Passive Electrical Properties of Toad Urinary Bladder Epithelium

Intercellular Electrical Coupling and Transepithelial Cellular and Shunt Conductances

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ABSTRACT The electrical resistances of the transcellular and paracellular pathways across the toad urinary bladder epithelium (a typical "tight" sodium-transporting epithelium) were determined by two independent sets of electrophysiological measurements: (*a*) the measurement of the total transepithelial resistance, the ratio of resistance of the apical to the basal cell membrane, and cable analysis of the voltage spread into the epithelium; (*b*) the measurement of the total transepithelial resistance and the ratio of resistances of both cell membranes before and after replacing all mucosal sodium with potassium (thus, increasing selectively the resistance of the apical membrane). The results obtained with both methods indicate the presence of a finite transepithelial shunt pathway, whose resistance is about 1.8 times the resistance of the transcellular pathway. Appropriate calculations show that the resistance of the shunt pathway is almost exclusively determined by the zonula occludens section of the limiting junctions. The mean resistance of the apical cell membrane is 1.7 times that of the basal cell membrane. The use of nonconducting materials on the mucosal side allowed us to demonstrate that apparently all epithelial cells are electrically coupled, with a mean space constant of 460 μm, and a voltage spread consistent with a thin sheet model.

INTRODUCTION

Sodium-transporting epithelia may be separated into two groups: "leaky" (e.g., kidney proximal tubule, upper intestine, gallbladder) and "tight" (e.g., kidney cortical collecting duct, frog skin, toad urinary bladder). Several lines of evidence indicate that both the high ionic conductance and the high hydraulic conductivity of leaky epithelia are the consequence of the existence

of a low resistance mucosa-to-serosa pathway (for review, see Diamond, 1971). By electrophysiological methods it has been demonstrated that the conductance of the shunt pathway in leaky epithelia is about 20 times higher than the transcellular conductance (Windhager et al., 1967; Frömter and Diamond, 1972; Frömter, 1972), and that the anatomical location of this shunt corresponds (in the *Necturus* gallbladder) to the tight junctions and intercellular spaces (Frömter, 1972).

It has also been shown that the presence in leaky epithelia of a cell potential negative to both bathing solutions does not require different polarities of the electromotive forces operating at the luminal and inner membranes. In fact, it is possible to have this kind of potential profile if, in the presence of a given ratio of the electromotive forces, the resistance of the shunt is low enough (Schultz, 1972). The whole conclusion might be that the differences between leaky and tight epithelia are the consequence solely of differences in the conductance of the shunt pathway, and thus do not reflect qualitative differences in the physiological properties of the cells.

The purpose of this work was to measure the resistance of the cellular and paracellular pathways in a tight sodium-transporting epithelium, the toad urinary bladder. This is an appropriate model because it is a tight epithelium

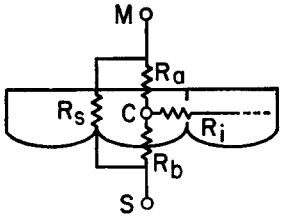


FIGURE 1

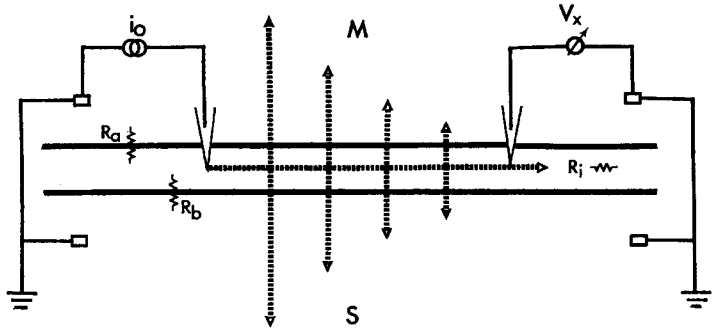


FIGURE 2

FIGURE 1. Equivalent circuit of the toad urinary bladder epithelim. R_a represents the resistance of the apical (mucosal) cell membrane. R_b is a lumped resistor of two pathways for current flow from the cell (C) to the serosal solution (the basal membrane, and the lateral membrane plus the intercellular space). R_s is the resistance of a shunt pathway; its location is not necessarily intercellular. R_i represents the resistance of the cytoplasm and the intercellular coupling structures. M and S are the mucosal and serosal solutions.

FIGURE 2. Illustration of the current spread in the epithelium, according to a thin sheet model with low resistance intercellular junctions. The current applied in a cell (i_o) flows radially away from the microelectrode, through the internal resistance (R_i), and leaks to the bathing solutions across the resistances of both the apical (R_a) and basal (R_b) cell membranes. The mathematical treatment of the steady-state voltage deflection (V_x) as a function of distance is given in the text.

(Frazier, 1962; Hays and Leaf, 1962), because its structural simplicity (Peachey and Rasmussen, 1961; Choi, 1963) makes electrophysiological analyses easy as compared to more complex epithelia such as renal tubule or frog skin, and because the results may be interpreted on the basis of considerable transport data available for this tissue. Although it has been suggested that there may be more than a single pathway in parallel across this epithelium (Civan et al., 1966), no data on the conductance of such pathways exist. An attempt has been made to estimate in a tight epithelium (the *Necturus* stomach) the conductances of the cellular and paracellular pathways, but the techniques used and the complex structure of the epithelium make difficult any interpretation of these results (Blum et al., 1971).

The simplest possible circuit analysis for the transepithelial flux of ions is shown in Fig. 1. R_a and R_b represent the resistances of the apical (mucosal) and basal (serosal) cell membranes, and R_s is the resistance of the shunt pathway. R_i, the cell-to-cell electrical resistance, is included because of the possibility of intercellular electrical coupling (Loewenstein et al., 1965) and its relevance in the following electrophysiological analysis.

METHODS

Colombian toads (*Bufo marinus*) were purchased from The Pet Farm, Miami, Florida, and kept at room temperature. Bladders were removed from pithed toads and placed in amphibian Ringer solution of the following composition (mM): NaCl 109.2, KCl 2.5, $NaHCO_3$ 2.38, $CaCl_2$ 0.8, glucose 5.5, pH about 7.8 when gassed with room air. The bladders were mounted as flat sheets, mucosal side upwards, between the two halves of a Lucite chamber, whose cross-sectional area is 3.14 cm^2. The bladder was supported in the center by a 3.5% agar-Ringer cylinder (cross-sectional area 2.27 cm^2), and in the periphery by a thin perforated plastic ring, below which Ringer was continually replaced. A negative pressure of 10–30 cm H_2O was applied to the serosal side in order to hold the tissue against the agar-Ringer cylinder and the plastic ring, and to reduce the spontaneous smooth muscle contractions. The mucosal solution was replaced at about 20-min intervals.

The electrical measurements were performed with macro- and micro-electrodes. Two Ag-AgCl electrodes were connected to each side of the chamber (mucosal and serosal) by means of polyethylene agar-Ringer bridges. One pair was used for the continuous measurement of the transepithelial potential (610 B electrometer, Keithley Instruments, Inc., Cleveland, Ohio), serosal side grounded, and the other for passing transepithelial DC current pulses. The microelectrodes were prepared by pulling 1 mm OD × 0.6 mm ID Pyrex glass (Drummond Scientific Company, Broomall, Pa.) with a horizontal two-stage puller (M-1, Industrial Science Associates, Ridgewood, N. Y.), and filled with 4 M potassium acetate by either the method of Tasaki, et al. (1954) or of Caldwell and Downing (1955). Their tip resistances ranged from 10 to 50 MΩ, and their tip potentials from 2 to 8 mV. For the intracellular application of current only low-resistance microelectrodes were used.

The preparation was stretched enough as to avoid macroscopic foldings and set up

horizontally on the stage of an inverted phase contrast microscope (Leitz Wetzlar, Germany). The cellular impalements were performed with mechanic micromanipulators, at a magnification of ×200 or 320. The interelectrode distance was measured with a calibrated micrometer eyepiece. Potentials were measured with M-4 electrometer probes (W-P Instruments, Inc., Hamden, Conn.), displayed on a Tektronix oscilloscope (Tektronix, Inc., Beaverton, Ore.), and photographed. Calibrated intracellular current pulses were delivered by a Grass SD9 stimulator (with stimulus isolation unit) through one of the probes (Grass Instrument Co., Quincy, Mass.). All measurements were performed in a shielded room on a heavy antivibratory table. The electrical noise of the system allowed the accurate measurement of a voltage deflection of 0.1 mV. Quick changes of the mucosal solution were obtained by suction followed by gravity superfusion through a broken tip micropipette placed close to the microelectrodes by means of a third micromanipulator.

Three kinds of measurements constitute the basis of these studies: (*a*) R_t, the total transepithelial resistance, was calculated as the ratio of the open circuit transepithelial potential to the current required to clamp the bladder potential at 0 (i.e., the short-circuit current), times the surface area of the preparation. This calculation is justified by our finding in this preparation that the current-voltage relationship is strictly linear over the range of −100 to +50 mV, when the voltage is measured 1 s after the initiation of the current pulse.

(*b*) $a = R_a/R_b$, the ratio of the apical to the basal membrane resistance, was measured as the ratio of the voltage deflections across each membrane with an intracellular microelectrode, when constant current pulses were passed across the preparation. Usually, identical pulses were passed with the microelectrode in the mucosal solution and in a cell, and the voltage deflections (ΔV_t and ΔV_b) were recorded and measured 1 s after the beginning of the current pulse. R_a/R_b was calculated as $(\Delta V_t - \Delta V_b)/\Delta V_b$. This determination is based on the assumption that the current flowing across each cell is the same, i.e., that if the cells are electrically coupled (see below), their electrical parameters are identical. As has been shown (Frazier, 1962), we have found that only about 5 % of the total resistance of the bladder remains ahead of the tip of the microelectrode when it is pushed from the cell across the basal membrane. As no correction was applied for this resistance, it is included in our calculation of R_b. For the calculation of R_a/R_b for a given bladder, at least eight different cells were impaled.

(*c*) The voltage spread into the epithelium was measured by passing depolarizing intracellular current pulses of 100–400-ms duration, and less than 5×10^{-8}-A intensity (to avoid microelectrode rectification), and recording the steady-state induced potential changes in other cells as a function of distance. At least eight cells, at various distances from the central cell, and roughly on the same radius, were explored in each study. The duration of the intracellular current pulses was as short as possible, within the limitation that it allowed the induced potential deflections to reach a steady state in every explored cell. As the voltage spread measured with Ringer on the mucosal side was altered by artifactual uncoupling of the cells induced by the impalements (see below), these measurements were performed after replacing the mucosal solution with mineral oil or Sylgard.

Only bladders with a spontaneous transepithelial potential higher than 30 mV,

and a total resistance higher than 2,000 ohm·cm² were used for these studies. The cellular impalements were considered adequate according to the following criteria: (*a*) The change from mucosal to cell potential was abrupt and reversed on removal of the microelectrode. (*b*) The cell potential was stable for at least 1 min and returned to its spontaneous value after the deflection induced by intracellular or transepithelial current. (*c*) The microelectrode-to-ground resistance increased after the impalement, as evidenced by unbalancing of the previously balanced potential recorded by the microelectrode while passing short 10^{-9}-A current pulses through it. (*d*) The potential recorded by the microelectrode was less than that recorded in the mucosal solution, when identical current pulses were passed transepithelially. Usually, a stable cellular potential, plus one of the other criteria, was considered enough to define a proper impalement.

THEORY

Two different methods were employed for the measurement of R_a, R_b, and R_s. Method A consisted of the measurement of R_t, R_a/R_b, and the voltage spread into the epithelium, as reported for the *Necturus* gallbladder (Frömter and Diamond, 1972; Frömter, 1972). Briefly, if the epithelium is considered to be a flat thin sheet of infinite dimension, whose internal resistance is low compared to the resistances of the apical and basal membranes (and is given by the combined resistance of the cytoplasm and intercellular junctions), the current injected into the epithelial sheet by a microelectrode will flow radially away from the source (through R_i), and leak out of the cells (through R_a and R_b), as shown in Fig. 2. If both external solutions are grounded, R_a and R_b may be treated as parallel resistors:

$$\frac{1}{R_z} = \frac{1}{R_a} + \frac{1}{R_b}, \tag{1}$$

where R_z is the resistance for the flow of current out of the cells. Within the limits of the previous assumptions, and if in addition the electrical resistances of the bathing solutions are neglected, the voltage spread into the epithelium may be described by the following differential equation (Eisenberg and Johnson, 1970; Shiba, 1971; Frömter, 1972):

$$\frac{d^2V}{dx^2} + \frac{1}{x}\frac{dV}{dx} - \frac{V}{\lambda^2} = 0, \tag{2}$$

where V is the steady-state voltage deflection recorded by the exploring microelectrode, x is the interelectrode distance, and λ is the space constant:

$$\lambda = (R_z/R_i)^{1/2}. \tag{3}$$

R_z is expressed in (ohm·cm²), and $R_i = \rho_s/h$ is expressed in (ohm), where ρ_s is the specific resistance of the sheet material (ohm·centimeter), and h is the thickness of the sheet (centimeters).

The general solution of Eq. 2 is

$$V_x = AK_o\left(\frac{x}{\lambda}\right) + BI_o\left(\frac{x}{\lambda}\right), \tag{4}$$

where A and B are integration constants, and K_o and I_o are zero-order modified Bessel functions of the second kind with imaginary argument. Since for $x \rightarrow \infty$, $V_x \rightarrow 0$, the value of B is 0, and the final solution of Eq. 2 becomes:

$$V_x = AK_o\left(\frac{x}{\lambda}\right). \tag{5}$$

By the method of Shiba (1971), the experimentally measured potentials are plotted as a function of the distance, and compared to a set of Bessel functions (Abramowitz and Stegun, 1965). The best fit, determined by eye, gives the values of A and λ. Once these values have been determined, R_z can be calculated according to (Frömter, 1972)

$$R_z = \frac{2\pi A\lambda^2}{i_o}, \tag{6}$$

where i_o is the applied intracellular current. The values of R_a, R_b, and R_s can be calculated from:

$$R_t = (R_a + R_b)\cdot R_s/(R_a + R_b + R_s), \tag{7}$$

$$R_z = R_a\cdot R_b/(R_a + R_b), \tag{8}$$

$$a = R_a/R_b, \tag{9}$$

since R_t, a, and R_z are experimentally determined.

If current flow through R_a is prevented by the use of a nonconducting material on the mucosal side instead of Ringer solution, R_z becomes equal to R_b. In this experimental condition (use of mineral oil or Sylgard instead of mucosal solution), Eq. 6 becomes

$$R_b = \frac{2\pi A\lambda^2}{i_o}, \tag{6'}$$

and the values of R_a and R_s are calculated from Eqs. 7 and 9. For complete discussions of the analysis of the voltage spread in thin cellular sheets, see Eisenberg and Johnson (1970), Shiba (1971), and Frömter (1972).

Method B was employed for an independent estimation of the resistances of the transcellular and paracellular pathways. It is well known that the apical membrane of this epithelium behaves as a sodium electrode (Leb et al., 1965; Gatzy and Clarkson, 1965), i.e., $P_{Na} \gg P_K$ (Finn and Nellans, 1972), and the inverse relationship holds for the basal membrane. Accordingly, if the sodium in the mucosal solution is replaced with potassium, the electrical resistance of the apical membrane (R_a) and

the electrical resistance of the transcellular pathway ($R_a + R_b$) will increase. If it is assumed that there is no change in R_b or R_s in these circumstances, an expression for R_s can be obtained from the application of Eqs. 7 and 8 to both experimental conditions:

$$R_s = \frac{R_t R_t'(a' - a)}{R_t(a' + 1) - R_t'(a + 1)}, \quad (10)$$

where $'$ represents determinations after replacement of Na with K. The validity of these assumptions and the magnitude of the possible errors involved will be discussed below. According to Eq. 10, R_s can be calculated from the measurements of the transepithelial resistance (R_t) and the ratio of the apical to basal membrane resistances (R_a/R_b) before and after replacing the sodium of the mucosal solution with potassium. Once the value of R_s is calculated, the values of R_a and R_b are calculated from Eqs. 7 and 9.

With both methods (A and B), R_a, R_b, R_t, and R_s are expressed in ohm·cm² of bladder surface. The results are given as means ± SE throughout the paper.

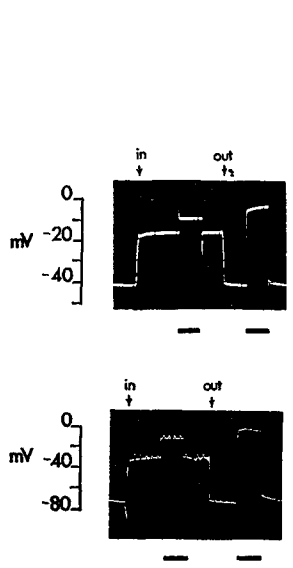
RESULTS

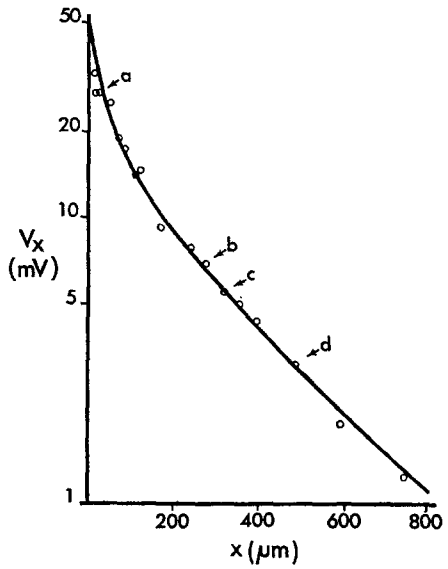
General

In 26 bladders, the transepithelial potential difference was −58 ± 4 mV, and 270 cellular impalements in these same bladders gave a cell potential of −29 ±1 mV (serosal potential = 0). Usually, the change from mucosal to cell potential is abrupt; two typical examples are shown in Fig. 3. When the transepithelial potential was clamped at 0, the cell potential was about 5 mV negative to both solutions. In several impalements, the microelectrode was pushed all the way across the epithelium; under these circumstances, only a second step was recorded. These results are similar to previous observations (Frazier, 1962; Civan and Frazier, 1968). The ratio of R_a/R_b under control conditions (Ringer solution on both sides) varied from 0.7 to 3.6, with a mean value of 1.7 ± 0.3.

Method A

Stable cellular potentials were easy to obtain for periods long enough for the measurement of R_a/R_b, but it was extremely difficult to obtain a stable potential in the central (current receiving) cell for periods long enough to get eight or more measurements of the voltage spread into the epithelium. Furthermore, the voltage changes with Ringer solution on the mucosal side rarely extended beyond 100 μm from the central cell, and usually did not fit the theoretically predicted spread. Even if a good fit was obtained, the calculated values for R_a and R_b were about one order of magnitude lower than the minimum predicted from R_t if R_s is assumed to be infinite. For example, from the data of one of those experiments (A = 1.8 mV; λ = 175 μm; i_o = 4·





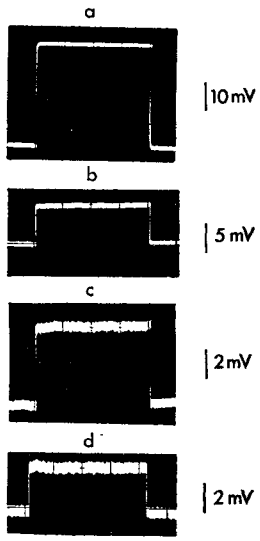


FIGURE 3

FIGURE 4

FIGURE 3. Two typical cellular impalements, with measurement of the voltage divider ratio. Starting on the left, the value of the transepithelial potential (serosal side grounded) is recorded. At the moment indicated by the arrow "in," the cell was impaled. The line between the two arrows is the intracellular record. The arrow "out" indicates that the microelectrode was pulled back to the mucosal solution, so the transepithelial potential is recorded again. The potential deflections indicated by the bars were generated by passing transepithelial DC current pulses of 1-s duration. The voltage deflection obtained with the microelectrode in a cell is ΔV_b, and with the microelectrode in the solution is ΔV_t. From these values, R_a/R_b was calculated. During all of the lower record, current pulses of 10^{-9} A and 5 ms were passed through the microelectrode and balanced before the impalement. The deflections observed during the impalement indicate an increase in the microelectrode-to-ground resistance (criterion for intracellular position, see text).

FIGURE 4. Typical experiment in which the voltage spread in the epithelium was measured with mineral oil (instead of Ringer solution) on the mucosal side. Current pulses of 2×10^{-8} A were applied in a cell, and the steady-state voltage deflections in other cells (V_x) are plotted on a logarithmic scale as a function of distance (x). The values were fitted by eye to the function K_o, and gave the values of $A = 9.9$ mV, and $\lambda = 400$ μm. Four records at several distances are shown on the right, where the distances and voltages correspond to the appropriately labeled points on the left. Pulses are 400 ms in duration.

10^{-8} A), the calculated value of R_s (Eq. 6) is 87 ohm·cm². The mean R_a/R_b was 1.22, and R_t was 2,850 ohm·cm². If $R_s = \infty$, $R_t = R_a + R_b$, and R_a can be no less than 1,570 ohm·cm². However, a value of 193 ohm·cm² was calculated from the voltage spread data from the same preparation.

These results suggest artifactual electrical uncoupling of the cells. However, this could be avoided by replacing the mucosal solution with a noncon-

ducting material (mineral oil or Sylgard). Under these conditions, the resistance of the mucosal side becomes essentially infinite, and the intracellularly applied current flows only through R_i and R_b. Repeated superfusion with the nonconducting material was used in order to prevent the formation of a layer of aqueous solution between the cells and the insulating material. Evidence for the absence of such a layer is provided by the observation of only one potential step (instead of the two steps observed in the presence of Ringer solution on the mucosal side) when the microelectrode was pushed across the epithelium. The values of the transepithelial potential, R_t, and R_a/R_b were the same before and after the use of the nonconducting material on the mucosal side. An example of the voltage spread measured in this condition is shown in Fig. 4. It can be seen that the measured voltage spread appropriately fits the theoretical prediction for a thin sheet model. In several experiments, the voltage spread was explored systematically around the central electrode. A typical example of the voltage recorded in several cells in each of the four quadrants around the current cell, as a function of distance, is shown in Fig. 5. Provided the impalements were adequate (vide supra), electrical coupling appeared to involve all cells, and did not follow "only particular paths" in the tissue (Loewenstein et al., 1965). In eight experiments, the space constant was 460 $\pm$ 90 μm (range 270–800 μm), and the value of A was 9.6 $\pm$ 3.6 mV, for an applied intracellular current normalized[1] to 2 $\times$ 10^{-8} A. R_a, R_b, and R_s in these eight bladders were calculated according to Eqs. 6′, 7, and 9, from the values of R_t and R_a/R_b measured with Ringer solution on the mucosal side and from the voltage spread measured with the nonconducting material on that side. The results are shown in Table I. It can be seen that there is a finite shunt across the tissue, and that its resistance is about 1.6 times the transcellular resistance ($R_a + R_b$).

Experiments were performed in order to show that the obtaining of adequate voltage spread with a nonconducting material on the mucosal side is a consequence of the absence of mucosal solution, and not of an effect of the insulating material on the properties of the bladder. For this purpose, small drops of Sylgard were placed by means of a micropipette on the mucosal surface of the bladder, at least 100 μm apart; Ringer solution covered the rest of the surface, as shown in Fig. 6. The cells were impaled with the microelectrode through the drops of Sylgard, and the voltage deflection was measured in one cell while passing current into the other. Significant deflections were obtained 400 μm away from the current source, results quite similar to those seen when the entire bladder was covered by this material, whereas this rarely occurred when Ringer solution was present in the mucosal chamber.

[1] As A is a function of the intracellularly applied current (Eq. 15, Discussion), for the calculation of its mean value each experimentally determined value of A was multiplied by 2 $\times$ $10^{-8}/i_o$, where i_o is the current intensity used in the experiment.

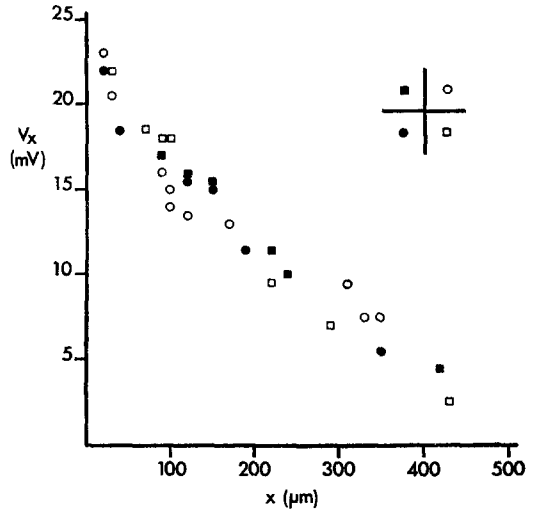


FIGURE 5. Typical experiment showing the radial spread of current in the epithelium. All the potentials were measured while the current passing microelectrode remained in the same cell. The deflections obtained (V_x) are plotted as a function of distance (x). The four symbols indicate the quadrant of the bladder (relative to the central cell) in which the explored cell was located.

TABLE I

TRANSCELLULAR AND SHUNT RESISTANCES, METHOD A (n = 8)

Total resistance	Apical membrane resistance	Basal membrane resistance	Cellular resistance	Shunt resistance
R_t, ohm·cm²	R_a, ohm·cm²	R_b, ohm·cm²	R_c, ohm·cm²	R_s, ohm·cm²
3,940±340	3,840±410	3,620±660	7,470±860	12,320±3,450

Method B

The replacement of the mucosal sodium with potassium causes a rapid reduction in the transepithelial potential and an increase in R_t. Both electrical parameters return to control values when the mucosal sodium is replaced. During the exposure to the high-K solution, the voltage deflection across the basal membrane decreases as compared to the voltage deflection across the whole tissue, when passing identical transepithelial current pulses. In other words, there is an increase in R_a/R_b. The cell potential (that in the control situation is positive with respect to the mucosal side) becomes negative to both sides in the presence of a high-K mucosal solution (Fig. 7). Mean values of the changes in transepithelial potential, cell potential, R_t, and R_a/R_b are shown in Table II. From the individual values of R_t and R_a/R_b before and after the change of mucosal solution was performed, the values of R_a, R_b, and R_s were calculated according to Eqs. 7, 9, and 10 for nine individual

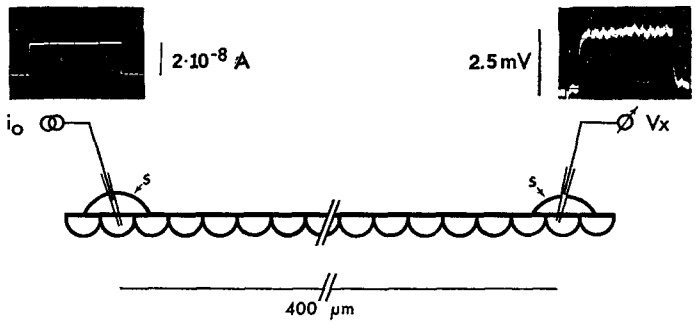


FIGURE 6. By means of a broken tip micropipette, drops of Sylgard (S) were placed on the mucosal surface of the epithelium. Most of the surface remained covered by Ringer solution. As shown, cells are then impaled with microelectrodes through the Sylgard drops. 2×10^{-8} A DC pulses (i_o) were applied into one cell, and the induced voltage deflections (V_x) recorded in the other cell. In a typical experiment, a V_x of 2.5 mV was obtained at a distance of 400 μm (compare to Figs. 4 and 5).

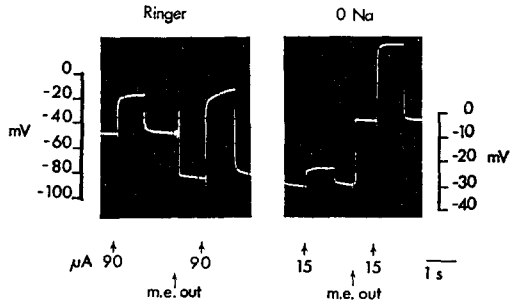


FIGURE 7

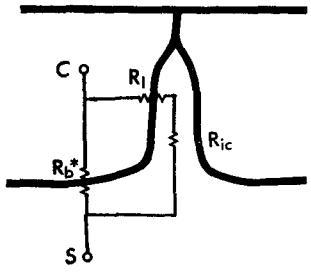


FIGURE 8

FIGURE 7. Typical records of cell potential and measurements of the voltage divider ratio under control conditions and after replacing the mucosal sodium by potassium. Each record starts on the left with the microelectrode in the cell. The arrow "m.e. out" indicates the moment when it was pulled back to the mucosal solution. The transepithelial current pulses used to measure the voltage divider ratio are indicated. The transepithelial potential falls nearly to 0, and the cell potential becomes negative to both sides after the change in mucosal solution. The increase in R_t is evident from the current intensities required to produce about the same ΔV_t in both situations. The in-increase in R_a/R_b is apparent from the comparison of the ΔV_b values in each case.

FIGURE 8. Equivalent circuit for R_b. C represents the cell, and S the serosal solution. R_l is the resistance of the lateral membrane; R_{ic}, the resistance of the intercellular space; R_b*, the resistance of the basal membrane. See text.

bladders. These results are shown in Table III. None of the calculated values is statistically different from those obtained for the same parameters by Method A. Thus, combining the results of both methods yields a mean trans-cellular resistance of 7,300 ohm·cm² and a shunt resistance of 13,200 ohm·cm². The mean ratio of conductances of the shunt and cellular pathways is 0.55.

TABLE II

EFFECTS OF THE REPLACEMENT OF MUCOSAL SODIUM WITH POTASSIUM ON POTENTIAL PROFILE AND RESISTANCE (n = 9)

Mucosal solution*	Transepithelial potential	Cell potential	Total resistance	R_a/R_b
	mV	*mV*	R_t, *ohm·cm²*	
Sodium	−64±8	−30±2	3,530±470	1.7±0.1
Potassium	−5±2	−20±2	5,430±850	5.5±1.0
P‡	<0.001	<0.001	<0.001	<0.005

* Sodium mucosal solution = standard Ringer solution (see Methods). Potassium mucosal solution = equimolar replacement of sodium with potassium.

‡ Paired t test (transepithelial potential, R_t, and R_a/R_b); t test (cell potential, n = 62 in sodium, 65 in potassium mucosal solution).

TABLE III

TRANSCELLULAR AND SHUNT RESISTANCES, METHOD B (n = 9)

Total resistance	Apical membrane resistance	Basal membrane resistance	Cellular resistance	Shunt resistance
R_t, *ohm·cm²*	R_a, *ohm·cm²*	R_b, *ohm·cm²*	R_s, *ohm·cm²*	R_g, *ohm·cm²*
3,530±470	4,240±1,080	2,810±910	7,050±1,980	14,170±4,680

DISCUSSION

Our results confirm previous observations with respect to the potential profile and the absolute value of the intracellular potential in toad urinary bladder epithelium, in both open and short circuit conditions (Frazier, 1962; Civan and Frazier, 1968). The use of Method A for the calculation of the transcellular and shunt resistances required the measurement of R_t, R_a/R_b, and the voltage spread in the epithelium. The values of R_t and R_a/R_b were relatively easy to obtain and reproducible for a given bladder, provided that the current used was of the proper intensity and the impalements were adequate, according to the criteria previously stated. Furthermore, both R_t and R_a/R_b increased, as expected, after replacing the mucosal sodium with potassium (Method B), after reducing the total ionic content of the mucosal solution (with isosmotic sucrose), or under the action of Amiloride (to be published). However, our third measurement, i.e., the voltage spread into the epithelium, proved to be extremely difficult when Ringer solution was bathing both sides of the tissue. Irregular results were obtained even in the same area of a given preparation, and usually intercellular electrical coupling was demonstrable only within about 100 μm of the current electrode. However, in some instances cells at higher distances showed significant voltage deflections, and in three cases it was possible to fit the measured values to the appropriate Bessel functions. Even in these cases, however, there was clearly an artifactual situation, according to the calculated values of R_a and R_b (v.s.). It is likely that the

smaller the cells, the more difficult the demonstration or quantification of intercellular electrical coupling. The relative size of the hole made by a microelectrode may be a factor that determines the degree of uncoupling (Oliveira-Castro and Loewenstein, 1971); furthermore, this effect appears to be dependent on the presence of calcium (or other divalent cations) in the bathing solution (Loewenstein et al., 1967). Preliminary experiments with calcium-free Ringer solution on the mucosal side have shown that the voltage spread in the epithelium increases. This observation is consistent with the possibility that the entry of calcium into the cell, around the microelectrode, is the factor that by raising intracellular free calcium concentration increases intercellular junction electrical resistance. Unfortunately, the removal of calcium from the mucosal solution also induces time-dependent changes of the electrical resistance and selectivity of the apical membrane, and probably reduces the resistance of the shunt pathway. Experiments for a full characterization of these effects are presently in progress.

On the other hand, the voltage spread measured with mineral oil or other nonconducting material on the mucosal side was shown to be radially distributed around the current microelectrode, and appeared to involve all the explored cells (within about 800 μm from the current source). The adequate fit to the theoretically predicted spread and the finding of a space constant almost two orders of magnitude higher than the thickness of the epithelium, provide a validation for the use of a thin sheet model for the analysis of the voltage spread (Eisenberg and Johnson, 1970). Our results differ from those reported by Loewenstein et al. (1965) in one of their initial investigations of electrical coupling in epithelial cells. They reported, for the toad urinary bladder epithelium, directional coupling (space constant 18 μm), and calculated, by cable analysis, nonjunctional membrane resistances of 5–130 ohm·cm^2. It is clear from their discussion that technical problems derived from the small size of the cells, as in our initial experiments. It is also possible that the fact that those experiments were performed at 8°C may have induced uncoupling of the cells by metabolic inhibition and a secondary increase of intracellular calcium concentration (Politoff et al., 1969). Similar to our initial results, their calculation of the resistance of the nonjunctional membranes gives values one to two orders of magnitude lower than the minimum predicted from R_t, if R_s is assumed to be infinite. We have observed, as has been previously shown for the frog skin (Snell and Leeman, 1957), that the transepithelial resistance of the toad urinary bladder increases on cooling. Thus, the low calculated values for the nonjunctional membrane resistance cannot be attributed to this factor. The measurement of the voltage spread with mineral oil or other insulating material instead of mucosal solution prevents the artifact already discussed. The theoretical analysis of the voltage spread under these circumstances requires two assumptions: (*a*) No current flows through the apical membrane; (*b*) the resistance of the basal membrane

(R_b) remains unchanged. The first assumption is valid only if no layer of solution is present between the cells and the insulating material. The presence of such a layer seems unlikely because of the absence of two potential steps when the potential profile across the epithelium was recorded, and because the section of the bladder where the voltage spread was investigated was repeatedly superfused with the insulating material. Furthermore, if this layer were present, its thickness would be small, and the pathway to ground for the current flowing from the cells across R_a would be: R_a, the resistance of the thin layer of solution (R_m), R_t, and the serosal solution. If the last is neglected, three factors indicate the low contribution, if any, of this possibility to an underestimation of R_b : (*a*) R_a is about 1.7 times higher than R_b. (*b*) R_m will have a high value (inversely proportional to the thickness of the layer). (*c*) R_t is relatively high (direct measurement). Thus, the pathway $R_a + R_m + R_t$ would have a total resistance several times higher than R_b, and would make a low contribution to the calculated value. This situation is different from that found for leaky epithelia (Frömter, 1972), where the low resistance of the shunt pathway allows current flow across the luminal membrane in the presence of only a thin layer of solution on that side. With respect to the second assumption, we do not have direct evidence showing that R_b remains constant with Ringer or a nonconducting material on the mucosal side, but the fact that R_t and R_a/R_b were the same before and after the replacement makes the possibility of a change in R_b unlikely. As shown, the presence of an insulating material on the mucosal side increases the voltage spread into the epithelium even if it only covers the small areas of the preparation where the impalements are performed. This indicates that the presence of the nonconducting material increases the voltage spread because it avoids cellular uncoupling, and not because it changes the properties of the bladder (e.g., by increasing R_b).

Resistances of the Transcellular and Shunt Pathways

The results of our calculations from the data obtained with Method A show the existence of a shunt of finite resistance, ranging from 0.4 to 5 times the resistance of the transcellular pathway (mean, 1.6). Although this variation may partly indicate additive errors in the measurements (mainly voltage deflections and distances in the determination of the voltage spread in the epithelium) and in the calculations (mainly the fitting procedure), it clearly may also represent true differences in the physiological condition of the tissue. The degree of stretch of the bladder may also be determining in part this variation. Macroscopic folding would result in an underestimation of the "true" space constant of the tissue, and consequently of R_a, R_b, and R_c, and would also lead to an underestimation of the surface area of the preparation, and consequently of R_t. The absolute magnitude of these potential errors cannot be determined; however, their influence on the relative value of R_s

when compared to R_c and R_t would be negligible (see Eq. 7), and none of the qualitative conclusions would be altered. In any event, such folding was avoided as much as possible, as stated in Methods. No simple correlation was found between the resistance of the shunt pathway and the transepithelial potential or the transepithelial resistance. These results are strikingly different from those reported for leaky epithelia. Boulpaep (1971) obtained for proximal tubule of *Necturus* a value of R_s/R_c of about 0.01, and the analogous ratio obtained by Frömter (1972) for the gallbladder of *Necturus* was 0.04. In other words, the conductance of the shunt in the toad urinary bladder is 40% of the total transepithelial conductance, in the proximal tubule of *Necturus* 99%, and in the *Necturus* gallbladder 96%.

Our second observation with Method A concerns the absolute value of the transcellular resistance, for which a mean value of 7,470 ohm·cm² was determined. This value may be compared again with those reported for proximal tubule and gallbladder of *Necturus*. These are 7,900 and 7,550 ohm·cm², respectively. Even though our results, and also those of Frömter, show important variations from one preparation to the other, the mean values are almost identical. This comparison clearly indicates that there are no significant differences in the conductance of the transcellular pathway of these epithelia, and that their different total conductances reflect differences in the conductance of the shunt. Although both Frömter's and our results show a higher mean resistance for the apical than the basal membrane, there is considerable variation from one animal to the other, and again this variation might have biological significance.

The value of R_s measured by Method A was very similar to that found with Method B. This last method is based on two explicit assumptions that require further discussion, namely, that both R_s and R_b remain constant with either sodium or potassium as the main mucosal solution cation. With respect to the first assumption, the shunt resistance with sodium on the mucosal side would be different from the shunt resistance with high potassium solution on the mucosal side if the shunt shows cation selectivity, i.e., if $g\mathrm{K} \neq g\mathrm{Na}$ (for the shunt). By the same derivation procedure used to obtain Eq. 10, it can be shown that the true resistance of the shunt (with sodium on the mucosal side) may be calculated from the same set of measurements, according to

$$R_s' = \frac{R_t R_t'[m(a' + 1) - (a + 1)]}{R_t(a' + 1) - R_t'(a + 1)}, \tag{11}$$

where m is the ratio of conductances of the shunt in the two conditions. The ratio R_s'/R_s is obtained by dividing Eq. 11 by Eq. 10.

$$\frac{R_s'}{R_s} = \frac{m(a' + 1) - (a + 1)}{a' - a}. \tag{12}$$

The variation of R_s'/R_s as a function of m may be calculated by introducing in Eq. 13 our mean values for a' (5.5) and a (1.6). The permeabilities of the toad urinary bladder (in the serosal to mucosal direction) for Na, K, and Cl are: PNa = 1.30, PK = 1.45, PCl = 1.33 cm·s^{-1}, and they are not affected by ouabain (Finn and Hutton, 1974; Finn, unpublished observations). These values can be used as an indication of the relative ionic conductances of the shunt pathway. If in the extreme situation it is considered that all the transepithelial depolarizing current is carried by Na and Cl under control conditions, and by K and Cl after the change in mucosal solution, m would be given by

$$m = \frac{g\mathrm{K} + g\mathrm{Cl}}{g\mathrm{Na} + g\mathrm{Cl}}, \tag{13}$$

and its value would be 1.06 leading, according to Eq. 12, to at most a 10% underestimation of R_s.

The second assumption for the use of Method B, i.e., that R_b is the same with sodium or potassium on the mucosal side, is based on the observation of a quick and reversible increase in the voltage divider ratio (R_a/R_b) when the change in solution is made. Our results differ from those reported by Cereijido and Curran (1965) in the frog skin. They found that the decrease in conductance of the tissue produced by replacing sodium with choline on the external side was due to decreases in the conductances of both the outer and inner barriers, with an actual decrease of the voltage divider ratio. Although we have always observed an increase in R_a/R_b when going from sodium to potassium as main cation of the mucosal solution, the possibility of an absolute increase in R_b in addition to the increase in R_a cannot be discarded. If this were the case, the value of R_s would be underestimated, and the true R_s (R_s'') would be given by

$$R_s'' = \frac{pR_tR_t'(a' + 1) - R_tR_t'(a + 1)}{pR_t(a' + 1) - R_t'(a + 1)}, \tag{14}$$

where p is R_b'/R_b, and R_b' is the resistance of the basal membrane when potassium is the main mucosal cation. From our mean values for a, a', R_t, and R_t', a mean value for R_s was calculated, and R_s'' was obtained from Eq. 14 for several values of p. Thus, the ratio R_s''/R_s as a function of p can be calculated. Even if R_b increases fivefold after the change in solution, the resulting error in the calculation of R_s is 35%, and does not invalidate the overall conclusion with respect to the resistance of the shunt pathway. Thus, the two initial assumptions for the use of Method B seem appropriate, and we may conclude that the values obtained by this method for the transcellular and shunt resistances provide an independent confirmation of the results obtained from the voltage spread experiments (Method A).

Horizontal Resistance of the Epithelium

The results obtained in the voltage spread experiments allow the calculation of the specific resistance of the sheet (including both the resistances of the cytoplasm and of the intercellular junctions), according to (Frömter, 1972):

$$R_i = \frac{2\pi A}{i_o}, \tag{15}$$

and

$$\rho_s = R_i \cdot h, \tag{16}$$

where R_i is the resistance for horizontal current flow of 1 cm² of the sheet (ohm), ρ_s is the specific resistance of the sheet material (ohm·cm), and h is the height of the cells (i.e., the thickness of the sheet). Our mean value for R_i is 1,500 kΩ. If h is assumed to be 5 μm, the value of ρ_s is 750 ohm·cm. This value is roughly 3 times that calculated for the specific resistance of frog skeletal muscle cytoplasm (Katz, 1948; Schanne, 1969), and within the range calculated for several epithelia by Loewenstein and coworkers (Loewenstein, 1966). From Frömter's results, a mean ρ_s of about 3,000 ohm·cm can be calculated for the *Necturus* gallbladder, i.e., a fourfold higher sheet specific resistance, even though the greater size of the cells in this epithelium would result in a lower number of intercellular junctions for a given distance, as compared with the toad urinary bladder. Assuming that the resistivity of the cytoplasm is similar in both tissues, this comparison indicates that the resistance of the cell-to-cell pathway is much higher in the epithelium of *Necturus* gallbladder than in the toad urinary bladder.

The anatomical location of the intercellular coupling elements is not clear. In some tissues, electrical coupling between cells has been attributed to septate junctions (Loewenstein and Kanno, 1964; Bullivant and Loewenstein, 1968). Recent studies indicate a correlation between the finding of high permeability between cells and the anatomical demonstration of gap junctions (Payton et al., 1969; Gilula et al., 1972; Friend and Gilula, 1972). Although gap junctions are occasionally found in toad bladder epithelium (DiBona, personal communication), no systematic freeze-fracture studies in this tissue have been published. A third possibility is the location of the cell-to-cell pathway in the junctional complex itself. If this were the case in this tissue, and if the location of the shunt were intercellular (vide infra), the transepithelial and the cell-to-cell pathways must be electrically insulated from each other, i.e., the flow of ions from cell to cell must bypass the intercellular space, given the relatively low electrical resistance of this space. Previous studies in other epithelia support this conclusion (Loewenstein and Kanno, 1964; Frömter, 1972).

Specific Resistances of the Cell Membranes

The values of R_a, R_b, and R_s are expressed, as is R_t, per square centimeter of tissue. The estimation of the specific resistance of each membrane requires a calculation of its true surface. The tissue is variably folded according to its degree of stretch (Gfeller and Walser, 1971). As this was not evaluated systematically in our experiments, only semiquantitative estimations can be made.

The equivalent circuit for R_b is represented in Fig. 8. R_l is the resistance of the lateral membrane, R_{ic} is the resistance of the intercellular space and R_b* is the resistance of the basal membrane. R_b represents the compound resistance given by the parallel disposition of R_b* and $(R_l + R_{ic})$. The value of R_{ic} may be calculated according to the assumptions and equations (4*a*–7*a*) stated in the Appendix, and is some 20 ohm·cm² of bladder. Thus, the contribution of the intercellular space to the resistance for current flow from the cell to the serosal solution is actually negligible. The specific resistance of the basal-lateral membrane can be calculated, according to the circuit represented in Fig. 8, from Eqs. 8 *a* and 9 *a* (Appendix), and its value is 40,000 ohm·cm². The specific resistance of the apical membrane can be calculated from Eq. 11 *a* (Appendix) to be 4,400 ohm·cm². Accordingly, the specific resistance of the basal-lateral membrane would be roughly one order of magnitude higher than the specific resistance of the apical membrane.

Location of the Shunt Pathway

The presence of a shunt pathway in the toad urinary bladder epithelium does not mean that its anatomical location is necessarily intercellular. At least two other possibilities must be considered: damage in the edge of the preparation, and a particular cell type.

Frömter, in his studies in *Necturus* gallbladder, measured the potential very close to the apical surface of the cells while passing high density current across the tissue, and was able to demonstrate an "electrical sink" when the tip of the microelectrode was close to an intercellular space. This is strong evidence for the intercellular location of the shunt pathway in that tissue (Frömter and Diamond, 1972; Frömter, 1972). Several such experiments in the toad urinary bladder were unsuccessful, i.e., no deformation of the electrical field was detected. A positive result could hardly be expected if one considers first, the close values of the resistances of the paracellular and cellular pathway (R_c/R_s = 0.55 in the toad urinary bladder, and about 25 in the *Necturus* gallbladder), and second, the relative sizes of the cells (3 to 5 times bigger in the *Necturus* gallbladder). However, this technique was useful in showing that there was also no electrical sink at the edge of the preparation. This observation, and the fact that a systematic investigation of edge damage in this

laboratory (Finn and Hutton, 1974) has shown that no such damage can be demonstrated in carefully mounted bladders, seem to rule out this possibility as the anatomical location of the shunt. The sensitivity of Frömter's method was tested by deeply impaling the microelectrode in order to damage two to five cells. In this case, a clear electrical sink could be demonstrated when the microelectrode approached the lesion, while being moved parallel to the epithelial surface.

It has been shown that increases of the osmolality of the external solution produce a decrease in the total electrical resistance of the frog skin, probably by "opening" a paracellular pathway (Ussing, 1963; Ussing and Windhager, 1964). The same results have been obtained with several solutes in the toad urinary bladder (Urakabe et al., 1970). Furthermore, the decrease in the resistance of the tissue is closely related to morphological alterations of the junctional complexes (DiBona, 1972; DiBona and Civan, 1973; Wade et al., 1973). Finally, we have observed that the conductance of the shunt pathway (measured by Method A) increases 10-fold after doubling the osmolality of the mucosal solution by adding urea (to be published). This result, and the observed morphological alterations of the tight junctions, strongly suggest that the location of the shunt pathway is intercellular.

We have no means of testing the hypothesis that a particular cell type might act as the shunt pathway. Since virtually all the cells are electrically coupled (vida supra), any cellular contribution to the shunt pathway must include only a very small proportion of the total number of cells. Since our measured R_s accounts for about 55% of R_t, each such cell would have a very low electrical resistance. Because of this, the cell electrical properties (potential, stability of the potential, membrane resistances) might be such as to fail to meet our criteria for an adequate impalement, and may have been systematically excluded from consideration. However, for the reasons given above (namely, that the evidence strongly favors an intercellular location for the shunt), we consider this possibility extremely unlikely. The same considerations may be made with respect to the possibility of damaged and desquamating cells as anatomical location of the shunt.

If the location of the transepithelial shunt pathway is intercellular, its total electrical resistance will be given by three elements in series: the zonula occludens, the zonula adherens, and the intercellular space. From the dimensions of these structures it is possible to get an estimation of their relative contribution to the resistance of the shunt. The equations and assumptions necessary for these calculations are detailed in the Appendix. They give for the intercellular spaces 40, and for the zonula adherens 2, ohm·cm^2 of bladder. Clearly, then, neither the intercellular space, nor the zonula adherens, contribute any significant amount to the transepithelial shunt resistance. Thus, the high resistance element must be the zonula occludens. This

conclusion is also supported by the observation that horseradish peroxidase penetrates the intercellular spaces from the serosal side, but does not penetrate the zonula occludens (Masur et al., 1971; Wade and DiScala, 1971). If a slit-like geometry is assumed for the zonula occludens, its width (see Appendix) would be approximately 0.02 Å, i.e., a value that is two orders of magnitude lower than the hydrated radius of sodium or potassium. This result indicates that the zonula occludens in this epithelium cannot be a slit-like structure, but must consist of discrete channels connecting the luminal solution and the zonula adherens. Such channels must be wide enough as to allow transepithelial flux of ions (conductance of the shunt pathway), but their resistance must be high enough (e.g., low total cross-sectional area of channels) to explain the high value of R_s. Further speculation indicates, for instance, that if the length of the channels is 5 times the length of the zonula occludens, their width 50 Å, and the resistivity of the solution 100 ohm·cm, the total cross-sectional area of channels (per cm^2 of bladder) would be 2×10^{-5} cm^2. If the mean width of the zonula occludens is considered to be 100 Å, the fractional area in the zonula occludens occupied by such channels would be about 1%. These calculations are in general agreement with those of Bennett and Trinkaus (1970) for the meroblastic egg of *Fundulus*. Although several uncertainties and simplifications (for instance, the resistivity of the solution) are involved in this analysis, it is clear that the information thus obtained agrees with recent electron microscopic findings on the fine structure of the zonula occludens in other tissues. Although this segment of the junctional complex was initially thought to consist of a complete fusion of the membranes of the adjacent cells (Farquhar and Palade, 1963), recent studies indicate that the contacts between the membranes occur only at some points, with a punctate appearance in cross sections; freeze-fracture studies seem to indicate that the zonula occludens is "a meshwork of branching and anastomosing threadlike contacts" (Goodenough and Revel, 1970). Conceivably, there are tortuous channels, within this meshwork, connecting the mucosal side and the zonula adherens. Thus, the available morphological information clearly indicates that the zonula occludens is not a slit-like space (as the zonula adherens and the intercellular space), and that it may act as a sieve, limiting the transepithelial passage of high molecular weight substances. This limitation is observed in tight and leaky epithelia (Farquhar and Palade, 1963; Masur et al., 1971). The striking difference in the electrical resistance of the transepithelial shunt pathway in these tissues indicates very different permeabilities of their zonulae occludentes; this difference can be dependent on the number, length (and tortuosity), and width of those channels, and on the degree of interaction between the molecular chains in the membrane and the ions contained in the space.

In summary, these calculations show that the resistive element of the para-

cellular shunt pathway of the toad urinary bladder epithelium is the zonula occludens. Furthermore, it is possible to conclude that an increase in the width of the intercellular space in this epithelium will have no measurable effects on the resistance of the shunt pathway if the limiting junction (specifically, the zonula occludens) remains unchanged, and that a reduction in the width of the intercellular space would produce a significant increase in the resistance of the shunt only at widths close to 3 Å. This situation is quite different from that observed in the *Necturus* gallbladder (Frömter, 1972), where it has been shown that the actual dimensions of the intercellular spaces are consistent with their participation as a regulatory element of the conductance of the shunt pathway.

APPENDIX

If the cell is considered a hexagonal cylinder (Danon et al., 1974), its apical (S_a), basal (S_b), and lateral (S_l) surfaces are given by

$$S_a = 3/2\, d^2 \sqrt{3} f_1, \tag{1 a}$$

$$S_b = 3/2\, d^2 \sqrt{3} f_2, \tag{2 a}$$

$$S_l = 6\, dhf_3, \tag{3 a}$$

where d is the length of each side of the cell, h is the height of the cell, and f_1, f_2, and f_3 are the microscopic folding factors for the three surfaces. The following values were used: $d = 5$ μm, $h = 5$ μm, $f_1 = 1.1$, $f_2 = 1.2$, $f_3 = 5$. For each cell, the calculated areas (in 10^{-8} cm²) are: $S_a = 71$, $S_b = 78$, and $S_l = 750$.

Calculation of the resistances of the intercellular spaces (R_{ic}) for the flow of intracellular current to the serosal solution: If one assumes (*a*) uniform length ($l_{ic} = h \cdot f_3$) and width (W_{ic}) of the spaces, and (*b*) uniform composition of the solution they contain, with the resistivity of Ringer solution (ρ_r), R_{ic} will be given by

$$R_{ic} = \frac{0.5\rho_r\, l_{ic}}{S_{ic}}, \tag{4 a}$$

where S_{ic} is the cross-sectional area of intercellular spaces per cm² of bladder. S_{ic} is calculated from

$$S_{ic} = W_{ic} \cdot L_{ic} \tag{5 a}$$

and

$$L_{ic} = \frac{6\, dN}{2} = 3\, dN, \tag{6 a}$$

where L_{ic} is the length of the network of cell boundaries (in the horizontal plane) per cm² of bladder, and N is the number of cells per cm² bladder:

$$N = (3/2\, d^2 \sqrt{3})^{-1}. \tag{7 a}$$

There is a small error involved in the use of Eq. 7 *a* since the cells at the perimeter are assumed to share all their borders with cells contained in the 1 cm² area of the bladder. For $d = 5$ μm, the error is less than 2%. The following values were used for the calculations: $\rho_r = 100$ ohm·cm, $W_{ic} = 0.03$ μm, and $d = 5$ μm.

Calculation of the specific resistances of the cell membranes:

$$\frac{1}{R_b} = \frac{1}{R_l + R_{ic}} + \frac{1}{R_b^*}, \tag{8 a}$$

(see Fig. 8). If it is assumed that the specific resistances of the basal and lateral membranes are equal:

$$\rho_b = R_b^* \cdot A_b = R_l \cdot R_l, \tag{9 a}$$

where ρ_b is the specific resistance of the basal-lateral membrane, A_b is the surface of basal cell membrane per cm² of bladder ($A_b = S_b \cdot N$), and A_l is the lateral cell surface per cm² of bladder ($A_l = S_l \cdot N$). From Eqs. 8 *a* and 9 *a*:

$$\frac{1}{R_b} = \frac{A_l}{\rho_b + R_{ic} \cdot A_l} + \frac{A_b}{\rho_b}, \tag{10 a}$$

ρ_b was calculated from Eq. 10 *a* for the following values: $R_b = 3{,}200$ ohm·cm², $R_{ic} = 20$ ohm·cm², the previously given areas of the membranes, and $N = 1.54 \cdot 10^6$.

$$\rho_a = R_a \cdot f_1, \tag{11 a}$$

where ρ_a is the specific resistance of the apical membrane, and R_a is the resistance of the apical cell membrane per cm² of bladder. ρ_a was calculated for $R_a = 4{,}000$ ohm·cm².

Calculations of the contributions of the tight junctions and intercellular spaces to the transepithelial flow of current:

$$R_{ic}^* = \frac{\rho_r \, l_{ic}}{S_{ic}}; \qquad l_{ic} = h \cdot f_3, \tag{12 a}$$

where R_{ic}^* is the resistance of the intercellular spaces for the flow of transepithelial current (per cm² of bladder).

$$R_{za} = \frac{\rho_r \, l_{za}}{S_{za}} = \frac{\rho_r \, l_{za}}{L_{za} \, W_{za}}, \tag{13 a}$$

where R_{za} is the resistance of the zonula adherens (per cm² of bladder), and the dimensions of the zonula adherens are: l_{za} = length, S_{za} = cross-sectional area per cm² of bladder, L_{za} = length of the network (equal to the length of the network of cell boundaries per cm² of bladder), and W_{za} = width.

Values used for the calculations: $f_3 = 5$, $l_{za} = 1$ μm, and $W_{za} = 0.02$ μm.

$$R_{zo} = \frac{\rho_r \, l_{zo}}{L_{zo} \, W_{zo}}, \tag{14 a}$$

where R_{zo} is the resistance of the zonula occludens (per cm² of bladder), and the dimensions of the zonula occludens are: l_{zo} = length, L_{zo} = length of the network of cell boundaries, and W_{zo} = width. If one assumes that $R_{zo} = R_s$, Eq. 14 *a* can be solved for W_{zo}. This value represents the width of the zonula occludens if it is a slit-like structure responsible for all the resistance of the shunt pathway.

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