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The Paracellular Pathway in Toad Urinary Bladder: Permselectivity and Kinetics of Opening

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Summary. Determination of serosa-to-mucosa fluxes of Na, K, and Cl yields information about the properties of the shunt pathway in toad urinary bladder. We show that measurement of these fluxes at 30-sec intervals following an abrupt increase in mucosal osmolality yields evidence on the rate of opening of the path and of its permselectivity. The relationship between the fluxes of any pair of these ions indicates that the shunt is paracellular both before and after the increase in conductance effected by hyperosmolality and that the transepithelial PD affects the permselectivity properties (at 0 mV, $P_K/P_{Na}/P_{Cl}=1:0.71:0.57$; at +25 mV, $P_K/P_{Na}/P_{Cl}=1:0.71:0.99$). The relationship between any of the fluxes and the total transepithelial conductance is linear and yields an estimate of cellular conductance (the intercept of this regression on the conductance axis) which is in accord with that measured electrically. These studies provide information on tight junction permeability to nonelectrolytes, as well. Finally, they provide new information about the role of the shunt path as a controlling influence on transepithelial sodium transport and raise the possibility that, in both leaky and tight epithelia, differences in transepithelial conductance from tissue to tissue, organ to organ, and species to species may be due, in the absence of edge damage, to changes in conductance of the paracellular pathway.

Since the early observations of Ussing and Windhager (1964) and of DiBona and Civan (1973), it has been clear that there are at least two parallel pathways for the movement of small molecules across epithelia, the transcellular and the paracellular, or shunt pathway. Epithelia of low resistance ("leaky"), such as the gallbladder, ileum, and proximal renal tubule, have an overall conductance which is largely determined by the conductance of the paracellular pathway. As has been shown by Frömter (1972), this pathway, at least in *Necturus* gallbladder, appears clearly to be between the cells. By inference, it has been assumed that such pathways in other epithelia are also intercellular, although unequivocal evidence on this point is hard to obtain. Nonetheless, in some tight epithelia the evidence is fairly compelling that the two pathways are constituted by a cellular pathway and a paracellular pathway, which

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includes the lateral intercellular spaces and the tight junctions. Electrophysiological data from our laboratory (Reuss & Finn, 1974, 1975) have shown that in toad urinary bladder the paracellular pathway accounts for approximately 30% of the total tissue conductance. Approximately similar results were found from the measurement of partial ionic conductances by Saito, Lief, and Essig (1974). It was shown that the serosa-to-mucosa fluxes of sodium, chloride, and potassium behaved as though they were traversing an extracellular path. In addition, it has been shown (Beauwens & Al-Awqati, 1976; Canessa, Labarca & Leaf, 1976; Macknight & McLaughlin, 1977) that little, if any, sodium enters the cells from the serosal side, again indicating that the serosa-to-mucosa sodium flux traverses only the paracellular path.

There are problems in evaluating this pathway in tight epithelia which do not exist in leaky epithelia. For instance, in the latter, since the conductance of the paracellular pathway is so high, measurements of transepithelial resistance and dilution potentials can be taken to indicate the properties of the shunt, and many such experiments have been done to study the nature of the shunt pathway and its behavior under various conditions (Moreno & Diamond, 1974). These studies have given considerable insight into the nature of the shunt path in these tissues, but have given no indication that this pathway plays a role in the control of transepithelial movement of sodium chloride.

On the other hand, in toad urinary bladder, increases in the mucosal solution osmolality lead to a marked increase in shunt conductance, with only slight changes in the cellular pathways (Reuss & Finn, 1976), and morphological alterations in the tight junction (DiBona & Civan, 1973; Wade, Revel & DiScala, 1973), whereas dilution of the mucosal solution leads to decreases in shunt conductance (Reuss & Finn, 1976). In fact, the latter authors suggested that the paracellular pathway might exert some control on the active pathway; this suggestion was confirmed and amplified by Civan & DiBona (1978), who showed that the conductance of the paracellular pathway was directly related to the osmolality of the mucosal bathing medium over the physiological range.

The purpose of the present experiments was to seek a measure of shunt conductance which could be followed as a function of alterations in the transport process. This represents a somewhat difficult task in the toad urinary bladder, for the electrophysiological assessment of the resistance of the various pathways is a relatively complicated procedure (Reuss & Finn, 1974) and requires at least 30 min of continual measurements. That is to say, one must measure the transepithelial resistance,

the relative resistances of the apical and basolateral membranes with an electrode in a cell, and repeat these measurements after the addition of amiloride, a potent diuretic which appears to decrease the conductance of sodium at the apical membrane (Bentley, 1968; Reuss & Finn, 1974). Such a technique, as stated above, requires a minimum of 30 min, and it would be far more satisfactory if a method could be devised to measure the shunt conductance more rapidly. Leaf (1965) suggested that serosa-to-mucosa fluxes of Na, K, and Cl traversed channel(s) separate from active sodium transport in the opposite direction. The suggestion was essentially proven by Saito *et al.* (1974), who showed that the determination of the transepithelial serosa-to-mucosa fluxes of sodium, potassium, iodide, and chloride could be used to determine the conductance of the paracellular pathway. They reasoned that, since the relationship between the fluxes of any two of these was linear and the regression line intersected the origin, the ions were traversing an identical extracellular pathway. The first object of the present studies, then, was to utilize this method with the modifications to be described below. We were mainly interested in measuring the fluxes rapidly in order to study the kinetics of opening and closing of the shunt under various conditions. As will be discussed below, this method yields useful new information on the nature of this pathway.

Materials and Methods

For these studies we utilized toads (*Bufo marinus*) purchased from the Pet Farm, Miami, Florida, or from Charles P. Chase Company, Miami, Florida. The toads were kept in running tap water at room temperature and were not ordinarily fed. They were pithed, and the bladders were removed and placed in a Ringer's solution containing (in mM): NaCl, 109; $CaCl_2$, 0.89; KCl, 2.5; $NaHCO_3$, 2.4; and glucose, 5.6. Solutions were gassed with air and had a pH at room temperature of approximately 8.0. Solution changes are described in the text. Bladders were subsequently mounted in a chamber designed for the rapid measurement of fluxes, as described previously (Finn & Rockoff, 1971). Briefly, the tissue is pinned to a cork ring, mounted between 2 rubber rings, and placed between Lucite chambers, each of which contains electrodes for the measurement of the transepithelial potential and short-circuit current, and Teflon impellers with internal magnets. The latter are rapidly rotated by means of motor-driven external horseshoe magnets. Tracer can be added to either bathing medium by way of a closed circulating pump system, and nonradioactive Ringer's solution is continually circulated through the other side by means of a Harvard pump and appropriate entrance and exit ports. The exiting fluid is then collected in test tubes mounted in a fraction collector for accurate timing. After tracer is allowed to reach equilibrium (that is, when the flux reaches a steady state as determined by successive transepithelial flux measurements) fluxes can be determined by collections at intervals as frequenctly as every 6 sec, depending on the rate of movement

of the tubes in the fraction collector. It was found, however, that 30-sec periods of collection were adequate. As described below, alterations in the osmolality of the mucosal solution were achieved by abrupt changes in the fluid passing through the mucosal side, while a constant tracer solution perfused the serosal side. This was done by using a switching device and appropriate valves to perform an instantaneous change from one delivery pump to another that contained a different solution. We have shown (Reuss & Finn, 1975) that changes in resistance occur within 1–2 sec after the change in media. However, because of the necessary lag time for transepithelial fluxes, the imposition of a nonsteady state, and the presence of unstirred layers, it was found that significant flux changes first appeared at 20–30 sec after the switch in solutions. All flux periods were therefore 30 sec in duration.

No bladder was used unless its transepithelial resistance was at least 2,000 $\Omega \cdot cm^2$, and its transepithelial potential at least 50 mV at the start of the experiment. As decribed below, experiments were performed with the bladder potential clamped at zero (short-circuit conditions) or at +25 mV (reference: mucosal solution)[1]. Resistance was measured by interrupting the voltage clamp for a few seconds and recording the transepithelial potential at frequent intervals, or by inermittently clamping the potential at +10 mV and measuring the current. Either method yields similar results (although transients occur during either voltage or current clamps (Finn & Rogenes, 1977) and affect the determination of resistance, such changes are fairly consistent and are minimal at the voltages and currents used here; furthermore, they are quire reproducible, so that the resistances, though perhaps not strictly correct, are internally consistent and correctly show relative changes as a result of the experimental pertubations). For simultaneous fluxes of more than a single isotope, ^{42}K was always one of the pair, the other being ^{36}Cl or ^{22}Na. On the day of the experiments, ^{42}K activity was determined on a gamma counter, and the activity of the other isotope was determined two weeks later on the gamma counter (^{22}Na) or a liquid scintillation counter (^{36}Cl).

Results

After substituting Ringer's + 100 mM NaCl for Ringer's on the mucosal side, the resistance falls in 1–2 sec and continues to decrease to a steady-state value in about 5 min. In these experiments, however, preparations were kept short circuited during the change in solution in order to determine the fluxes at a constant transepithelial voltage. Resistance was therefore determined immediately before the addition of hypertonic solution to the mucosal medium and again 10 min later, just prior to return to Ringer's. As shown below, repeated solution changes may be performed on a single preparation, and there is generally a return to or toward the control measurements. Because of this, and because it was considered necessary to show reproducibility as well as to control

1 The voltage clamp compensated for the voltage drop across the solution resistance when the bladder was mounted in Ringer's solution. However, we did not change this compensation with changes in the mucosal bathing medium (DiBona & Civan, 1973). Since the addition of 100 mM NaCl changed the measured resistance of the solution from 36 to 25 Ω, it can be easily shown that maintaining the original compensation resulted in an error in resistance calculation (and in transepithelial voltage during clamping) of less than 10%; this error is small and has no effect on our conclusions.

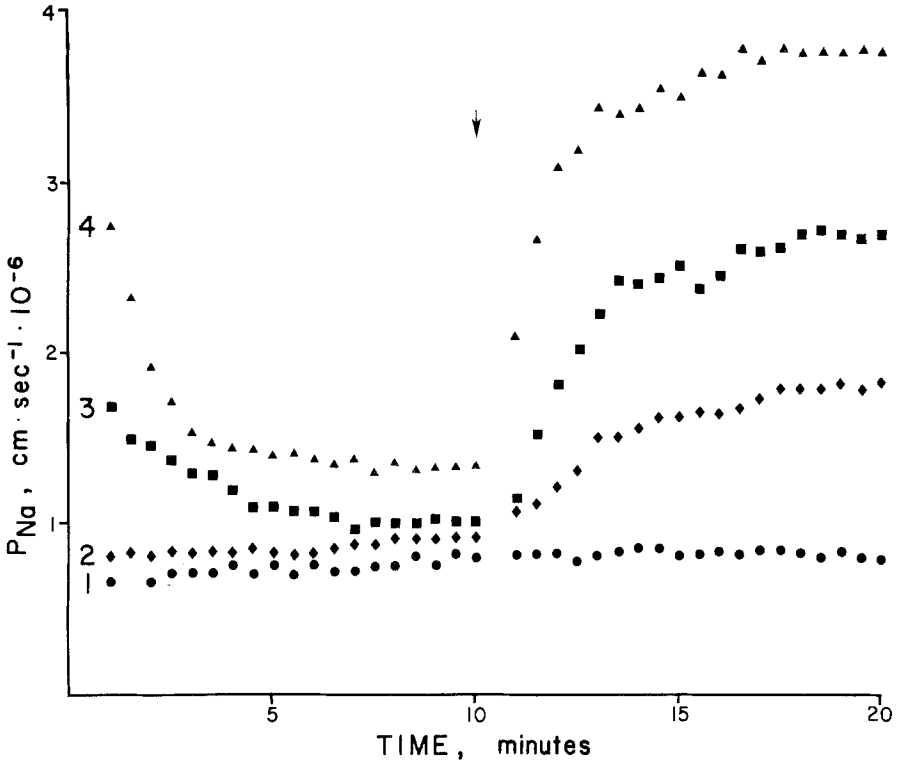

Fig. 1. Effect of hypertonicity on fluxes in a single bladder. The numbers adjacent to the data represent the order in which the studies were done and all are consecutive; that is, the total elapsed time on the figure is 80 min. The bladder is first set up in normal Ringer's (curve 1, circles), and at the arrow the mucosal solution is changed to Ringer's +50 mM NaCl; at 20 min Ringer's is replaced. Subsequent changes at the arrow are: curve 2, diamonds, Ringer's +100 mM NaCl; curve 3, squares, Ringer's +150 mM NaCl 10; curve 4, triangles, Ringer's +200 mM NaCl. The fluxes are plotted as permeability ($P_{Na}=J_{Na}/C_{Na}$, where C_{Na} is the concentration in the mucosal solution)

each experiment, bladders were rejected in which the resistance did not return to at least 75% of the control value following return of the mucosal solution to normal Ringer's. In each case, as shown below, the increase in transepithelial flux following the change in mucosal solution reached a steady state within 5 or 6 min; normal Ringer's was therefore returned to the mucosal medium 10 min after the original change to the hyperosmotic medium. Exposure to hypertonicity for over 30 min generally resulted in an irreversible decrease in resistance (*see* Reuss & Finn, 1975). The time course of the transepithelial flux is shown in Fig. 1. In this experiment, successively increasing concentrations of sodium chloride were added to the mucosal medium, interspersed by

periods during which normal Ringer's solution was reintroduced into the medium. Fluxes were determined at 30-sec intervals throughout the entire study. When 50 mM sodium chloride is abruptly added to the Ringer's solution, there is no detectable change in the transepithelial flux, resistance, or short-circuit current. However, at higher concentrations of added NaCl there is an increase in transepithelial flux and a decrease in resistance which are roughly proportional to the concentration added. The rate at which the flux rises to its new level also is a function of the sodium concentration. In each case, after return to Ringer's, the transepithelial flux returns to a value close to the control level, although there is a slight but progressive increase with repeated exposure to hypertonic solutions. As shown in Fig. 1, the transepithelial flux rises rapidly at first and then reaches a plateau, with a half time of about 2–3 min. In order to estimate the initial rate of rise, the first four points after the change in solutions were fit to a straight line by the method of least squares. Although this procedure is arbitrary, it does provide an objective way to quantitate the change in conductance. In addition, we determined the percent change from baseline to plateau by averaging the values for the last 3 min of the 10 min exposure to the hyperosmotic solution. When sodium chloride was used as the agent to induce the change in conductance, 100 mM was the concentration used routinely, since the reversibility was most nearly complete with this solution. In four preparations, 100 mM sodium chloride was added three consecutive times, with return to Ringer's after each 10-min exposure. There were no significant changes in the effects of the hyperosmotic solution as a function of time on any of the parameters measured.

As stated above, some experiments were performed at a transepithelial potential of 25 mV. The purpose of this was to determine whether or not clamping the transepithelial potential at some value other than zero was associated with any change in the kinetics of opening of the shunt pathway. As shown in Table 1, the rate of the rise of the transepithelial flux is greater at 25 mM than at zero. These experiments were done by clamping the preparation at 25 mM, serosa positive, for 10 min prior to the change is osmolality and again throughout the osmolality change. As shown in the table, and as expected from consideration of the driving force, the resting transepithelial serosa-to-mucosa flux is higher when the preparation is clamped at 25 mV. The absolute increase in steady-state flux is higher at 25 than at 0 mV, whereas the relative increase is unchanged.

Figure 2 shows an experiment in which simultaneous determinations

Table 1. Effect of Voltage Clamp on Shunt Kinetics

	$R(\Omega \cdot cm^2)$	J_{Na} ($\mu Eq \cdot cm^{-2} \cdot hr^{-1}$	$\%\Delta J_{Na}$	dJ_{Na}/dt
1. Short circuit				
Ringer's control	2126±127	0.157±0.018	310±60	0.097±0.022
Ringer's+100 mM NaCl	765±67	0.575±0.074		
ΔNaCl	−1361±129	0.419±0.069		
2. Clamp +25				
Ringer's control	1878±125	0.226±0.028	311±50	0.186±0.040
Ringer's+100 mM NaCl	740±72	0.846±0.100		
ΔNaCl	−1138±114	0.620±0.086		
Δ (+25–0)	223±152	0.202±0.038	1±28	0.088±0.027
p	NS	<0.01	NS	<0.01

R = transepithelial resistance.

J_{Na} = transepithelial (serosa-to-mucosa) flux.

$$\%\Delta J_{Na} = \frac{J_{Na}(\text{hypertonic}) - J_{Na}(\text{control})}{J_{Na}(\text{control})} \times 100.$$

dJ_{Na}/dt = initial rate of rise of sodium flux, determined as the least squares slope of a plot of J_{Na} *vs.* time, for the first 4 points (2 min) after change to the hypertonic solution. Units are flux ($\mu eq \cdot cm^{-2} \cdot hr^{-1}$)/time (min).

NS = not significant.

of potassium and sodium fluxes were made in a single bladder. In this experiment, sodium chloride, sucrose, and urea were used successively as the osmotic agents added to the mucosal side. As shown in the figure, the fluxes vary over a 25-fold range. There is a remarkable degree of linearity between them, and it should be noted that this linearity holds over the entire range. In fact, the regression line plotted for those points obtained under control conditions (with normal Ringer's in the mucosal solution) is insignificantly different from the regression line for the entire set of data. Thus, the relationship between potassium and sodium is not altered by changes in the overall conductance pathway. The intercept of this regression line on the ordinate is not significantly different from zero. Furthermore, the slope of the line indicates the relative permselectivity of the pathway for the two ions. In 6 experiments of this type, the slope (mean ± SEM) was 1.409±0.14 and the intercept was −0.020±0.056. From the limiting ionic conductance of potassium and sodium (Harned & Owen, 1967), the free solution ratio is 1.467, a value not significantly different from that observed. Thus, these data indicate that the permselectivity of this pathway for K and Na is not different from that in free solution. It is also clear from Fig. 2 that the clamping

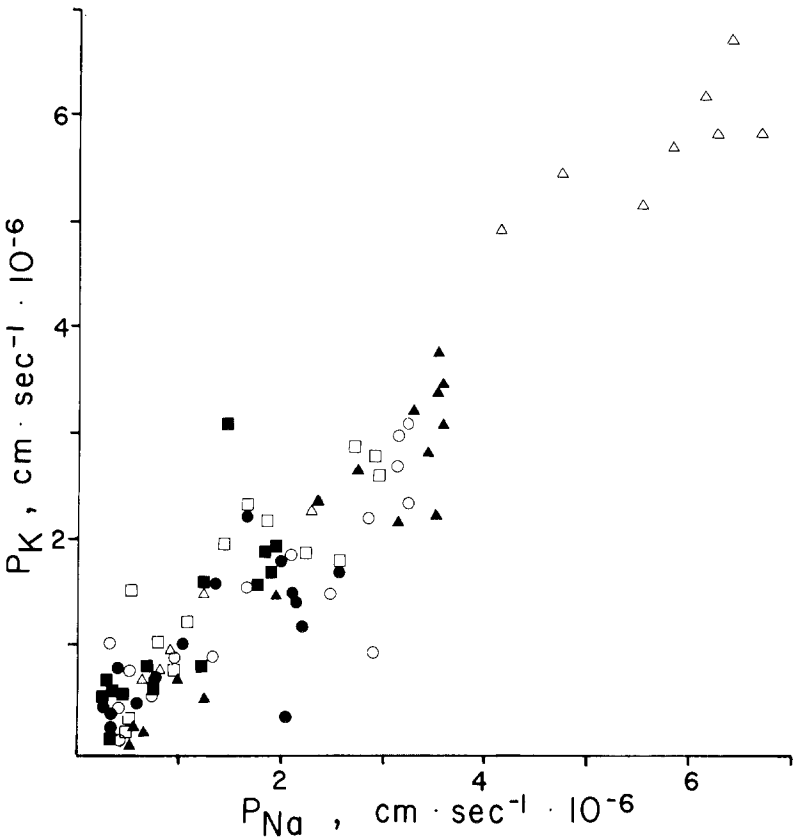

Fig. 2. Relationship between potassium and sodium fluxes in a single bladder. Again, fluxes are plotted as permeabilities for ease of comparison. Variations in fluxes are due to hypertonic addition. Values in the lower left (P_{Na} and $P_K < 1$) are in Ringer's solution, while others are during early and late times after addition of 100 mM NaCl (○, ●) sucrose (□, ■) or urea (△, ▲). Filled symbols are data obtained during 0 mV clamping, open symbols during 25 mV (serosa positive) clamping

of the preparation at a positive potential had no effect on the relationship between potassium and sodium permeabilities.

As shown in Fig. 3, similar studies were performed in which potassium and chloride fluxes were measured. In three such studies at 0 mV, the slope was 1.766 ± 0.096 and the intercept 0.027 ± 0.103. The ratio of limiting conductances in this case is 0.963, indicating a significantly higher (1.83-fold) permeability of this pathway to K (and therefore to Na) than to Cl.

Of further interest is the effect of a change in potential on the relationship between the fluxes of K and Cl. As has been shown, (Saito *et al.*, 1974), if one assumes that there is no significant coupling of isotope flows and that each measured backflux is passive and uncoupled to

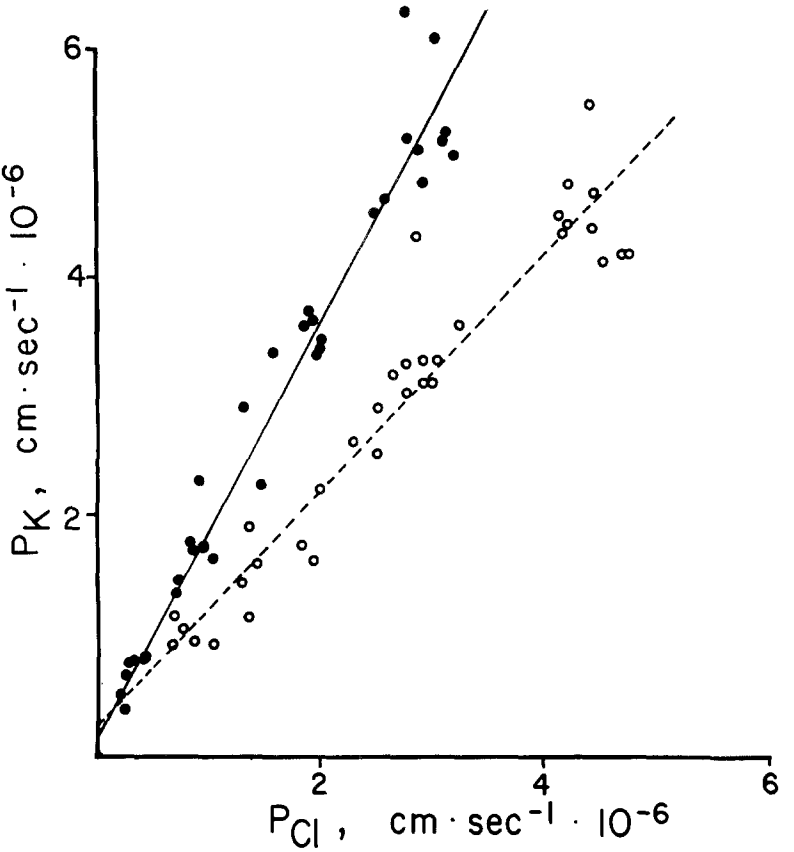

Fig. 3. Relationship between potassium and chloride fluxes in a single bladder. In this preparation changes in permeabilities were brought about by adding 100 mM NaCl to the mucosal solution at 0 mV (solid line, filled circles) or 25 mV (dashed line, open circles). The lines are the least squares fits to the data. All data at 25 mV have been corrected for the effect of the potential as follows:

$$\frac{J_i(25\,\text{mV})}{J_i(0\,\text{mV})} = (zF\Psi/RT)/(\exp[zF\Psi/RT] - 1)$$

where J_i is the *S-M* flux of K or Cl, z the valence, F the Faraday, ψ the potential, R the gas constant, and T the absolute temperature. Thus, the effect of potential alone would increase J_K and decrease J_{Cl} and obscure the comparison; the calculation utilized assumes that the ions move in this pathway by diffusion, and corrects for the differing effect of the driving force on the two ions

flows of other species, cation outflux (or anion influx) at 25 mV should exceed that at short circuit by 1.57; on the other hand, the effect of Cl outflux is in the reverse direction, and at 25 mV the anion outflux should be 0.59 times that at 0 mV. In Fig. 3, all fluxes at 25 mV have

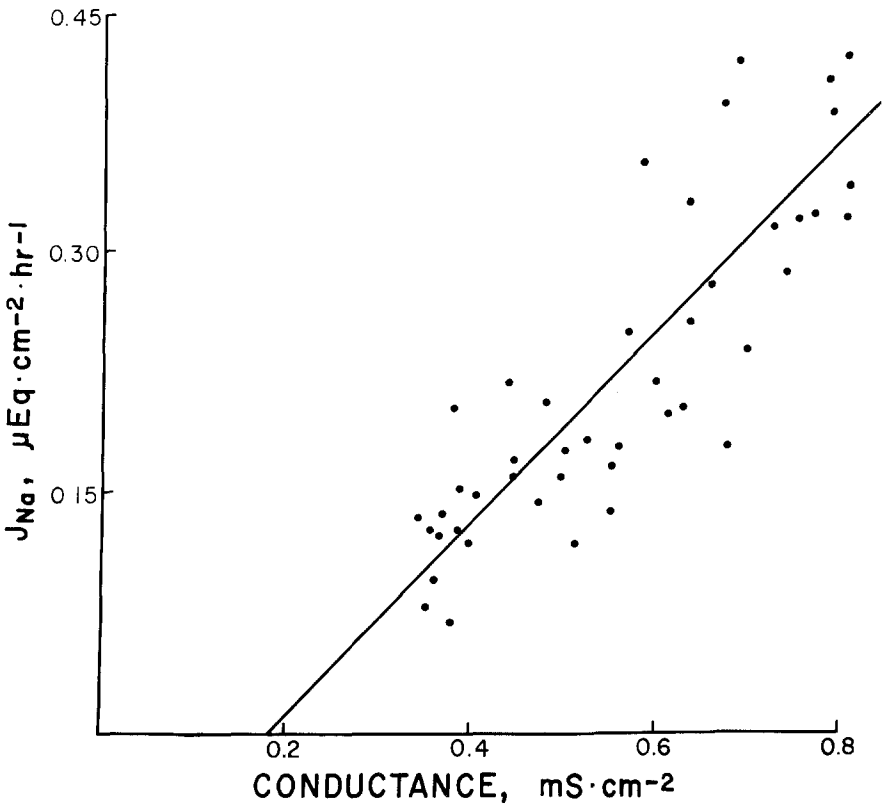

Fig. 4. Relationship between total tissue conductance and J_{Na} in 18 bladders. Points represent steady-state measurements of fluxes in the presence of normal or hypertonic mucosal solutions. Data during the transient stages are not available since resistance (1/conductance) was determined only in the steady state (*see* text). These data are from the bladders of Table 1. The least squares regression line is shown

been corrected for the effect of potential, and the mean slope in these experiments was 1.010 ± 0.025, a value not significantly different from the ratio of the limiting ionic conductances of 0.963. Again it is important to note that in these experiments the ratio of K to Cl permeabilities was the same (at either 25 or 0 mV) before and after the addition of a hyperosmotic solution to the mucosal medium. Thus it appears that (i) the shunt pathway clearly prefers cations to anions under short-circuited conditions, (ii) if the preparation is clamped at 25 mV positive (in the direction of the spontaneous open-circuit PD) the selectivity ratios for Na, K, and Cl are identical to those in free solution, (iii) the imposition of an osmotic gradient "opens" the shunt pathway but does not affect its permselectivities at either potential.

Table 2. Effects of Different Solutes on Paracellular Pathway

Concentration	ΔR ($\Omega \cdot cm^2$)	ΔJ_{Na} ($\mu eq \cdot cm^{-2} \cdot hr^{-1}$)	$\%\Delta$	dJ_{Na}/dt
		Sodium chloride addition ($n=3$)		
50	413±175	−0.004±0.008	−1±3	0.023±0.023
100	−1616±134	0.674±0.144	609±137	0.125±0.049
200	−1644±303	2.214±0.438	1173±184	1.281±0.369
		Urea addition ($n=3$)		
50	−232±29	0.004±0.002	0.6 ±0.8	0
200	−1294±372	0.974±0.088	649±146	0.231±0.090
300	−1234±254	1.569±0.167	1067±285	0.480±0.149
		Sucrose addition ($n=4$)		
50	−28±45	0.026±0.006	19±3	0.049±0.020
200	−262±116	0.050±0.020	32±14	0.056±0.023
300	−330±175	0.024±0.002	12±1	0.071±0.021

$\Delta R = R$ (hypertonic) − R (Ringer's).
$\Delta J_{Na} = J_{Na}$ (hypertonic) − J_{Na} (Ringer's).
Other symbols are as in Table 1.

As was shown in Fig. 2, the addition of sucrose and urea to the mucosal medium also effects a rise in shunt conductance. However, the magnitude of the effect of these substituions is quite different. Table 2 indicates the changes in resistance and backflux and the % increase and rate of rise of the backflux upon addition of each solute in varying concentrations. In these experiments a single solute was used in all three concentrations on a given bladder. Despite the fact that the number of preparations is small, it is clear that sucrose is relatively ineffective, even at a concentration of 300 mM, in changing the resistance of the shunt pathway or of the entire preparation. That the effect of NaCl is not due to changes in ionic strength alone, however, is shown by the fact that urea appears to be about as effective as NaCl.

Figure 4 is a plot of the sodium backflux, expressed as the permeability, against the total tissue conductance of the 18 tissues of Table 1. Although, as stated under *Methods*, preparations were not used unless their initial transepithelial resistance was over 2000 $\Omega \cdot cm^2$, there was a sizable variation in this parameter during the course of an experiment, as well as in its initial value. In particular, repeated exposures to hypertonic solution resulted in a fall in resting resistance (i.e., resistance in Ringer's solution) although the response to hypertonic solutions did not change. In each case there were several measurements made, for,

as stated above, repeated experiments were performed in the same preparations. Note that there is a high degree of linearity, and that the intercept of this line on the abscissa yields the value of the transepithelial conductance when the flux is zero. The fact that this intersection is at a finite value for the transepithelial conductance suggests that, once again, the sodium is traversing the paracellular path. If one assumes, furthermore, that *all* of the sodium is traversing this path, then extrapolation to zero flux would indicate the transepithelial conductance when the shunt conductance is zero. The remaining conductance would then be due entirely to the cellular path, and the reciprocal of the intersection yields the value for the cellular resistance, $5500 \pm 520\ \Omega \cdot cm^2$, not very different from that observed in microelectrode studies from this tissue (Reuss & Finn, 1974).

If we plot the flux data in the same preparations, after opening the shunt with hypertonic saline, there is no significant change in slope (0.589 to 0.475), although the scatter is greater, or in the x-intercept ($6485\ \Omega \cdot cm^2$). Data from either K or Cl fluxes fall in the same range when plotted in this fashion.

Discussion

In these experiments we have described a method for the determination of transepithelial fluxes of the major ionic constituents in the medium under conditions in which there is a rapid change in transepithelial resistance. Because it has been known for some time (DiBona & Civan, 1973; Reuss & Finn, 1975; Urakabe, Handler & Orloff, 1970; Wade *et al.*, 1973) that increases in mucosal osmolality result in a marked decrease in transepithelial resistance, and because there is good evidence that this change in resistance appears to be limited to the paracellular pathway, we thought that it would be of interest to determine the kinetics of the opening and closing of this pathway. The tissue resistance changes within seconds (Reuss & Finn, 1975), but measurement of shunt resistance with microelectrode techniques takes far longer. However, with the present technique it is possible to measure the rate of opening of the shunt, although the technique has some limitations. A major problem with this determination relates to changes in the steady state and to the presence of unstirred layers. If one adds isotope suddenly and determines the rate of build-up of the flux on the opposite side, one can determine the rate at which a simple change in concentration will relax to a steady-state value. Several experiments of this type were performed, and the

halftime was found to be approximately 2 min. Since this value must be in some way superimposed on the measurements of flux following a sudden opening of the paracellular pathway, it is impossible to calculate the exact rate at which the shunt is opening. We therefore can obtain only a relative rate of opening and closing of this path, but it is obvious from the data that these rates of opening can be used to characterize this pathway.

As described above, the measurements of the fluxes yield evidence of the relative permselectivities of this pathway. Under short-circuit conditions the sodium-to-potassium permeability ratio is not different from that in free solution, whereas there is a strong preference for cations over anions. Both of these results are somewhat similar to those observed previously by Saito *et al.* (1974). The slight differences which do exist may result from the larger number of flux periods studied in the present experiments but are more likely due to the fact that many fluxes were determined on each preparation.

Of considerable interest is our finding of an effect of a change in transepithelial voltage on the permselectivity of this pathway. At a voltage of 25 mV the cation/anion selectivity becomes equal to the free solution mobility ratio. At the present time, there is no obvious reason why hyperpolarizing the epithelium should result in a decrease in the selectivity for cations over anions, but one possible explanation is that the shunt is lined with negative charges under normal conditions, and that the application of a transmembrane potential affects the distribution of these charges. It is clear that the application of a potential of 25 mV does not affect total shunt conductance, because in the steady state sodium efflux increases almost exactly as predicted (for passive diffusion) under a voltage clamp of 25 mV. That is, the mean flux in the 18 experiments shown in Table 1 was 0.157 $\mu eq \cdot cm^{-2} \cdot hr^{-1}$ at a voltage clamp of zero and 0.226 at a voltage clamp of +25 mV. As stated above, the clamp alone should have increased the flux by a factor of 1.57 to a value of 0.246. Furthermore, total tissue resistance is not different at 25 or 0 mV.

The shunt pathway can thus be conveniently studied. In tight epithelia, evidence that this pathway is between the cells has always been circumstantial. Previous data (DiBona & Civan, 1973; Reuss & Finn, 1975; Urakabe *et al.*, 1976; Wade *et al.*, 1973) have made it clear that when hyperosmotic solution is added to the mucosal side there is a large decrease in resistance and the appearance of morphologic changes in the tight junction. Although these changes do not appear for some time

after the imposition of the osmotic gradient, it would appear that this failure to see them early is simply a manifestation of the insensitivity of the electron-microscope techniques. In the present study, as well as that reported previously by Saito *et al.* (1974), the fact that the relationship between the fluxes of pairs of ions is quite linear and passes through the origin strongly suggests that these ions traverse a common path. As stated before, the evidence that sodium does not enter the cells from the serosal medium is compelling (Beauwens & Al-Awqati, 1976; Canessa *et al.*, 1976; Macknight & McLaughlin, 1977). The question as to whether or not potassium traverses the mucosal barrier is not entirely convincing, though electrophysiologic and isotope exchange data certainly suggest that this is the case (Finn, 1974; Gatzy & Clarkson, 1965; Leb, Hoshiko & Lindley, 1965; Robinson & Macknight, 1976). In addition, Macknight (1977) presented evidence that little, if any, chloride exchanges across the mucosal barrier. In any case, since all three ions appear to traverse the same pathway under control conditions, it is extremely likely that this pathway is between the cells. Furthermore, the observation that the imposition of an osmotic gradient, which is known to affect the paracellular pathway (*see* above) not only increases all of the fluxes by 15- to 25-fold but also does not change their linearity or the slope of the relationship between them strongly suggests the conclusion that these ions traverse the extracellular pathway.

This pathway opens and closes at different rates, depending upon the conditions. As shown in Table 1, a hyperpolarizing voltage does not affect the percentage change in shunt conductance, whereas the rate of change is affected. It is of considerable interest to determine whether or not transport rate affects or is affected by the shunt pathways. As has been previously shown (Civan & DiBona, 1978); Reuss & Finn, 1975), decreases in transport rate brought about by reduction in mucosal sodium concentration result in an increase in the shunt resistance. These changes led us to suggest that perhaps there was some control exerted between the shunt pathway and the transport pathway; the present studies represent an effort at measuring the nature of the shunt pathway and its kinetics. In separate studies (*manuscript in preparation*), we have shown that vasopressin results in a marked decrease in the rate of shunt opening, and ouabain in an increase. Again, these data are consistent with the idea that the shunt pathway is a function in some way of the transporting pathway.

Of interest are the effects of the different substances used to raise the osmolality. As shown in Table 2, urea has an effect approximately

similar to that of sodium chloride, whereas sucrose is far less effective in opening the shunt. These data are consistent with the idea that the shunt opens as a consequence of the entrance of solute from the mucosal medium into the tight junction, followed by water inflow either from the lateral spaces or from the cells. Thus, despite a similar osmotic gradient in the sucrose studies, no change in the paracellular path occurred; these kinds of studies can therefore give us some insight into the nature of the barrier represented by the tight junction.

Finally, these studies also provide an independent method of determining the conductance of the cellular pathway, as shown by the data in Fig. 4. Of interest is the fact that multiple determinations of sodium backflux and transepithelial conductance in a single tissue will also generate a regression (from spontaneous or induced changes in shunt resistance) which will allow computation of cell as well as shunt conductance.

From these latter data, it is of interest to speculate on the causes of the well-known variation in transepithelial resistance and potential which is encountered with different bladder preparations from time to time. From Fig. 4 it can be seen that transepithelial conductances varied over a wide range, yet the relationship between the backfluxes and the conductance is quite consistent. Furthermore, in studies reported before from this laboratory (Reuss & Finn, 1974, 1975), although the mean transepithelial resistance was almost twice as high as that in the present study, the transcellular resistance was not significantly different from that computed from the data of Fig. 4. It may therefore be the case that the difference between low and high resistance preparations is due only to differences in shunt resistance. The variations do not appear to be due to "edge damage" (Finn & Hutton, 1974; Helman & Miller, 1971; Walser, 1970) since one would not expect such a pathway to be affected by changes in osmolality. Somewhat similar observations have also been made in toad skin (Bruns, Kristensen & Hviid Larsen, 1976). Thus, the cellular resistance pathway may be relatively constant from bladder to bladder just as seems to be the case between leaky and tight epithelia. For instance, cell resistances are similar in toad urinary bladder (5–8 $k\Omega \cdot cm^2$) and *Necturus* gallbladder (Fromter, 1972; Reuss & Finn, 1976). These findings have potentially great significance regarding the control of sodium transport in epithelia. Although it has been suggested (Lewis, Eaton & Diamond, 1976) that there may be an element of feedback control of transport between the apical and basolateral membrane, any such loop presumably includes the paracellular pathway in its effector mechanism. As previously suggested, then

(Reuss & Finn, 1975), and recently confirmed (Civan & DiBona, 1978), the paracellular pathway may play an important or even dominant role in the control of sodium transport in epithelia.

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