
p_{diff}	N.S.	N.S.	N.S.	<0.05
Control	3640±370	3800±740	3830±1470	10870±1700
230 mM NaCl	1130±190	2680±640	3290±920	1690±330
△[a]	2500±460	1120±750	540±1770	9180±1590
p_{diff}	<0.001	N.S.	N.S.	<0.001

Resistances measured in the same bladders under control conditions and after exposure to the high NaCl mucosal solution. The concentrations of the other solutes were kept constant. 170 mM NaCl, n=7; 230 mM NaCl, n=9.

[a] △=control-experimental.

From these experiments we conclude that the diminution of transepithelial resistance produced in the toad bladder by increasing the osmolality of the mucosal solution is mainly secondary to a reduction in the resistance of the shunt pathway. These changes are similar when NaCl, urea or, in other experiments not shown, KCl or sucrose are added at concentrations adequate to produce the same total osmolality change. Thus, this is a nonspecific osmotic effect.

Effects of Increases in Mucosal Solution Osmolality on the Potential Profile of the Epithelium

Fig. 5 shows the values of the potentials at both cell borders before and after the addition of NaCl to the mucosal solution. The effects of 240 mM urea and 120 mM NaCl additions are very similar. The transepithelial potential drops to 15% of control, and the potential across the apical membrane reverses. Thus, the normal two-step potential profile across this epithelium changes to a negative cell profile. An intermediate concentration

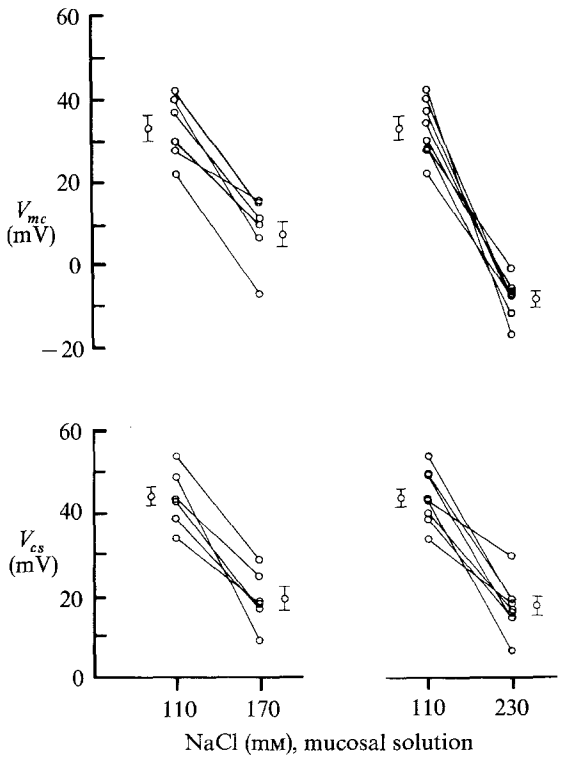

Fig. 5. Effect of high mucosal NaCl concentrations on apical and basal-lateral membrane potentials. Abbreviations as in Fig. 2. Each value is the mean of at least 6 impalements in a given bladder. Means ± SEM are given in addition to the individual results

of NaCl reduces the transepithelial and cell potentials by a smaller amount. Usually, the cell remains positive to the mucosal and negative to the serosal solution. These changes, like the resistance changes, are reversible if Ringer's solution replaces the hypertonic solution within 30 min.

Discussion

We have shown that the resistance of the shunt pathway increases after reduction of mucosal sodium chloride concentration to 11 mM, while the cell membrane resistances remain unchanged. This effect is secondary to the reduction of ionic concentration and not to the decreased osmolality, because the addition of sucrose to the low NaCl solution does not alter this result. Such a process might provide a mechanism to reduce the back leak of electrolytes into the lumen when the urine of the animal has a low sodium concentration. The observation of a reduction in mucosal membrane potential at low mucosal NaCl concentrations might seem inconsistent with the absence of measurable changes in the mucosal membrane resistance (which would be expected to increase). However, there are several possible explanations for this observation. First, the sodium conductance of the membrane might increase at low external Na concentrations: Finn (1971) has shown that the decrease in sodium influx (from mucosal medium to cells) is less than predicted (for a constant P_{Na}) when mucosal sodium is reduced from 111 to 10 mM, and Lindemann and Gebhardt (1973) have shown in frog urinary bladder that fast changes in mucosal sodium concentration produce electrical potential transients consistent with a higher P_{Na} at low Na concentration. Second, the membrane conductance for other ions might increase under these circumstances. Third, it is unlikely that the potential across the luminal membrane is due to diffusion alone (Finn, 1974).

We have previously shown that, under control conditions, almost all of the shunt resistance across this tissue is located in the zonula occludens of the junctional complexes (Reuss & Finn, 1974). In very simple terms, an increase in the shunt resistance may be due to a decrease of the conductivity of the solution that fills the transepithelial paracellular channels, to a reduction of the total width of these channels per unit area of bladder, to an increase of their length, or to a combination of these factors. As the paracellular pathway is constituted by the series arrangement of the zonula occludens, the zonula adherens, and the lateral intercellular space, a decrease in width at any of these levels might produce an increase in the shunt resistance. However, such a change seems unlikely, at least for the zonula

adherens and the lateral intercellular space, since they would have to be almost completely obliterated to account for the observed change (Reuss & Finn, 1974). Therefore, the change must occur in the zonula occludens. Although a reduction of the width of the channels presumably contained in this structure (Goodenough & Revel, 1970) would explain our observations, they are also consistent with a reduction of the conductivity of the solution within these structures. Its composition would then be dependent on the composition of the mucosal solution.

The transepithelial potential at low mucosal NaCl concentration was similar to observations with ionic substitutions (Gatzy & Clarkson, 1965; Leb, Hoshiko & Lindley, 1965). The unexpected finding is that the drop of transepithelial potential is due to similar reductions of the potential differences across both cell membranes, and not across the apical membrane alone, as might be expected. This observation is similar to those reported in frog skin (Cereijido & Curran, 1965) and *Amphiuma* distal tubule (Wiederholt & Giebisch, 1974). An obvious explanation would be that changes of intracellular ionic concentration took place (e.g., decrease in K activity), thus changing, under steady-state conditions, the diffusion potential across the serosal membrane. However, other experiments (Reuss & Finn, 1975) show that this and other changes of serosal membrane potential after changes of mucosal solution take place with a delay of only 10 to 15 msec (measured from the onset of the change in mucosal membrane potential to the onset of the change in serosal membrane potential). This rules out the possibility of significant changes in intracellular ionic concentrations mediated by diffusion. Further, it can be shown that the measured reduction in the shunt resistance (when mucosal Na increases) should change the serosal membrane potential in the direction opposite to that observed (Schultz, 1972).

The addition of urea, NaCl, KCl or sucrose to a normal mucosal solution produces a quick reduction of the transepithelial potential and the transepithelial resistance, which appears to be independent of the solution used. Thus, doubling the mucosal osmolality gives about the same reduction in transepithelial resistance with any of these solutes after a new steady-state is reached. The time courses of the resistance changes differ somewhat: with KCl the change is the fastest, and with sucrose, the slowest. It has been reported that hyperosmolality of the mucosal solution produces localized dilatations of the zonulae occludentes, and that the "tight junctions" become permeable to barium, sulfate, colloidal lanthanum, and ruthenium red (Wade *et al.*, 1973; Erlij & Martinez-Palomo, 1973). This strongly suggests opening of a paracellular shunt pathway. Our results, shown in Fig. 3

and Table 2, confirm this hypothesis. However, urea addition also brings about a decrease in the resistance of the cellular pathway. This is consistent with observations of ultrastructural alterations of the cells (Erlij & Martinez-Palomo, 1973) and increased permeability to lipoid-soluble substances presumed to go across the cells (Bindslev *et al.*, 1974) when the bladder is exposed to hyperosmolal mucosal solutions. If it is assumed that the solution within the zonula occludens has the conductivity of Ringer's, the magnitude of the shunt resistance change cannot be explained by a change of its composition alone since at most the change of ionic concentration on the mucosal side was twofold, and about the same R_s change took place after the addition of the nonionic molecule urea. Thus, to explain the reduction of the shunt resistance, an increase of width of the channels is necessary, and this is consistent with electron-microscopic observations by others. Previous calculations allow us to rule out any significant contribution of the zonula adherens and the lateral intercellular spaces to this resistance change (Reuss & Finn, 1974).

The potential profile across the epithelium was drastically changed after exposure to hyperosmotic mucosal solutions. With either NaCl or urea added in order to double the osmolality, the cell potential became negative to both solutions. The steady-state profile shows a reduction of the potential across the basal-lateral membrane and a change in polarity of the apical membrane potential. Such changes can occur solely as a consequence of the opening of the shunt pathway, since, in a tight epithelium in which the electromotive forces at each cell border are oriented in a stepwise fashion (i.e., cell positive to the mucosal solution and negative to the serosal solution), a decrease in shunt resistance can reverse the potential across the luminal membrane without changes in the electromotive forces themselves (Schultz, 1972). However, our measurements allow us to calculate the magnitude of the effect of the change in shunt resistance on the cell potentials at both borders, and this calculation shows that the drop in shunt resistance alone cannot quantitatively explain the membrane potential changes. This doubtlessly indicates that exposure to hyperosmotic solution on the mucosal side not only "opens" a shunt pathway, but also alters the cellular pathway. This alteration may be a change in intracellular ionic activities, membrane permselectivity, or pump activity.

In conclusion, our experiments indicate that the paracellular shunt pathway across the toad urinary bladder epithelium changes as a function of the sodium concentration – and in some instances osmolality – of the mucosal solution, in a way that might provide a regulatory mechanism for net sodium transport (Fig. 6). For instance, at low urinary sodium con-

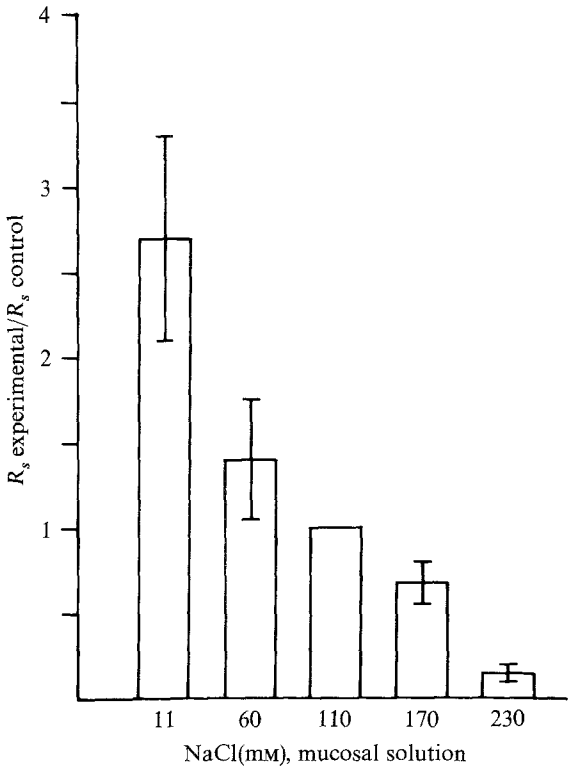

Fig. 6. Changes of shunt resistance as a function of mucosal NaCl concentration. Results normalized to the value of R_s with Ringer's solution bathing both sides of the tissue. Means ± SEM. *See* Tables 1 and 2

centration (i.e., under normal in vivo conditions), the high shunt resistance would prevent significant back leak of sodium, make the pump more efficient, and hence provide sodium conservation at minimal energy expenditure. Such control of shunt conductance may also have wide physiological implications in other tissues (Boulpaep, 1972; Lifschitz, Garcia & Earley, 1973; DeBermudez & Windhager, 1974).

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