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EFFECTS OF CHANGES IN THE COMPOSITION OF THE SEROSAL SOLUTION ON THE ELECTRICAL PROPERTIES OF THE TOAD URINARY BLADDER EPITHELIUM

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SUMMARY

1. The potential profile and the cellular and paracellular transepithelial resistances of the toad urinary bladder were measured, by means of micro-electrode techniques, as functions of the osmolality of the serosal solution.

2. Reductions in serosal osmolality (that increase the rate of active sodium transport) produced proportional decreases in the electrical resistances of the apical and basal–lateral cell membranes, while the changes in resistance of the paracellular pathway were more complex. The apical membrane potential increased.

3. Increases in serosal osmolality (that decrease sodium transport) produced increases in the electrical resistances of both cell membranes, and moderate reduction in the paracellular resistance. The polarity of the apical membrane potential reversed.

4. These results indicate that reductions in serosal solution osmolality stimulate sodium transport by increasing both the sodium permeability of the luminal cell membrane (thus increasing sodium entry), and the electromotive force generated at the serosal border of the cell, thus enhancing the rate of sodium pumping. Conversely, increases in osmolality reduced sodium transport by reducing both the sodium permeability of the luminal membrane and the serosal membrane electromotive force.

INTRODUCTION

In addition to hormonal influences, physical and/or chemical factors operating at the inner barrier of sodium-transporting epithelia can influence their transport rate. This has been shown to be the case in the

urinary bladder of the toad: moderate reductions in the osmolality of the solution bathing the serosal side increase the rate of sodium transport, and increases in the osmolality of that solution decrease it (Lipton, 1972; Bentley, Candia, Parisi & Saladino, 1973). It has also been shown that the total transepithelial electrical resistance of this tissue falls after exposure to hyposmotic serosal solutions, and simultaneously localized dilatations ('blisters') appear in the zonula occludens of the junctional complexes (DiBona & Civan, 1973; Wade, Revel & DiScala, 1973). Increases in the serosal osmolality also produce reductions in the transepithelial resistance. However, a higher concentration is required (as compared to the effect of the same solute from the mucosal side) and no structural alterations of the junctional complexes are evident (Bindslev, Tormey, Pietras & Wright, 1974).

These effects are not peculiar to the toad urinary bladder, since the relationship between inner solution osmolality and sodium transport rate was first demonstrated in the frog skin, and related to changes in cell volume (Ussing, 1965), and the relationship between inner solution osmolality and total transepithelial resistance has been also found in the distal tubule of rat kidney (DeBermudez & Windhager, 1974).

The interpretation of these observations is difficult on several grounds.

(1) Though in some experimental conditions there is a close correlation between osmotically induced reductions in transepithelial resistance and the presence of 'blisters' in the junctional complexes, this relationship is by no means constant (Bindslev *et al.* 1974).

(2) It is generally assumed that significant reductions in total transepithelial resistance reflect, in tight epithelia, diminutions in shunt resistance. However, it must be considered that the cellular and shunt resistances are of the same order of magnitude in the toad urinary bladder (Reuss & Finn, 1974) and thus possible contributions of the cellular pathway to drops in total resistance cannot be ruled out *a priori*. Furthermore, we have shown that the low transepithelial resistance observed in the toad urinary bladder after exposure to urea on the mucosal side is due to reductions of both shunt and cellular resistances (Reuss & Finn, 1975*b*) and this observation agrees with permeability determinations of Bindslev *et al.* (1974).

(3) The available information on effects of changes in the composition of the serosal solution on the electrical properties of tight epithelia does not distinguish between changes of the cellular and shunt transepithelial pathways, and the experiments that demonstrate an inverse relationship between serosal solution osmolality and net sodium transport do not evaluate the role of each cell membrane in these effects.

The use of micro-electrode techniques previously described (Reuss &

Finn, 1974, 1975*b*) allows us to measure the epithelial potential profile and the resistances of the cell membranes and shunt pathway under different experimental conditions in the same preparation. These techniques were employed to measure the cell membrane potentials and the resistances of the transepithelial pathways of the toad urinary bladder at several sodium concentrations and osmolalities of the serosal solution.

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 a Sylgard cylinder on the centre and on a plastic perforated ring on the periphery. Only those with a control transepithelial potential higher than 50 mV were used. The control electrophysiological measurements were performed with Ringer solution bathing both sides of the tissue (composition, mM/l.: 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). The serosal solution was changed to one containing different NaCl and sucrose concentrations (see Results), while the other solutes were kept constant. After the transepithelial potential and short-circuit current reached a new steady-state following the change of solution, the electrophysiological measurements were repeated. The intracellular potentials and the cellular potential changes produced by the transepithelial pulses were measured with intracellular micro-electrodes, as previously described. Ag–AgCl electrodes connected to the solutions by means of agar–Ringer bridges were used to measure the transepithelial potential and to apply transepithelial d.c. pulses. All potentials were measured relative to that of the serosal solution, taken as ground. The criteria for successful impalement were the same as previously described (Reuss & Finn, 1974).

Determination of the resistances. The total transepithelial resistance (R_t) was calculated from

$$R_t = (E/i)s,$$

where E is the transepithelial voltage deflexion 1 sec 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 ratio of the apical (R_a) to the basal–lateral (R_b) cell membrane resistance was calculated from the voltage divider ratio (produced by a transepithelial pulse with the micro-electrode in a cell). Usually, the apical membrane voltage deflexion (ΔV_a) was calculated by subtracting the basal membrane deflexion (ΔV_b) from the transepithelial voltage deflexion (ΔV_t):

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

The value of R_a/R_b was calculated as the mean of at least six impalements in each condition.

The values of R_t and R_a/R_b were measured before and after the addition of 10^{-5} M amiloride to the mucosal solution, either with Ringer or an experimental solution on the serosal side. We have shown that amiloride increases R_a without altering R_b or R_s; therefore the measurements before and after the use of amiloride allow the calculation of R_s, R_a and R_b (Reuss & Finn, 1974, 1975*b*).

RESULTS

Effects of low serosal NaCl concentrations on the transepithelial resistances

Exposure of bladders to a serosal NaCl concentration of 83 or 60 mM (osmolalities reduced, as compared to control, by 25 and 40 %, respectively) produces increases in transepithelial potential and short-circuit current, as previously shown (Lipton, 1972), while the total transepithelial resistance falls.

TABLE 1. Effects of low serosal NaCl concentration on transepithelial cellular and shunt resistances

	Total transepithelial resistance ($\Omega.cm^2$)	Apical membrane resistance ($\Omega.cm^2$)	Basal membrane resistance ($\Omega.cm^2$)	Shunt resistance ($\Omega.cm^2$)
Control	4890 ± 470	4850 ± 1110	4110 ± 640	10280 ± 1250
83 mM-NaCl	3420 ± 340	2310 ± 380	1690 ± 260	27840 ± 4500
Difference	1470 ± 240	2550 ± 770	2420 ± 410	17560 ± 5130
P	< 0·005	< 0·05	< 0·005	< 0·05
Control	4760 ± 500	5740 ± 1120	4520 ± 860	12890 ± 1870
60 mM-NaCl	3340 ± 350	3230 ± 700	1920 ± 370	24860 ± 7270
Difference	1420 ± 280	2500 ± 900	2610 ± 690	11970 ± 7230
P	< 0·001	< 0·025	< 0·005	n.s.

Resistances measured in the same preparations under control conditions and after exposure to the low NaCl serosal solution. The concentrations of all other solutes were kept constant. 83 mM-NaCl, $n = 5$; 60 mM-NaCl, $n = 10$.

The effects on the resistances of the cellular and shunt pathways are summarized in Table 1. In both experimental conditions, the resistances of the apical (R_a) and basal–lateral (R_b) membranes fell significantly. The resistance of the shunt pathway (R_s) increased by 170 % at 83 mM-NaCl, and by 90 % at 60 mM-NaCl, although at the latter concentration this change was not significant. In any event, the data clearly indicate that the reduction in total transepithelial resistance at these two low serosal NaCl concentrations is the consequence of the diminution in the resistances of the cell membranes.

To get additional information on the shunt resistance as a function of serosal NaCl concentration, the resistances were measured after exposing the bladder to an 11 mM-NaCl serosal solution. R_a and R_b were reduced to 12 and 17 % of control, respectively, while R_s fell to a mean value of 13 % of control. These changes, and the accompanying reduction in total transepithelial resistance are only partially reversible,

while reversibility of the same parameters was complete within 2 hr of exposure to 83 or 60 mM-NaCl on the serosal side.

These results indicate that the resistance of the cellular pathway is reduced at low serosal NaCl concentrations, while the shunt resistance increases and then falls when the NaCl concentration is reduced further. To distinguish between the effects of reduced serosal osmolality and NaCl concentration *per se*, the serosal NaCl was reduced to 83 mM and

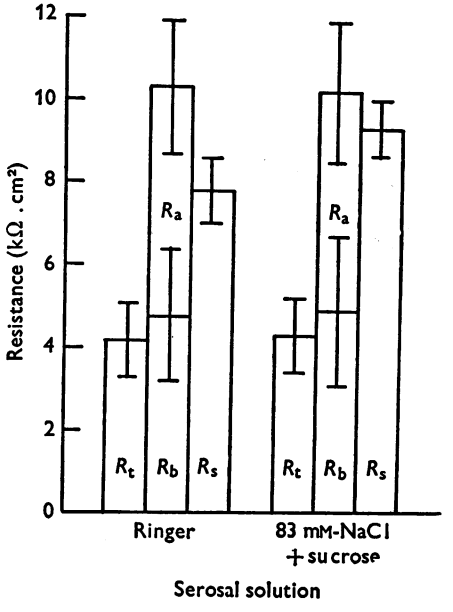

Fig. 1. Effects of low serosal NaCl concentration on the resistances of the cellular and paracellular (shunt) transepithelial pathways. The resistances were measured at different times in the same preparations exposed to Ringer or the experimental solution (83 mM-NaCl plus sucrose to isosmolality) on the serosal side. The order in which the measurements were performed was randomized. R_t = total transepithelial resistance, R_a = apical membrane resistance, R_b = shunt resistance. Data are shown as means ± S.E. of mean. There were no significant changes in any parameter (compare Table 1). n = 5 expts.

the osmolality kept constant by addition of sucrose. These results are shown in Fig. 1. No significant differences were observed as compared to Ringer. Similar lack of changes in the resistances was also demonstrated in three experiments in which serosal NaCl was reduced to 60 mM, with sucrose added to isosmolality. These results indicate that the low serosal solution osmolality, and not the low NaCl concentration *per se*, is the factor necessary to alter the resistances of the cellular and shunt pathways.

Effects of low serosal NaCl concentrations on the cell membrane potentials

The changes in potential across the apical and basal–lateral membranes after exposure to 83 or 60 mM-NaCl on the serosal side are shown in Fig. 2. No significant changes of the basal membrane potentials (V_{cs}) were observed. However, the apical membrane potential (V_{mc}) was significantly increased at both low NaCl concentrations. Note that this

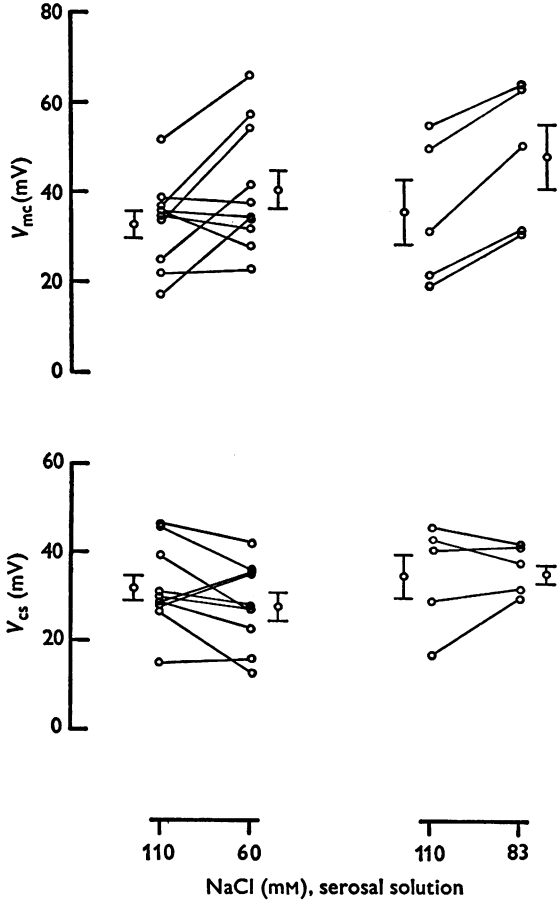

Fig. 2. Effects of low serosal NaCl concentration and osmolality on the cell membrane potentials. Potentials measured in the same bladders before and after replacing Ringer by the low NaCl solution on the serosal side. Each value corresponds to one preparation (mean of at least six impalements). V_{mc} = apical membrane potential (cell − mucosal solution), V_{cs} = basal–lateral membrane potential (serosal solution − cell). Means ± S.E. of mean are shown for each condition. The changes in V_{mc} are significant ($P < 0{\cdot}025$ at 60 mM-NaCl, $P < 0{\cdot}0025$ at 83 mM-NaCl, paired analysis). No significant changes in V_{cs}.

increase in apical membrane potential is measured at a time when the resistance of the membrane is decreased (Table 1), thus indicating an increase in the Na permeability of the apical membrane.

At 11 mM-NaCl, the potentials across both membranes fell: V_{mc} from 30 ± 3 to 2 ± 8 and V_{cs} from 32 ± 6 to 19 ± 2 mV. These observations are consistent with the measured change in R_s (see above).

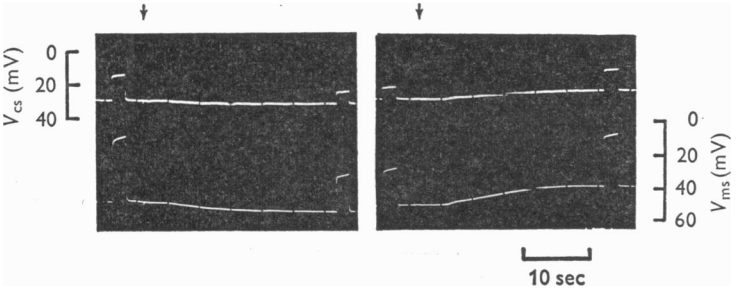

Fig. 3. Time course of the effect of the reduction of serosal NaCl concentration (and osmolality) on cell potential and transepithelial resistances. V_{cs} = basal-lateral membrane potential, V_{ms} = transepithelial potential. The record starts on the left with the micro-electrode in a cell. At the first arrow, the serosal solution was changed to 60 mM-NaCl, and at the second arrow to standard Ringer. Identical transepithelial d.c. pulses were applied to monitor the resistance of the tissue. See text.

The time course of the change in short-circuit current has been reported in a previous study (Lipton, 1972). As changes in cell volume produced by osmotic gradients are presumed to be fast, it seemed interesting to study the changes in potential and resistances during the serosal solution substitution, mainly to compare the direction of any observed changes with the results obtained from steady-state determinations. Fig. 3 shows that these changes may occur within seconds and were in the same direction as observed after prolonged exposure. It can also be seen that the apical membrane potential changed more than the basal–lateral membrane potential.

The change in basal–lateral membrane potential was in general smaller than the change in apical membrane potential, and sometimes absent. The time course of the changes was also variable, the rate of change being roughly proportional to the magnitude of the imposed osmotic gradient. At 60 and 83 mM, the maximum effect was reached at about 30 and 40 min, respectively. The resistance change is shown by the magnitude of the voltage deflexions produced by identical transepithelial d.c. pulses: after exposure to low NaCl concentrations the deflexions across the whole tissue and across the basal membrane are reduced.

From records such as those shown in Fig. 3 it is possible to compute the short-circuit current, which increases *pari-passu* with the increases in potential and tissue conductance. Although measurements of this kind are not adequate to allow calculation of the relative changes in the cellular and paracellular transepithelial resistances (see Methods), the simultaneous observation of hyperpolarization suggests that the drop in R_t is not the consequence of diminished shunt resistance, but secondary to the reduction in R_a and R_b, as was shown to be the case in the steady state (Figs. 1 and 2, Table 1).

The potentials at both cell borders were also measured after exposure of the tissue to low NaCl serosal solutions to which sucrose was added to isosmolality. At 83 mM-NaCl there were no significant changes in V_{mc} or V_{cs}; at 60 mM-NaCl, V_{cs} remained unchanged, while V_{mc} decreased slightly; this change is in the opposite direction to that seen at the same NaCl concentration when the osmolality was kept low. These experiments indicate, as noted for the resistance changes, that the low serosal osmolality and not the low NaCl concentration by itself is the factor that increases the potential at the apical cell border.

Effects of high serosal NaCl concentrations and osmolalities on the transepithelial resistances

Exposure of bladders to 170 or 230 mM-NaCl on the serosal side (50 and 100% increases in osmolality) produces a fall in both transepithelial potential and short-circuit current, which reach a new steady state in about 30–45 min. The total transepithelial resistance rises slowly during this period.

The effects on the resistances of the cellular and shunt pathways are shown in Table 2. At the two high NaCl concentrations, the resistances of both cell membranes are increased; at 170 mM-NaCl, R_a increases by 180% and R_b increases by 300%; at 230 mM-NaCl, the relative changes are about six- and nine-fold, respectively. In both situations, the increase in R_b was greater than the increase in R_a, and the voltage divider ratio (R_a/R_b) was in every experiment less than 1. The resistance of the shunt pathway was reduced, at the two high serosal NaCl concentrations, to about 50% of control. This fact explains why the increase in total transepithelial resistance was only moderate.

Another series of experiments was performed to distinguish between the effect of high osmolality and high NaCl concentration by itself on these parameters. 120 mM sucrose was added to Ringer solution in order to raise the serosal osmolality to a value similar to that at 170 mM-NaCl. The results, shown in Fig. 4, are very similar to those obtained with

TABLE 2. Effects of high serosal NaCl concentration on transepithelial cellular and shunt resistances

	Total transepithelial resistance ($\Omega.cm^2$)	Apical membrane resistance ($\Omega.cm^2$)	Basal membrane resistance ($\Omega.cm^2$)	Shunt resistance ($\Omega.cm^2$)
Control	3900 ± 410	3200 ± 360	2730 ± 290	12420 ± 1780
170 mM-NaCl	4900 ± 530	8880 ± 2030	10920 ± 2010	6980 ± 1200
Difference	1000 ± 200	5680 ± 1900	8180 ± 1830	5450 ± 1400
P	< 0·005	< 0·025	< 0·005	< 0·01
Control	4510 ± 390	3740 ± 170	2930 ± 480	14460 ± 1510
230 mM-NaCl	6590 ± 470	22610 ± 5840	27820 ± 4510	7840 ± 670
Difference	2080 ± 160	18880 ± 5670	24890 ± 4340	6620 ± 1360
P	< 0·001	< 0·05	< 0·02	< 0·01

Resistances measured in the same preparations under control conditions and after exposure to the high NaCl serosal solution. The concentrations of all other solutes were kept constant. 170 mM-NaCl, $n = 7$; 230 mM-NaCl, $n = 4$.

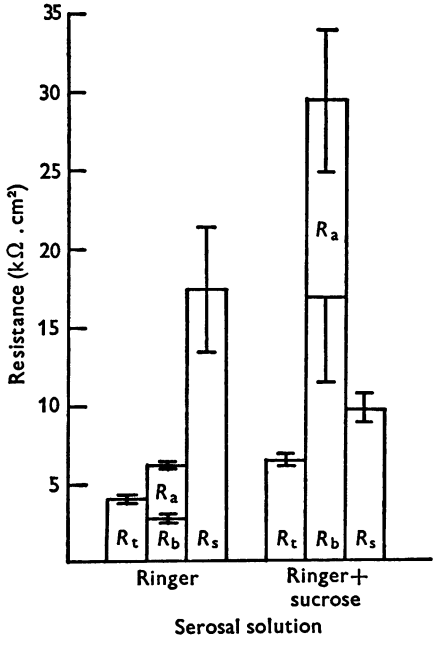

Fig. 4. Effect of a hyperosmotic serosal solution (Ringer plus 120 mM sucrose) on the resistances of the cellular and shunt pathways. Resistances measured in the same preparations under exposure to serosal standard and experimental solutions. Abbreviations as in Fig. 1. All changes are significant ($P < 0·05$, paired analysis). $n = 5$ expts. Compare with Table 2.

NaCl, i.e. increase in the resistances of both cell membranes (R_b more than R_a) and decrease in R_s to about 50% of its control value. These observations indicate that the changes in passive electrical properties observed after exposure of the bladder to high serosal NaCl concentration are the consequence of the hyperosmolality of the solution, and not of the high NaCl concentration by itself.

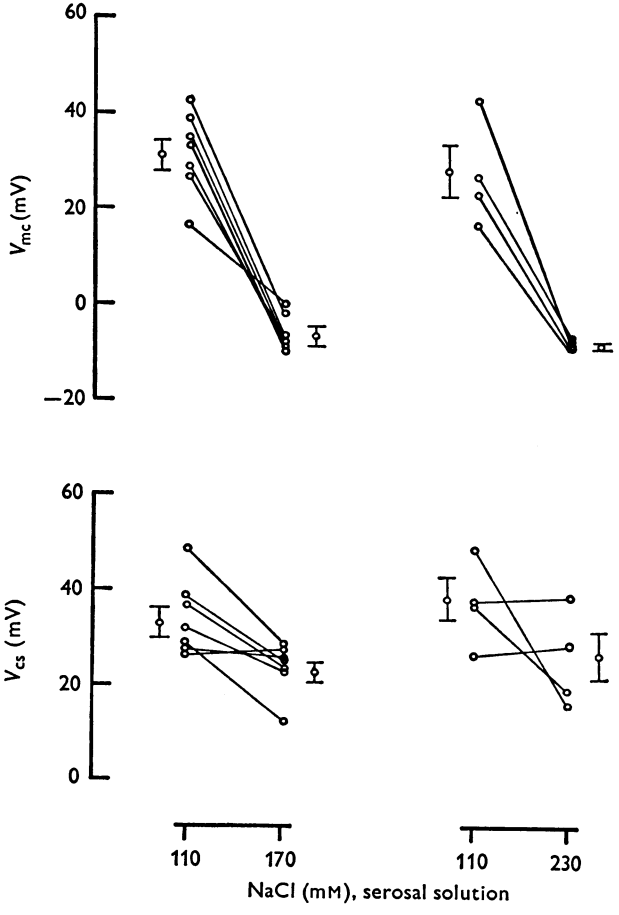

Fig. 5. Effects of high serosal NaCl concentration and osmolality on the cell membrane potentials. Experimental procedure and abbreviations as in Fig. 2. The changes in V_{mc} are significant ($P < 0{\cdot}001$ at 170 mM-NaCl, $P < 0{\cdot}01$ at 230 mM-NaCl). Change in V_{cs} significant at 170 mM-NaCl ($P < 0{\cdot}01$).

Effects of high serosal NaCl concentrations and osmolalities on the cell membrane potentials

Fig. 5 summarizes the changes in electrical potential at both cell borders after exposure to high NaCl serosal solutions. The most striking changes occur at the luminal membrane, whose potential is reversed (i.e. the cell becomes negative to the mucosal solution). The basal membrane potential is somewhat reduced (significantly at 170 mM-NaCl). Again, these changes are due to a non-specific osmotic effect, as shown by the fact that the addition of sucrose to the serosal solution gives the same results (Fig. 6).

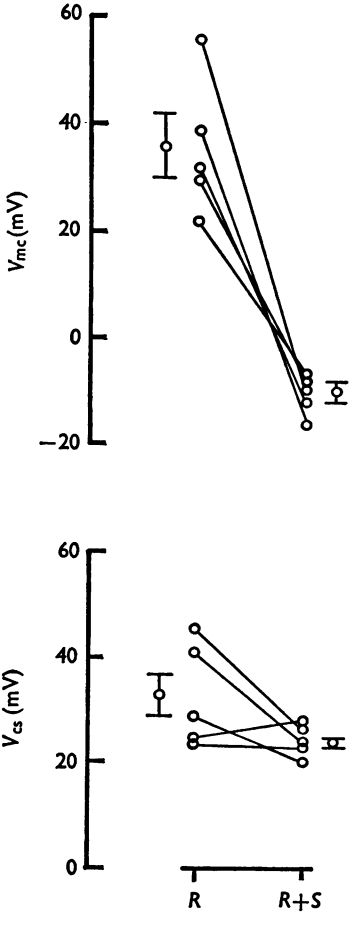

Fig. 6. Effects of a hyperosmotic serosal solution (Ringer plus 120 mM sucrose) on the cell membrane potentials. Experimental procedure and abbreviations as in Fig. 2. The change in V_{mc} is significant ($P < 0{\cdot}005$).

In experiments similar to that shown in Fig. 3, the potentials across both cell borders were monitored continuously during the change in solution. Although a slight increase in resistance was evident within a few seconds, no significant changes in cell potentials were observed within this short time.

DISCUSSION

Changes in the osmolality of the serosal solution produce alterations in both the cellular and paracellular transepithelial pathways of the toad urinary bladder. Significant changes are observed in the resistances of the cell membranes and the shunt pathway, and in the potential profile across the epithelium.

The effects of high and low serosal osmolalities on the resistances of these pathways are summarized in Fig. 7. The shunt resistance varies

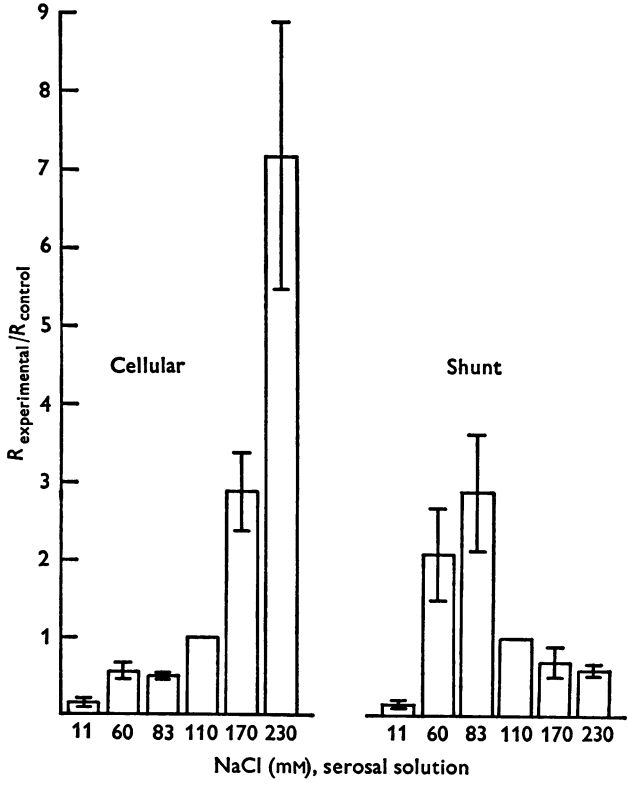

Fig. 7. Summary of the effects of several serosal NaCl concentrations (in the absence of sucrose) on the resistances of the cellular ($R_a + R_b$) and shunt (R_s) pathways. All values are normalized to that measured in the same preparation with standard Ringer on both sides. Means ± S.E. of mean.

inversely with the osmolality of the serosal solution, in the range of 75% (83 mM-NaCl) to 200% (230 mM-NaCl) of the osmolality of Ringer solution. Experiments in which sucrose was used to replace NaCl or added to the control solution prove that these effects, as the effects on the resistances of the cell membranes and on the potential profile, are due to the osmolality changes and not to the serosal NaCl concentration by itself. At a serosal solution osmolality below 75% of control, R_s starts to decrease as the osmolality is reduced further. At 11 mM-NaCl (osmolality = 15% of control) the drop in R_s and R_t is only partially reversible, and the cell potentials frequently unstable, suggesting cell damage that might contribute to the calculated conductance of the shunt. However, it is possible that the decrease in R_s in the low osmolality range may partly correspond to an increase in the conductance of the paracellular pathway itself, as indicated by the observation of 'blisters' in the tight junctions when the serosal solution is diluted by 50% (DiBona & Civan, 1973). With the exception of very low serosal osmolalities, the changes in shunt resistance take place in the same direction as previously shown for changes in the osmolality of the mucosal solution (Reuss & Finn, 1975*b*). However, it is clear that the magnitude of the decrease in R_s at high osmolalities is significantly less when the solute is added to the serosal solution. This is the case, in our experiments, with both NaCl and sucrose. This observation is consistent with the absence of reductions in total transepithelial resistance when the serosal osmolality is raised by 240–300 m-osmole/kg (Urakabe, Handler & Orloff, 1970; Bindslev *et al.* 1974). The latter authors added 500 mM sucrose to the serosal solution in order to obtain a similar effect to that produced by 300 mM urea from the mucosal side.

Within a twofold increase in serosal osmolality, the shunt resistance decreased only to about 50% of control, while the resistance of the cellular pathway increased several-fold, thus raising significantly the total resistance. The fact that the decrease in R_s is observed with the addition of both ionic and non-polar molecules rules out the possibility of a reduced shunt resistance mediated by increased conductivity of the solution contained in paracellular transepithelial aqueous channels. Previous calculations (Reuss & Finn, 1974) have shown that the fraction of the shunt resistance located in the zonula adhaerens and the lateral intercellular space is negligible; thus, the drop in R_s seems more likely to be due to a reduction of the resistance of the zonula occludens, probably via widening of the channels contained in this segment. The absence of blisters under this experimental situation (Bindslev *et al.* 1974) does not invalidate this explanation because the required increase in radius of these channels would be a few Å (e.g. *ca.* 10 Å if 1% the total area of

zonulae occludentes is assumed to be occupied by aqueous channels). Furthermore, the magnitude of the changes in R_s that we measure would allow one to predict that no gross dilatations of the zonulae occludentes need have occurred.

As shown also in Fig. 7, the electrical resistance of the cellular pathway varies directly with the osmolality of the serosal solution .The experiments shown in Figs. 1 and 4 indicate that the effects are not due to the changes in NaCl concentration by itself. The cellular resistance changes are more marked than the changes in shunt resistance. Consequently, the total transepithelial resistance changes in the same direction as the cellular resistance (Tables 1 and 2). These changes in transepithelial resistance are comparable to those observed by Bentley *et al.* (1973) at high serosal osmolality. However, Lipton (1972) did not observe changes in total resistance at low serosal osmolality. Unfortunately, no absolute values were reported. If, in those experiments, the shunt resistance were low enough under control conditions, the contribution of the cellular resistance to the total resistance would be minimized, and thus the latter might remain apparently constant even with important variations in the cellular resistance. Both the apical and basal resistances increased or decreased together as a function of the osmolality of the serosal solution, but the change in R_b was greater than the change in R_a. Thus, the ratio R_a/R_b decreased at high osmolalities and increased at low osmolalities. As net sodium transport takes place via the transcellular pathway, the increase at low serosal osmolality and the decrease at high serosal osmolality are due to changes in the resistance of this pathway. The likelihood that the volume of the cells changes inversely with the effective osmolality of the serosal solution (Lipton, 1972; Bentley *et al.* 1973), suggests that the resistances of the cell membranes, and thus the rate of active sodium transport, might be a direct function of cell volume, as initially suggested by Ussing (1965).

The changes in potential profile across the epithelium took place mainly at the apical membrane: its potential increased at low serosal osmolality and decreased at high serosal osmolality. In theory, several mechanisms might explain the increase in apical membrane potential at low serosal osmolality. As illustrated in Fig. 8, V_{mc} (the measured potential across the apical membrane) is a direct function of E_a, the electromotive force generated at this membrane, an inverse function of E_b (electromotive force generated at the basal–lateral membrane), provided that there is a finite shunt resistance in parallel with the cells, and depends also on the values of the resistances of the cell membranes and the shunt. Although this equivalent circuit cannot be presently subjected to complete analysis, three possible mechanisms for the increase

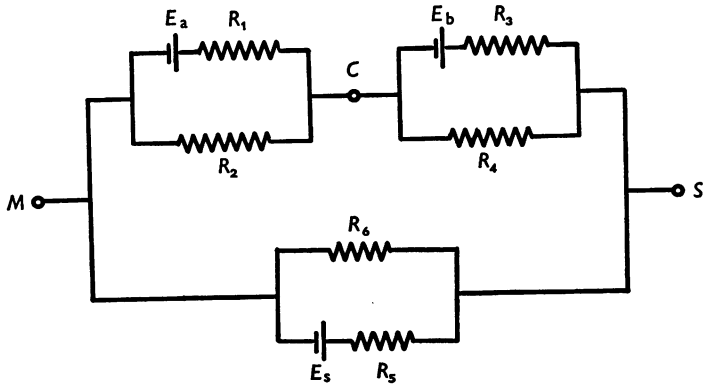

Fig. 8. Equivalent circuit for the toad urinary bladder epithelium. M = mucosal solution, C = cell, S = serosal solution. E_a, E_b and E_s represent the electromotive forces generated at the apical membrane, basal–lateral membrane and shunt pathway, respectively; R_1, R_3 and R_5 the internal resistances of the batteries; R_2, R_4 and R_6, their external resistances. R_a, R_b and R_s (equivalent resistances of the apical membrane, basal–lateral membrane, and shunt, respectively) result from the parallel disposition of the corresponding internal and external resistances. The significance of E_s is discussed in the text. See Schultz (1972).

in V_{mc} could be an increase in R_s, a decrease in E_b, or an increase in E_a. The increase in R_s was demonstrated at 83 and 60 mM serosal NaCl. However, two arguments indicate that the contribution of this mechanism is probably small: (1) the lack of a symmetric effect on V_{cs} (which would be expected to increase also); (2) the fact that R_s is high (as compared to the resistances of the cell membranes) under control conditions, and thus the depolarization produced by one membrane potential on the other (via the shunt) is necessarily small. The second possibility, i.e. a decrease in E_b, is also unlikely, because the short-circuit current was increased and V_{cs} remained unchanged, even though R_b was diminished. As discussed below, this suggests that E_b actually increased. Finally, the most likely possibility is that the increase in V_{mc} is the consequence of an increase in E_a. If we accept that the conductance of this membrane is mainly its sodium conductance (Leb, Hoshiko & Lindley, 1965; Gatzy & Clarkson, 1965; Finn & Nellans, 1972), the simultaneous observation of increased potential and increased conductance (i.e. decreased resistance) indicates an increase in sodium permeability. In theory, the decrease in R_a under these conditions could be secondary to increased apical membrane conductance to ions other than sodium, but such a mechanism (e.g. increased g_{Cl} or g_K) would, by itself, reduce V_{mc}. Thus, if this effect actually takes place, the increase in g_{Na} is even

greater. An alternative explanation would be that, as two pathways for sodium entry have been clearly identified (Finn, 1975), one of them might be electrogenic and was stimulated by serosal hyposmolality. The magnitude of the change in R_a under these circumstances would be difficult to predict. Finally, the apical potential increase could be the consequence of reduced electrogenic transport of H^+ from the cells to the mucosal solution. Two arguments seem to rule out this possibility: first, at the pH of the solutions employed in these experiments, H^+ secretion is negligible (Ludens & Fanestil, 1972), and second, if such were only mechanism of the increase in V_{mc}, R_a would not be expected to decrease. In summary, the simultaneous observations of increases in short-circuit current, apical membrane potential and apical membrane conductance indicate an augmentation of apical g_{Na}, even though additional mechanisms might effect less important contributions to either the change in potential or electrical resistance.

The serosal membrane potential remained essentially unchanged at low serosal osmolality. For the reasons already discussed, the contribution of changes in E_a or R_s to V_{cs} is probably small. The fact is that V_{cs} did not change even though R_b decreased by about 50%. According to the interpretation of the basal membrane potential as being a potassium diffusion potential, this would indicate an increase in potassium conductance, and consequently in E_b. However, this interpretation of the serosal membrane potential has been challenged by observations that indicate that the sodium pump may be electrogenic (Frazier & Leaf, 1963; Finn, 1974). Again, a decreased resistance of the active transport pathway, at the serosal border of the cell, would explain the higher rate of sodium transport, the lower resistance of the membrane, and the constancy of the membrane potential. Independently of the mechanisms involved, our observations indicate that both the basal sodium pump and the sodium entry into the cells are stimulated by hyposmolality of the serosal solution.

At high serosal osmolality, again the main change in potential profile took place at the apical membrane. In almost every case, the cell potential, normally positive to the mucosal solution, became negative. As the resistance of the apical membrane increased several-fold, it is safe to conclude that its sodium permeability was drastically reduced. The potential reversal might correspond to the contribution of the basal membrane to the apical potential through a decreased R_s, or to a true reversal of E_a (e.g. increased g_{Cl} or H^+ secretion). Similarly to the previous analysis, the concordant directions of the changes in short-circuit current, apical membrane potential and apical membrane resistance make certain the conclusion of decreased apical sodium permeability.

The basal–lateral membrane potential was moderately reduced at high serosal osmolality. The change was significant only in the series at 170 mM-NaCl, but the same tendency was observed at 230 mM-NaCl, and 120 mM sucrose. According to the possible interpretations of this potential (see above), either the potassium conductance of the membrane is reduced (diffusion potential hypothesis) or the resistance of the active sodium transport pathway is increased (electrogenic pump hypothesis). Both alternatives would account qualitatively for the higher basal–lateral membrane resistance and the somewhat reduced potential.

In this discussion we have implicitly assumed that no current is generated at the shunt, and thus the shunt does not contribute to V_{mc} or V_{cs}, except as a passive conductive pathway (i.e. $E_s = 0$). However, if the shunt were permselective, the exposure to bathing solutions asymmetric in ionic concentrations would generate paracellular diffusion potentials capable of changing V_{mc} and V_{cs} (Fig. 8). No evaluation of the permselectivity of the shunt in tight epithelia is presently possible, the main difficulty being the predominant role of the cellular pathway in the generation of the transepithelial potential. However, it is possible to calculate, from the equations describing the circuit shown in Fig. 8, the maximal possible contribution of E_s to V_{mc} during exposure to hyposmolal serosal solutions, namely by assuming that sodium is the sole permeant species across the shunt. The results are 1·7 mV at 60 and 0·5 mV at 83 mM-NaCl. Even these extreme values are smaller than the measured V_{mc} changes. In addition, the comparison of the changes in V_{mc} during exposure to serosal solutions made hyperosmolal by addition of NaCl or sucrose shows that they were very similar, even though a shunt NaCl concentration gradient was present only in the former situation.

In summary, our experiments show that exposure of the toad urinary bladder to low serosal osmolality produces, possibly by cell swelling, increases in both the sodium permeability of the apical membrane and the rate of sodium pumping at the serosal border. High serosal osmolality, that produces cell shrinkage, decreases the sodium permeability of the apical membrane and the rate of active sodium extrusion. Although our experiments do not provide evidence for dependence or independence between these two sites of action, previous observations have shown a similarly dual action of vasopressin (Finn, 1971) and close dependence of the basal membrane potential on apical potential changes induced by changes of the composition of the mucosal solution (Reuss & Finn, 1975*a*). Together, these observations suggest that the two cell membranes do not behave as if they were independent of one another.

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