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Cellular and Paracellular Resistances of the *Necturus* Proximal Tubule

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Summary. Individual resistances of the apical cell membrane, R_a, the basolateral cell membrane, R_{bl}, and the paracellular shunt, R_s, were determined in the *Necturus* proximal tubule using a set of three electrical parameters. Four electrical parameters were measured: the transepithelial resistance, (R_{te}), the apical and basolateral cell membrane resistance in parallel, (R_Z free-flow tubules), the basolateral cell membrane resistance in oil-filled tubules, (R_Z oil-filled), and the ratio of apical and basolateral cell membrane resistance (R_a/R_{bl}). R_{te} was determined from an analysis of the spatial decay of luminal voltage following luminal current injection. R_Z free-flow and R_Z oil-filled were measured by the analysis of the spatial decay of intracellular voltage deflections following cellular current injection in free flow and oil-filled tubules, respectively. R_a/R_{bl} was estimated from the ratio of voltage deflections across the apical and basolateral cell membranes following transepithelial current injection. In addition, the magnitude of cellular and luminal cable interactions was evaluated, by comparing the spatial decay of voltage deflections in the cell and in the lumen following intracellular current injection. The combined cell membrane resistance (R_a+R_{bl}) is between one to two orders of magnitude greater than the paracellular resistance. This result supports the view that the *Necturus* proximal tubule is a leaky epithelium.

Key words proximal tubule · cable analysis · membrane resistances

Introduction

Measurements of transepithelial and cell membrane potential differences in leaky epithelia are used frequently to describe the nature of transport processes. Each of these measured potential differences has two components, the total electromotive force generated from the diffusion potentials of all permeant ions and the voltage resulting from current flow across either the particular cell membrane resistance or the paracellular shunt resistance.

In a leaky epithelium such as the *Necturus* proximal tubule, the voltages resulting from current flow across the cell membrane and shunt resistances may contribute significantly to measured potential differences. Consequently, measured membrane potential differences are not good estimates of the electromotive forces across either the cell membranes or the paracellular shunt. Since the electromotive force is the parameter which is directly related to the electrochemical potentials of ions, it is essential to the study of individual ion conductances. Therefore it is important to be able to estimate from an equivalent circuit the true electromotive force using measurements of individual cell membrane and paracellular resistances in conjunction with the measured potential differences.

Because of the small size and tubular structure of renal tubules, the experiments which are used to measure resistances involve passing current from intraluminal or intracellular point sources. The experiments thus require the use of complicated single or double cable analyses to describe the current decay from point sources. In spite of these complications, measurements of cell membrane and paracellular resistances can be performed on renal tubules with reliable results. Windhager et al. [22] reported the resistance of the apical and basolateral membrane and the shunt resistance in the *Necturus* proximal tubule cell. They observed that the resistance of the cell membranes is an order of magnitude higher than the transepithelial resistance and provided the first direct evidence for a leaky paracellular shunt in the *Necturus* proximal tubule. Similar conclusions were made by Hoshi and Sakai [15] in the proximal tubule of the newt kidney (*Triturus pyrrhogaster*) and Anagnostopoulos and Velu [2] in proximal tubule of the *Necturus* kidney.

The aim of this study is: (i) to evaluate several techniques for measuring cellular and paracellular resistances, (ii) to provide a mathematical analysis to describe the current spread in the cell cable following intraepithelial current injection in the proximal tubule, (iii) to determine the magnitude and importance of luminal and cell cable to cable interactions,

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and (iv) to define the ranges of apical and basolateral cell membrane and paracellular resistances in *Necturus* of proximal tubule obtained by different experimental techniques.

Methods and Materials

Kidney Preparation

Adult *Necturus maculosus* were obtained from Connecticut Valley Biological Co. (Southampton, Mass.) and held in an aquarium at 10 °C for a least one month prior to use. The kidneys were doubly perfused with control Ringer's as described previously [10]. The perfusion solution contained 100.5 mM Na^+, 2.5 mM K^+, 1.8 mM Ca^{++}, 1.0 mM Mg^{++}, 98.1 mM Cl^-, 10 mM HCO_3^-, 0.5 mM $H_2PO_4^-$, 2.2 mM glucose, 15 g·l^{-1} PVP and 2000 U·l^{-1} heparin. All solutions were bubbled with 99% O_2 – 1% CO_2 to a pH of 7.6. The lumen of experimental tubules was perfused by means of a double barreled pipette inserted in the Bowman's capsule. Only early proximal tubules were chosen in this study [16].

Experimental Protocol

In order to determine the apical (R_a), basolateral (R_{bl}), and shunt (R_s) resistances of the *Necturus* early proximal tubule, four sets of experiments were performed. These experiments, illustrated in Fig. 1, included the measurement of transepithelial resistance (R_{te}), method *a*; apical and basolateral cell membrane resistance in parallel (R_Z free-flow), method *b*; the resistance of the basolateral cell membrane in oil-filled tubules (R_Z oil-filled), method *c*; and the ratio of apical to basolateral cell membrane voltage changes (α) following luminal current injection, method *d*. In addition a fifth type of experiment was performed, method *e*, in which the magnitude of transepithelial and basolateral voltage changes following intracellular current injection was determined.

Microelectrode Preparation

Conventional microelectrodes were drawn on micropipette puller (Model PD-5, Narishige Scientific Instruments, Tokyo, Japan) from 1.2 mm OD – 0.6 mm ID capillary tubing (Frederick Haer and Co., Brunswick, Me.), and filled with 1 M KCl. In order to minimize impalement damage, electrodes which were used for transepithelial impalements were beveled to a resistance of 10–20 MΩ (Model #1300 Beveler, W.P. Instruments, New Haven, Conn.). Electrical potential differences were measured by means of two electrometers (Model 725, W.P. Instruments) and current pulses generated by a constant current source (Model KS 700, W.P. Instruments). All microelectrode connections to the electrometer were made with Ag/AgCl half cells. Potential differences were measured with respect to a 3 M KCl agar bridge in the solution on the surface of the kidney connected to ground by a Ag/AgCl half cell and recorded on a strip chart recorder (Brush 220, Gould Inc., Cleveland, Ohio).

Method a) Transepithelial Resistance

One current passing and two voltage-measuring microelectrodes were placed and maintained in the lumen of a straight portion of each early proximal tubule analyzed for transepithelial resistance. The distance down the lumen between each voltage measuring electrode and the current electrode was measured with an ocular micrometer.

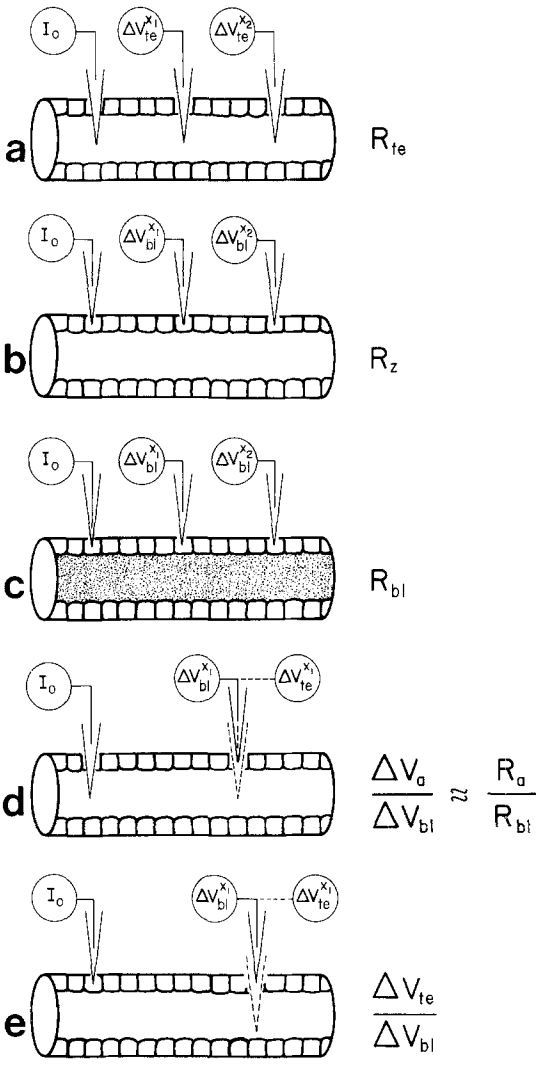

Fig. 1. Five experiments were performed on the early proximal tubule of the *Necturus* kidney. Method *a*; Three microelectrodes were placed into the lumen of the tubule. One electrode was used to pass current (I^0), and the other two were used to measure the change in intraepithelial voltage (ΔV_{te}) at two distances, x_1, and x_2, from the current electrode. Method *b*; The current electrode was placed in a cell, and voltage deflections (ΔV_{bl}) were measured at two or more distances x_1, x_2, x_n from the current source by means of a series of cell impalements with a second microelectrode. Method *c* is similar to method *b* except the lumen is filled with oil to inhibit current leaking across the apical cell membrane. Method *d*; Current electrode was placed in the tubule lumen and voltage deflections were measured by a second electrode in a cell and in the lumen at the same distance (x_1) from the current electrode. Method *e*; A current electrode was placed in the cell, and voltage deflections were measured at the same distance (x_1) in a cell and in the lumen by a second microelectrode.

Because of the difficulty of placing and maintaining three electrodes in the lumen of the proximal tubule, it was necessary to verify that the electrodes were indeed in the lumen. The luminal position of electrodes was verified by monitoring the transepithelial potential differences (V_{te}) during a rapid reduction in the Cl^- activity of the luminal perfusion solution from 74 to

6 mM. Cl was replaced by gluconate [12]. Because the paracellular shunt has a high Cl^- permeability [12], perfusion of a low Cl^- solution in the lumen of the *Necturus* proximal tubule resulted in a hyperpolarization of V_{te}. Resistance measurements were performed only if all three microelectrodes in the lumen of the tubule detected the same control V_{te}, the same change in V_{te} during luminal low Cl^- perfusion, and the same V_{te} when control solution was returned to the lumen.

Current pulses of 2×10^{-7} A and 0.5 sec duration were passed through the most distally placed microelectrode. Electrotonic potential differences were recorded by the other two microelectrodes. At the end of each experiment a control solution stained with 0.5% Hercules Green Shade #2 (H. Kohnstamn and Co., New York) was perfused through the tubule lumen to outline the apical surface. The diameter of the lumen was then estimated by an ocular micrometer. In these experiments the lumen of the tubule was considered to be a simple cylinder without inclusion of the brush border membrane surface area amplification.

Treating the renal tubule as a single unbranched infinite cable, the length constant (λ_l, μm) of the luminal cable was determined from the following equation [4]:

$$\Delta V_{te}^x = \Delta V_{te}^0 e^{-x/\lambda_l} \tag{1}$$

where ΔV_{te}^x and ΔV_{te}^0 are the electrotonic voltage deflections measured in the lumen at distances x and 0 from the current electrode. λ_l was calculated from the inverse of the slope of a logarithmic plot of ΔV_{te}^x versus distance from the current electrode and ΔV_{te}^0 from the $x=0$ intercept. ρ_l(Ω cm), the volume resistivity of the luminal fluid was obtained from conductivity measurements (100 Ω cm for control solution) and R_l^{input}, the input resistance, from the ratio $\Delta V_{te}^0/I^0$ where I^0 is the applied current.

The transepithelial specific resistance, R_{te}, (Ω cm^2) can be calculated in four different ways by using different combinations of the parameters, ρ_l, λ_l, R_l^{input}, and the optical radius, a_0 [4]. In this study R_{te} was estimated from the equation:

$$R_{te} = \frac{2\rho_l \lambda_l^2}{a_0}. \tag{2}$$

The electrical radius, a_l, was calculated from the equation

$$a_l = \sqrt{\rho_l \lambda_e / 2\pi R_l^{\text{input}}}. \tag{3}$$

The electrical radius was compared to the optical radius as a check of the internal consistency of the cable analysis technique.

Method b) Apical and Basolateral Cell Membrane Resistances in Parallel

The parallel resistance of the apical and basolateral cell membrane, R_Z free-flow, was determined in free-flow tubules from the electrotonic voltage spread in the cell cable resulting from intracellular current injection. Depolarizing current pulses of either 2.0×10^{-8} A, 2.5×10^{-8} A or 5×10^{-8} A were injected through an intracellular microelectrode. Voltage deflections were detected at several points along the tubule by means of sequential cell impalements with a measuring microelectrode (Fig. 1*b*). The distance between current and measuring electrodes was always greater than one tubule diameter and less than 700 μm.

Assuming that the proximal tubule is a sheet of cells wrapped into an infinite tube, the voltage attenuation resulting from intracellular current injection is approximated by the following equation (*see* Appendix for a detailed mathematical treatment of the cable equations used in this study).

$$\Delta V_{bl}^x = \Delta V_{bl}^0 e^{-x/\lambda_c} \tag{4}$$

where ΔV_{bl}^x is the voltage deflection at a distance, x, from the current source, ΔV_{bl}^0 is an empirical intercept for $x=0$, and λ_c is the length constant of the cellular cable. λ_c, was determined from the slope of a logarithmic plot of ΔV_{bl}^x *vs.* distance and ΔV_{bl}^0 from the intercept. The input resistance R_c^{input} was calculated from the ratio $\Delta V_{bl}^0/I^0$ where I^0 is the input current. The cell cable specific resistance, ρ_c, (Ω cm), was calculated from the following equation:

$$\rho_c = \frac{4\pi a_0 d R_c^{\text{input}}}{\lambda_c} \tag{5}$$

where a_0 is the tubule radius and d, the thickness of the cell layer estimated as 20 μm. R_Z free-flow is then given by

$$R_Z \text{ free-flow} = \frac{\rho_c \lambda_c^2}{d}. \tag{6}$$

Method c) Basolateral Cell Membrane Resistance Oil-Filled Tubules

The resistance, R_Z oil-filled, was measured by a technique similar to the one described in method *b*. However, to minimize current flow across the lumen membrane and thereby obtain a direct estimate of basolateral cell membrane resistance, the tubule lumen was filled with mineral oil dyed with Sudan Black. This technique is illustrated in Fig. 1*c*.

Method d) Ratio of Transepithelial to Basolateral Cell Membrane Voltage Deflections Resulting from Intra-Luminal Current Injection

Current pulses of 2×10^{-7} A and 0.5 sec duration were injected into the lumen of the tubule with a microelectrode (Fig. 1*d*). At another site along the tubule a second microelectrode was inserted sequentially into a cell and then into the lumen of the proximal tubule. The distance between the current electrode and the measuring microelectrode was kept constant during the insertion of the measuring electrode first into the cell and then into the lumen.

The ratio of apical to basolateral cell membrane voltage deflections, α was determined from the equation:

$$\alpha = \frac{\Delta V_{te}^x}{\Delta V_{bl}^x} - 1 \tag{7}$$

where ΔV_{te}^x and ΔV_{bl}^x are electrotonic potential differences with the measuring microelectrode in the lumen, and in the cell, respectively. The voltage ratio α was used as an estimate of the ratio of the apical and basolateral cell membrane resistances, R_a/R_{bl}.

Method e) Ratio of Transepithelial to Basolateral Cell Membrane Voltage Deflections Resulting from Intracellular Current Injection

The degree to which current injected into the cell cable enters into the luminal cable was estimated by the following technique (Fig. 1*e*). A microelectrode was placed in a cell across the basolateral cell membrane through which current pulses of 5×10^{-8} A were passed. At a different location along the tubule a second microelectrode was placed first into a cell and then into the lumen. Thus the electrotonic voltage deflections resulting from current pulses injected in the cell cable were recorded at the same point (x_1) along the tubule first within the cell cable and then within the luminal cable.

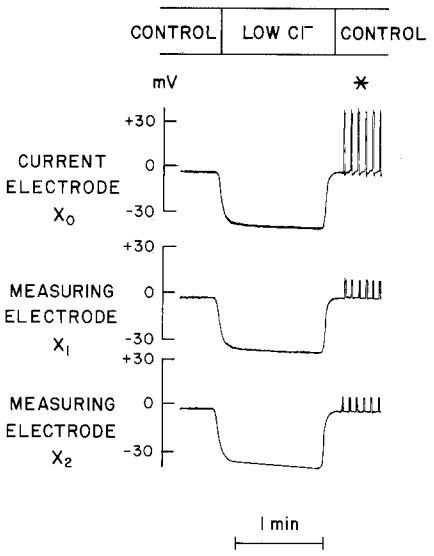

Fig. 2. Three tracings showing the voltage recorded by three microelectrodes in the lumen of the proximal tubule. In order to verify the location, the activity of Cl^- was reduced only in the luminal solution from 74 to 6 mM, resulting in a hyperpolarization of V_{te}. Note that each electrode records the same −38 mV hyperpolarization when Cl^- was lowered, indicating that each electrode was in the lumen. After testing the position of the electrodes, current pulses were passed through the current electrode (upper tracing) and the voltage deflections detected by the measuring electrodes (lower traces). *Note that deflections from the current electrode were off the scale of the recording

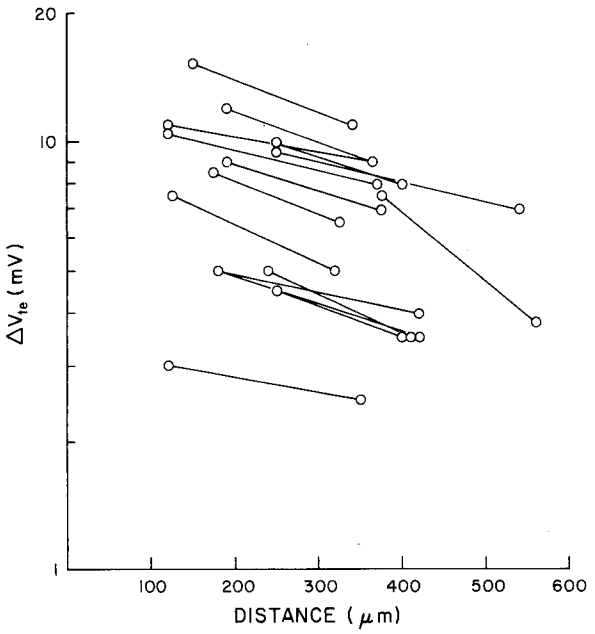

Fig. 3. Luminal cable. Changes in transepithelial potential difference detected by the measuring electrodes *vs.* distance from the current electrode. Each pair of points connected by a line indicates an experiment on an individual tubule

Apical and Basolateral Cell Membrane and Paracellular Shunt Resistances

According to a simple equivalent model of the *Necturus* proximal tubule in which all apical cell membrane resistances are lumped into a single resistance, R_a, the basolateral cell membrane resistances into a single R_{bl} and the paracellular shunt resistances into a single resistance, R_s (Ref. #3 and Appendix, Fig. 8), R_{te}, R_Z free-flow, R_Z oil-filled and α can be described by the following equations:

$$R_{te}=\frac{(R_a+R_{bl})R_s}{R_a+R_{bl}+R_s} \tag{8}$$

$$R_Z \text{ free-flow}=\frac{R_a \cdot R_{bl}}{R_a+R_{bl}} \tag{9}$$

$$R_Z \text{ oil-filled}=R_{bl} \tag{10}$$

$$\alpha=\frac{R_a}{R_{bl}}. \tag{11}$$

The four values R_{te}, R_Z free-flow, R_Z oil-filled, and α were obtained from methods *a*, *b*, *c* and *d*. Equations (8) through (11) were used for solving for the unknows R_a, R_{bl}, and R_s.

Results

Transepithelial Resistance

Figure 2 shows a typical recording in which three microelectrodes were placed in the lumen of the *Necturus* proximal tubule. The proper electrode position was checked by reducing the Cl^- activity in the luminal perfusion, only, from 74 to 6 mM and determining if the electrodes detected the same change in V_{te}. Because the shunt is highly selective to Cl^- [12], lowering Cl^- in only the luminal perfusion hyperpolarizes V_{te}. In the experiment shown in Fig. 2 reducing luminal Cl^- hyperpolarized V_{te} by −38 mV. All three electrodes in the lumen recorded the same hyperpolarization during Cl^- reduction and returned to the same V_{te} when control solution is replaced. The similarity of V_{te} recorded by the microelectrodes indicates that the electrode tips were indeed in the same tubule lumen.

After verifying the position of the electrode tips, current pulses (off scale deflections, upper tracing Fig. 2) were passed through the current electrode and the electrotonic voltage deflections recorded (lower tracings Fig. 2). As expected from Eq. (1), voltage deflections diminish with distance from the current source. The electrotonic voltages from 15 experiments are plotted in Fig. 3 as a function of distance from the current source. Values for λ_l, ρ_l, a_0, and R_{te} from each tubule separately are summarized in Table 1, column 1.

Using the average of the intercepts (ΔV_{te}^0) calculated from Eq. (1) for each tubule, the electrical ra-

Table 1. Lumen and cellular cable properties of the proximal tubule

	Luminal cable Method *a*	Cellular cable (free flow) Method *b*	Cellular cable (oil filled) Method *c*	Voltage ratio Method *d*
λ (μm)	729 ±67	248 ± 17	304 ± 9	–
R_{input} (Ω)	5.3± 0.56 ×10^4	1.0± 0.09 ×10^6	1.4± 0.1 ×10^6	–
ρ (Ω cm)	100	7000 ±873	8227 ±883	–
a_o (μm)	57 ± 3	63 ± 3	70	–
R_{te} (Ω cm^2)	260 ±58	–	–	–
R_Z (Ω cm^2)	–	1784 ±149	–	–
R_{bl} (Ω cm^2)	–	–	3750 ±220	–
R_a/R_{bl}	–	–	–	2.9±0.3
n	15	17	5	10

dius radius was calculated from Eq. (3). The calculated radius, a_l, of 51±5 μm (n=15) is not significantly different (P=0.2) from the optically measured inner tubule radius, a_0, (Table 1, column 1). The similarity of electrical and optical radii indicates that different combinations of the parameters ρ_l, λ_l, R_l^{input} and a_0 can be used to calculate R_{te} with the same result and also that the main site of the transepithelial resistance is located near the inner tubule circumference.

Apical and Basolateral Cell Membrane Resistance in Parallel, R_Z Free-Flow

The electrical properties of the cell layer were studied by analyzing the spatial decay of voltage in the cells along the tubule resulting from intracellular current injection from a point source according to Method *b*.

As pointed out above, proper placement of the microelectrode is an important aspect of these experiments. In this experiment, the intracellular position of the current electrode was verified by comparing the basolateral cell membrane potentials recorded by both the current and measuring microelectrodes in the absence of current pulses. Similar recordings by both microelectrodes during intracellular impalements were indicative of the location of the microelectrode tips (*see* Reference [12] for an example of this localization technique). Figure 4 is a plot of electrotonic voltage deflections *vs.* distance from an intracellular current source in free flow tubules. Values for λ_c, ρ_c, a_0, R_c^{input}, and R_Z free-flow are given in Table 1, column 2. When current is injected into a continuous sheet of cells, it can leave the cells both across the apical and the basolateral cell membrane. Therefore the value, R_Z free-flow, is the resistance of apical and basolateral cell membranes in parallel.

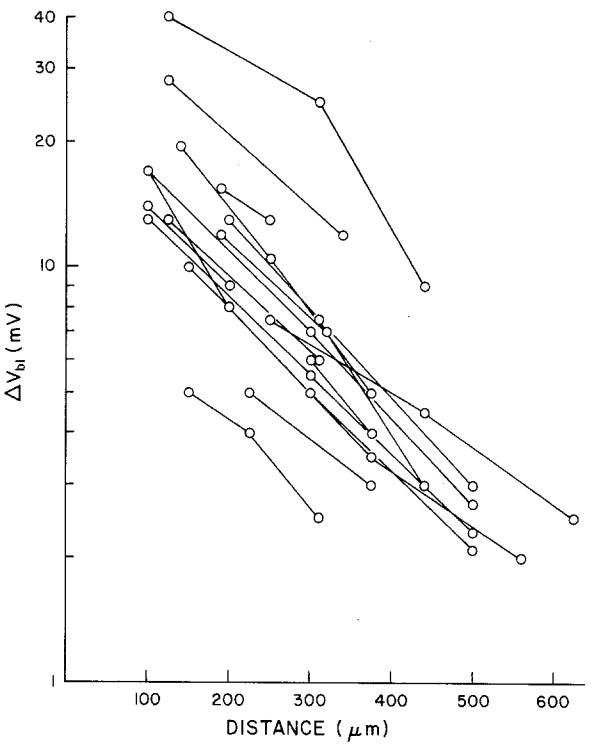

Fig. 4. Changes in potential across the basolateral cell membrane resulting from current injection into the cellular cable of free-flow tubules. Each point is the voltage change detected by measuring electrode in cell at different distances along the tubules. Lines connect measurements made in the same tubule. The currents used in these experiments were either 2.0, 2.5, or 5.0 ×10^{-8} A

Basolateral Cell Membrane Resistance, R_Z Oil-Filled

A technique of estimating only the resistance of the basolateral membrane is to analyze the spatial decay of voltage in the cells along the tubule with the lumen of tubule filled with a nonconducting oil. Filling the lumen with oil will considerably reduce current leaving across the apical cell membrane. Therefore R_Z oil-filled represents only the resistance of the basolateral cell membrane, assuming that the

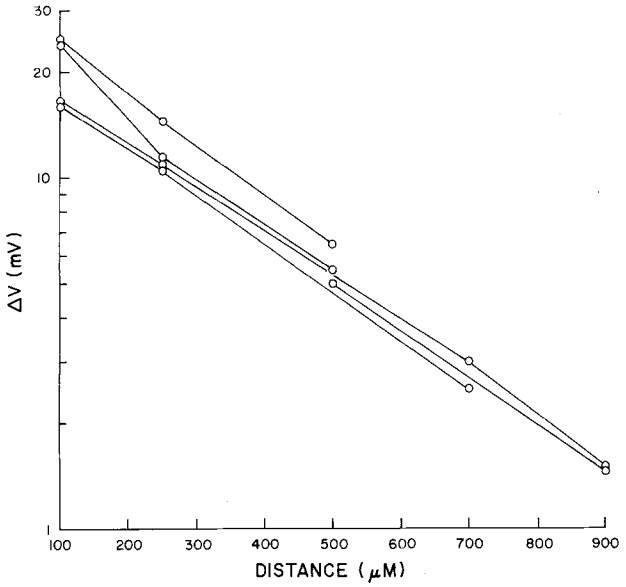

Fig. 5. The same plot as in Fig. 4 except that in these experiments the tubule lumen was filled with oil

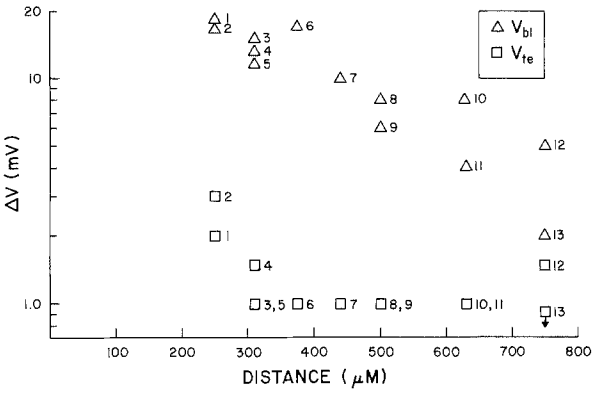

Fig. 6. A plot of the voltage changes in the cell (triangles) and in the lumen (squares) resulting from current injection into the cell cable. Each number represents paired measurements at the same distance from the current electrode. Only one pair of measurements was made on each tubule

oil makes the apical cell membrane resistance infinitely high.

Figure 5 is a plot of voltage deflections versus distances from an intracellular current source in oil-filled tubules. The results of five experiments are summarized in Table 1, column 3.

As expected, the λ_c of oil filled tubules is greater than λ_c of free-flow tubules, mainly because current cannot leave the cells across the apical membrane. There is no significant difference in ρ_c from free flow to oil filled, indicating that filling the lumen with oil does not affect the specific resistance of the cell cable, e.g., by uncoupling cells.

Resistance Ratio

As discussed in the Methods, resistance ratio was estimated from the voltage ratio. The data are given in Table 1, column 4.

Cable-to-Cable Interactions

The analysis of the spatial decay of voltage in the cells along the tubule resulting from current injected intracellularly from a point source (R_Z free-flow) assumes that there are no cable-to-cable interactions or "cross talk" between luminal and cellular cables (*see* Appendix). It assumes that during intracellular current injection both luminal and peritubular compartments along the tubule remain at ground isopotential with respect to space and time. However, if along the tubule a significant amount of current enters into the finite luminal volume conductor, the intraluminal potential may be raised above ground. The electronic voltage in the lumen would exhibit spatial decay with length of the tubule. If the luminal electrotonic potential is large and if the cable property of the lumen, λ_l, is much greater than the length constant of the cell, λ_c, the result would be that the lumen would act as another source of current for the cell cable. At sufficient distance from the current source, current instead of leaving the cells across both apical and basolateral cell membranes would in fact be entering the cells from the lumen across the apical cell membrane. Measurements of the electrotonic voltage deflections at distances beyond such current reversal point would not be indicative of the spatial decay of current in the cell cable but would reflect the degree of current entering the cell cable from the lumen. This would result in a significant error in the measurement of R_Z free-flow. In addition, at distances shorter than the current reversal point the cell cable electrotonic decay will be affected by the non-uniform potential in the lumen such that the measured λ_c would not reflect the cable properties of the cell layer but a complex combination of cellular and luminal cable properties.

In order to test the magnitude of cellular and luminal cable "cross talk," current pulses were injected in the cell cable and electrotonic voltage deflections were measured in the cell ΔV_{bl}^x and in the lumen ΔV_{te}^x at the same distance from the current source. A logarithmic plot of ΔV_{bl}^x and ΔV_{te}^x as a function of distance for 13 tubules is shown in Fig. 6.

It is clear that between 250 and 750 μm from the current source the electrotonic voltage deflections in the luminal cable resulting from current injection in

Table 2. Apical and basolateral cell membrane and paracellular shunt resistances

Experimental parameters	*a, b, d*	*a, b, c*	*a, c, d*
R_a $\Omega\,cm^2$	6957	3403	10885
R_{bl} $\Omega\,cm^2$	2399	3750	3750
R_s $\Omega\,cm^2$	267	270	265

Method $a=R_{te}$, Method $b=R_Z$ free-flow, Method $c=R_Z$ oil-filled, and Method $d=R_a/R_{bl}$. The parameters R_a, R_{bl}, and R_s were calculated from Eqs. (8) through (11).

the cell cable are only a small fraction of the voltage deflections in the cell cable. In fact, voltage deflections in the luminal cable beyond 250 μm approach the minimum sensitivity of our measurements, 1 mV. Because the voltage deflections in the lumen are small compared to those along the cell cable, the interference of the luminal cable to the measurement of cell cable properties will also be small. This point will be discussed in more detail below.

Cellular and Paracellular Resistances

Four measurements, methods *a*, *b*, *c* and *d* were made to determine the individual cell membrane and paracellular resistances, R_a, R_{bl}, and R_s. There are three unknown parameters, R_a, R_{bl}, and R_s, and four experiments to evaluate them; however, only one, method *a*, R_{te}, contains information on the paracellular resistance. Therefore there are three different ways to calculate R_a, R_{bl}, R_s from Eqs. (8) through (11). The data and the method of analysis are summarized in Table 2.

The first method (colum 1 in Table 2) uses results from experiments *a*, *b*, and *d* (R_{te}, R_Z free-flow, and α) and Eqs. (8), (9) and (11); the second method (column 2, Table 2) uses experiments *a*, *b* and *c* and Eqs. (8) through (10), and the third method (column 3, Table 2) uses experiments *a*, *c* and *d* and Eqs. (8), (10) and (11) to calculate the parameters R_a, R_{bl} and R_s.

It is clear that R_a, R_{bl} and R_s differ according to the method of analysis. There are small differences in R_{bl} and R_s and large differences in R_a. Some of the reasons for these differences will be discussed below.

Discussion

One of the main conclusions of this paper is that, irrespective of the method used to calculate R_a, R_{bl} and R_s, the resistance of the shunt pathway is between one and two orders of magnitude less than the sum of the cell membrane resistances, R_a+R_{bl}. It is this relative magnitude of resistances $(R_a+R_{bl})/R_s$ which indicates that the *Necturus* proximal tubule is a leaky epithelium. A leaky epithelium is defined as one in which the resistance to the flow of ions is less through the paracellular pathway than through the cellular pathway irrespective of the absolute magnitude of the resistance of a given pathway.

Transepithelial Resistance

Reported values of transepithelial resistances for all segments of the *Necturus* proximal tubule vary over a considerable range, 43 to 641 $\Omega\,cm^2$ [8, 22]. In general, the measured values in the *Necturus* proximal tubule are much higher than transepithelial resistances of mammalian proximal tubules which are about 5 $\Omega\,cm^2$ [6, 13, 19].

A large part of the variation in reported values of R_{te} from this and other laboratories could originate from three sources: the physiological status of the kidney, the segmental localization along the tubule, and the nature of the experimental technique.

The sensitivity of R_{te} to changes in the physiological status of the kidney is well documented. For example, volume expansion in both blood perfused and doubly perfused kidneys causes a reduction in R_{te} [4, 11]. In addition, experiments in the doubly perfused kidney have demonstrated that differences in the composition of the perfusion solutions affect R_{te} [11]. Furthermore, values of transepithelial resistance measured in the blood perfused kidney are lower than those reported for the doubly perfused kidney [11]. The reason for this latter difference is probably related to differences in both flow through the kidney and fluid composition between blood perfused and doubly-perfused kidney preparations. Consequently, in order to compare reported values of transepithelial resistance of the *Necturus* proximal tubule, careful consideration should be given to the method of kidney preparation (blood perfused or doubly-perfused), the flow rate, and composition of either infusion or perfusion solutions.

Another source of variability in reported values of transepithelial resistance is between segments of the proximal tubule. Morphologically, the *Necturus* proximal tubule can be divided into two segments, an early and a late proximal tubule [16]. Transepithelial resistances measured in the early portion of the proximal tubule are lower than those of the late proximal [8].

A third source of variability is the experimental technique. As stated in the methods, R_{te} can be obtained from any combination of three parameters out of four experimentally determined values ρ_l, λ_l, R_l^{input} and a_0. Each parameter has its own experimental error. As a result R_{te} is a derived value depending on the measurement of voltage deflections, interelectrode distance, and tubule radius. One source of experimental error in measurements of R_{te} is impalement damage. It should be recognized that, because of the method for measuring R_{te}, impalement damage or similarly poor electrode tip localization could result as well in higher values for R_{te} as easily as lower ones. For example, if excess damage is caused by an intraluminal impalement or if the electrode tip is not in the lumen at distances close to the current electrode while good impalements are made at distances away from the electrode, then ΔV_{te} measured close to the current electrode would be underestimated. The result would be an increase in the measured λ_l and R_{te} (*see* Eqs. (1) and (2)). Impalement damage or poor tip localization would result in lower measured values of R_{te} only if the damage were uniform along the tubule or if good impalements were made close to the current electrode and poor impalements at greater distances.

In this study care was taken to identify and minimize experimental errors. Electrodes were beveled to minimize impalement damage. Because a collapse of the tubule lumen could artifically change the measured R_{te} the lumen of the tubule was perfused with control solution by means of a pipette in the glomerulus which maintained an open lumen. Therefore changes in R_{te} due to impalements collapsing the lumen were avoided. The lumen was perfused with a dyed solution to aid in the measurement of tubule radius. In addition, the location of the three microelectrode tips were verified during each experiment to confirm its luminal position.

Apical and Basolateral Cell Membrane Resistances in Parallel R_Z free-flow, and Cable to Cable Interactions

Although subject to similar variation in the physiological status of the kidney and in the experimental technique, reported values of λ_c in free-flow tubules are less variable than reported values of λ_l. For example, Windhager et al. [22] and Anagnostopolous and Velu [2] reported values for λ_c of 200 μm and Anagnostopolous et al. [1] values of λ_c from 214 to 250 μm for the *Necturus* proximal tubule and a value of 248 μm is reported in the present study.

To analyze the spatial decay of voltage in the cell cable, the proximal tubule can be mathematically modeled as two concentric cables, an inner luminal and an outer cellular cable. The present mathematic model assumes that cellular and luminal cables are independent of each other. Spatial decay of current in one cable is unaffected by the other cable. Recently, Anagnostopolous et al. [1] have suggested that the measurements of cellular cable properties in the *Necturus* proximal tubule by means of intracellular current injection as shown in method *d* of this paper may be inaccurate because of a significant cable-to-cable interaction or "cross talk."

The phenomenon of "cross talk" can be a problem in the measurements of cellular cable properties only if a significant amount of current injected into the cell cable enters into the lumen of the tubule. If the amount of current which leaks into the lumen is large and λ_l is much greater than λ_c, it is possible that the current in the lumen could leak back into the cell cable and act as an additional extracellular current source. The interference from back leak of current into the cellular cable would become especially severe at large distances from the current source where the voltage displacements at a given site in the luminal cable would exceed those in the cellular cable.

Anagnostopolous et al. using a theoretical model of cable to cable interactions claimed that at distances of 1000 μm from the current source the error in the measurement λ_c is large (*see* Fig. 2 Ref. [1]) because of current back leak from the luminal cable to the cell cable. At distances less than 700 μm where electrotonic voltage deflections in the cell cable are much higher than the luminal cable, the error is not significant. At distances much greater than 900 μm or 3 times λ_c, according to Anagnostopolous et al., the current entering the cell cable is significant. However, at such distances the amplitude of voltage deflections for current strengths between 2.0 and 5.0×10^{-8} A becomes too small to be measured experimentally with accuracy.

In direct studies (Fig. 6) we have addressed the question of cable-to-cable interaction. Current was injected into the cell cable and electronic voltage deflections in the luminal cable and in the cellular cable were measured at the same distance along the tubule. If there is significant "cross talk" then the electronic voltage decay in the cell cable should reflect the decay of voltage in the luminal cable. However, the experiments illustrated in Fig. 6 indicate that the voltage deflections in the luminal cable between 250 and 750 μm are only a small fraction of the voltage in the cellular cable. We conclude that cross talk is not a problem in the

measurement of R_Z free-flow of proximal tubule of *Necturus* kidney.

Basolateral Cell Membrane Resistance
Oil-Filled Tubules

The basolateral cell membrane resistance can be measured directly from the intracellular spatial decay of voltage resulting from an intracellular current source in tubules with the lumen oil filled. A major assumption with the method for estimated R_{bl} directly is that filling the lumen with oil effectively increases the resistance of the luminal cell membrane during intracellular current injection. Continued transport of fluid by the tubule would contribute to the removal of fluid. However, the usefulness of this technique may be limited for the following reasons.

Firstly, it is difficult to ascertain that oil completely fills the lumen without leaving a conductive film between the oil droplet and the brush border of the cells. Removing a film of fluid from the brush border of the cell is difficult and could in turn cause a change in the thickness of cell layer, resulting in changes in R_{bl}.

Secondly, similar to many Na^+ transporting epithelia, the apical membrane of the *Necturus* proximal tubule is an important site of Na^+ entry into the cell. Removing Na^+ from the luminal solution of the proximal tubule in both the doubly perfused *Necturus* kidney and the isolated *Ambystoma* tubule preparation results in a reduction in intracellular Na [7, 17, 20]. The apical cell membrane of the proximal tubule is also an important site of glucose and amino acid transport. In the *Necturus* proximal tubule the addition of amino acids to the luminal solution increases intracellular Na^+ activity [7].

Thirdly, the perfusion of organic anion containing luminal solution in the *Necturus* proximal tubule increases the transepithelial resistances [8]. Likewise the addition of glucose to the perfusion solution of the newt kidney results in changes in transepithelial as well as in cell membrane resistances [14].

As a result, making the apical cell membrane inaccessable to electrolyte by filling the lumen of the tubule with oil must cause large changes in intracellular ion activities which could change the resistance of the basolateral membrane. Clearly, because filling the lumen of the proximal tubule with oil results in completely different physiological conditions from the other techniques mentioned above, it is probably the least desirable technique of estimating R_{bl}.

Resistance Ratio

The ratio of apical to basolateral cell membrane resistances was estimated in this study from the ratio of voltage deflection across the apical and basolateral cell membranes following current injection into the lumen of the tubule. The results indicated that the apical cell membrane represents 2.9 times higher resistance to current flow than the basolateral cell membrane. A similar situation is present in the rat proximal tubule which has an apical to basolateral cell membrane resistance of 2.1 [9].

Two potential problems with estimating the ratio of apical to basolateral cell membrane resistances from the ratio of apical to basolateral voltage deflections have been pointed out by Hoshi et al. [14] and by Boulpaep and Sackin [5]. Hoshi et al. [14] found in newt kidney that the voltage ratio is not constant but varies along the tubule. The variation with distance is particularly acute at distances of $<400\,\mu m$ from current source. At distances greater than $500\,\mu m$ the voltage ratio was independent of distance. In contrast to the newt proximal tubule, the results in both the rat [9] and the *Necturus* proximal tubules [1] suggest that the voltage ratio is independent of distance both near to and far from the current source. The apparent dependence of the voltage ratio, α, on distance from the current microelectrode results from the problem of cell cable and luminal cable interaction. When apical to basolateral voltage deflections resulting from transepithelial current injection are used to estimate the ratio of resistances, it is assumed that the voltage deflections measured across the basolateral cell membrane result from current entering the cell only by way of its own apical cell membrane. However, if λ_c is long compared to λ_l and if a considerable amount of current enters the particular cell from its neighboring cells, then a portion of the voltage deflection measured across the basolateral cell membrane of that cell with be the result of two components. This potential error is expected to be small in the measurement of α in the *Necturus* proximal tubule, since λ_l is about three times greater than λ_c.

In addition, the equivalence of the voltage ratio, α, and the resistance ratio, R_a/R_{bl} depends upon the assumption either that the lateral intercellular space is isopotential with ground or that the tight junction makes up the entire paracellular resistance. Boulpaep and Sackin [5] have pointed out that if the intercellular space resistance is appreciable compared to R_s there will be a potential drop along the interspace when current is injected into the lumen of the tubule resulting in α being an underestimate of R_a/R_{bl}. The magnitude of the discrepancy between α and R_a/R_{bl} was estimated in the isolated *Ambystoma* proximal tubules to be 28%. This information is not available at this time for the *Necturus* proximal tubule.

Individual Cell Membrane and Paracellular Resistances

As outlined in the Results four different experiments can be used to determine R_a, R_{bl} and R_s. However, transepithelial resistance is the only parameter which contains information on the shunt as well as the individual cell membrane resistances. Therefore, there are only three combinations of the four parameters which can be used to solve for R_a, R_{bl} and R_s (*see* Eqs. (8) through (11), and Table 2).

Each method has assumptions and experimental errors such that different combinations of parameters will result in somewhat different results. The largest variations, as illustrated in Table 2, are in the measured apical cell membrane resistance and the smallest variations are in the measured shunt resistances. From the discussion above, we believe that the best combination out of the three is to use the measurements R_{te}, R_Z free-flow, and R_a/R_{bl}. This combination is preferred over those which estimate R_{bl} directly in oil-filled tubules because filling the lumen with oil leads to uncertainties as to physiological status of the tubule. Indeed, in this study, the resistance of the basolateral cell membrane obtained from R_Z oil-filled experiments was higher than if R_{bl} was estimated from R_{te}, R_Z-free flow, and R_a/R_{bl} (Table 2).

Despite the differences in measured values R_a, R_{bl} and R_s the conclusion is the same; the combined cell membrane resistance R_a+R_{bl} is much greater than the paracellular resistance of the *Necturus* proximal tubule. It is this ratio $(R_a+R_{bl})/R_s$ which determines the degree of leakiness of the epithelium. Consequently, the paracellular pathway of the *Necturus* proximal tubule represents a low resistance shunt pathway and the cell membrane a high resistance pathway for ion movement. This finding supports the view that the *Necturus* proximal tubule is a leaky epithelium.

Cell-to-Cell Coupling

It is well established that the cells of many biological structures are interconnected by membrane channels with diameters between 16 and 20 Å in mammalian cells and between 20 to 30 Å in insect cells [21]. The existence of these channels in the *Necturus* proximal tubule can be inferred from the observations that current pulses injected into a cell of the proximal tubule can pass from cell to cell along the tubule (Figs. 3 and 7) and that the decay of voltage deflections in the cells along the tubule resulting from intracellular current injection behaves like a cable with a continuous cytoplasmic core bounded by insulating membranes (*see* Appendix).

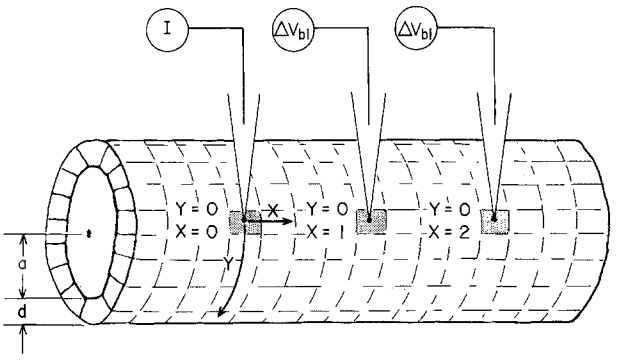

Fig. 7. Tubule model for double core infinite cable analysis. Inner tubule radius $=a$, cell thickness $=d$. The x axis is a coordinate within the cell wall, parallel to the tubule axis. The y axis is a circumferential coordinate in a plane perpendicular to the x axis. Current is injected from a point source in one cell at $x=0$, $y=0$. Voltage deflections ΔV_{bl} are recorded on the x axis at two distances from the current source $x=1$ and $x=2$

An estimate of the resistance of the cell-to-cell pathway can be obtained from the volume resistivity of the cell cable, ρ_c (Table 1), assuming (i) that the resistance of the cytoplasm is approximately equal to Ringer's solution (100 Ω cm); (ii) that the cell-to-cell junctional transition along the x axis (*see* Fig. 7) occurs in series every 20 μm; (iii) that there is no current flow along the y axis (*see* Fig. 7); and (iv) that the effective area can be represented by an annulus of area $\pi[(a+d)^2-a^2]$ without area amplification. The cell-to-cell junctional resistance calculated in this way will probably be an overestimate of the actual resistance since the volume resistivity of cytoplasm is most likely higher than Ringer's solution.

The estimated cell-to-cell junctional resistance is 14 Ω cm^2 where the specific resistance refers to the geometric area of the annulus perpendicular to the axis of the tubule. This is a surprisingly low value when compared to R_a, R_{bl}, and R_s (Table 2). However, low values for the intercellular junctional resistance are consistent with those reported for the *Drosophila* and *Chironomus* salivary glands which are in the range of 0.3 to 12 Ω cm^2 [18]. This points out that the cell-to-cell junction in *Necturus* proximal tubule represents a rather low resistance pathway for ion flow when compared to the resistance to flow across the apical and basolateral cell membranes.

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Appendix

The determination of individual cell membrane resistances in a cylindrical tubule relies on the assumption that the tubule can be treated as a double core infinite cable, consisting of an inner core constituted by the tubule lumen and an outer core constituted by an annulus of tubule cells which are all electrically coupled to each other. The annulus of tubule cells is seen as of uniform volume resistivity and bounded by an inner cylindrical insulating layer, the luminal or apical cell membrane, and by an outer cylindrical insulating layer, the pertibular of basolateral cell membrane. If one assumes, as shown in Fig. 7, a sheet of cells of thickness d wrapped into an infinite tube along the coordinate x, with an inner radius a, it is possible to describe the spread of current from a point source within that sheet located at $x=0$ and $y=0$, where x is a coordinate parallel to the tubule axis and y is the circumferential coordinate of a circular plane normal to axis x and having a radius $>a$ and $<(a+d)$.

In the extreme case of a perfectly insulated and flat sheet of cells, in the x and y plane, coupled to each other in all directions including x and y, but insulated from the outer solutions, the two-dimensional voltage spread in a radial direction $V(r)$ around the point source along an infinite flat sheet of cells is described by a solution of the following differential equation, written as the divergence of a gradient:

$$\nabla^2 V_{(r)} = -\left(\frac{\rho_c}{d}\right) S_{(r)} \tag{A1}$$

where ρ_c is the bulk volume resistivity of cell cytoplasm and lateral coupling resistances among cells in Ω cm, d is the thickness of the cell layer, $S_{(r)}$ represents the local intensity of current from externally applied source of current.

For all points outside the locus of injection $r>0$, and if there are no other sources except at $r=0$, the solution to Eq. (A1) is:

$$V_{(r)} = -V^* \ln\left(\frac{r}{r_0}\right) \tag{A2}$$

where V^* is a characteristic voltage determined by the point current source and r_0 is a characteristic radial distance from the source.

If the total amount of current applied is I^0 and I^r is the radial current density, the differential form of Ohm's law together with (A2) gives

$$I^0 = 2\pi r I^r = -2\pi r \left(\frac{d}{\rho_c}\right) \operatorname{grad} V_{(r)} = 2\pi r \left(\frac{d}{\rho_c}\right)\left(\frac{V^*}{r}\right). \tag{A3}$$

Hence

$$V^* = \frac{I^0 \rho_c}{2\pi d}. \tag{A4}$$

However, the epithelium is not perfectly insulated from its external solutions. As shown in Fig. 8, the proximal tubule epithelium can be represented by a double parallel array of resistors, apical cell membrane resistances R_a, and basolateral membrane resistances R_{bl} connected by a core bulk resistance R_c, illustrating both cytoplasmic and coupling membrane resistance. The tubule lumen resistance R_1 and peritubular outside solution resistance R_0 are considered negligible. If R_a and R_{bl} are finite, the parallel resistance of the two cell membranes is given by R_Z as defined in Eq. (9). Therefore everywhere along the sheet of cells there is an additional negative current source (current sink) so that the source term $S_{(r)}$ in Eq. (A1) is the sum of the total leakage of current from the cells to the external solutions $V_{(r)}/R_Z$ plus the current injected at point source S^0

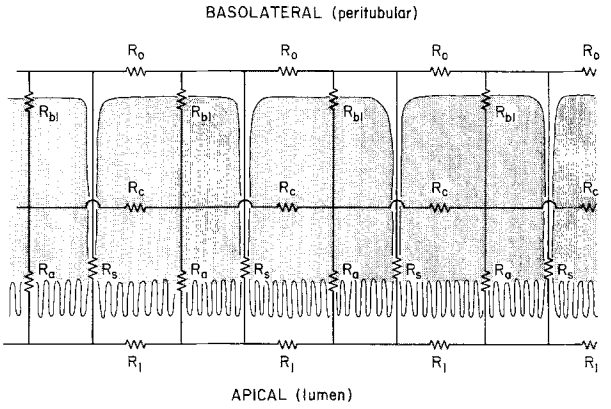

Fig. 8. Equivalent electrical circuit for the renal tubule cells with array of discrete resistor elements for each cell. R_a = apical or luminal cell membrane resistance; R_{bl} = basolateral cell membrane resistance; R_s = paracellular shunt resistance; R_c = lumped cytoplasmic and cell to cell coupling resistance; R_1 = luminal fluid resistance; R_0 = peritubular fluid resistance

$$S = -\frac{V_{(r)}}{R_Z} + S^0. \tag{A5}$$

Writing Eq. (A1) in rectangular coordinates x and y defined in Fig. 7 and substituting the source term from Eq. (A5) one obtains the two dimensional cable expression:

$$\frac{\delta^2 V}{\delta x^2} + \frac{\delta^2 V}{\delta y^2} - \frac{\rho_c}{R_Z d} V = -\left(\frac{\rho_c}{d}\right) S^0. \tag{A6}$$

By analogy with single-core cable analysis, an effective cellular length constant λ_c can be defined such that

$$\lambda_c^2 = \frac{R_Z d}{\rho_c}. \tag{A7}$$

Equation (A6) is a modified Bessel equation describing voltage distribution in a two-dimensional infinite flat sheet of cells. Since in the tubule the y coordinate shows a periodicity of $2\pi a$, the Fourier series solution for the simple case that all electrodes for current injection I^0 and voltage deflections ΔV_{bl} are on the x-axis (*see* Fig. 7) yields for the voltage spread along the axis x, when current is injected at $x=0$, $y=0$:

$$V_{(x,y=0)} = \frac{V^*}{2} \sum_{n=-\infty}^{+\infty} \frac{\exp -\left(\frac{x}{a}\sqrt{\left(\frac{a}{\lambda c}\right)^2 + n^2}\right)}{\sqrt{\left(\frac{a}{\lambda c}\right)^2 + n^2}}. \tag{A8}$$

The term V^* comes from the ideal case solution considered in (A4) where an infinite flat conducting sheet is perfectly insulated from its environment. It can be shown that if x/a becomes large, the higher order terms in Eq. (A8) decay faster with increasing x than does the $n=0$ term. Thus, in particular for $n=0$, Eq. (A8) can be approximated by

$$V_{(x,y=0)} = V^* \frac{\lambda_c}{2a} e^{-x/\lambda_c}. \tag{A9}$$

Substituting (A4) into (A9) yields:

$$V_{(x,y=0)} = \frac{\rho_c \lambda_c I^0}{4\pi a d} e^{-x/\lambda_c}. \tag{A10}$$

In practice, current is injected at $x=0$, $y=0$, and voltage deflections are recorded which are superimposed upon the resting membrane potential across the basolateral membrane, V_{bl}. Therefore the term ΔV_{bl}^x or the changes in basolateral membrane potential may be substituted for $V_{(x,y=0)}$ as stated in Eq. (4) of the methods.

A logarithmic plot of ΔV_{bl}^x against x provides enough information to obtain empirical values for λ_c and ρ_c using Eq. (A10). The slope of the plot is $-(1/\lambda_c)$. The intercept for $x=0$ is equal to an empirical value ΔV_{bl}^0 as stated in Eq. (4) of the Methods. From ΔV_{bl}^0 the value of ρ_c may be calculated as in Eq. (5) of the methods.

Finally, Eq. (A7) allows us to calculate from λ_c and ρ_c the total leakage conductance $(1/R_z)$ which is equal to $(1/R_a)+(1/R_{bl})$.

In conclusion, it should be noted that the following implicit assumptions have been made in the present treatment:

1) $d \ll R_z/\rho_c$, i.e., that the main limiting resistances are at the level of the insulating layer R_a and R_{bl}.

2) $d \ll a$, i.e., that the current spread, as in a prefectly flat sheet, is only two-dimensional along axis x and y and not along the thickness (radial axis of the tubule).

3) R_1 and $R_0 \ll R_c$, (in Fig. 8), i.e., that ρ_l, the resistance per unit tubule length of the lumen, or the resistance of the bath per unit length tubule is negligible compared to ρ_c the cellular cytoplasmic resistance per unit length tubule.

4) $\Delta V_{te}^x = 0$ along the entire tubule length, i.e., that the tubule lumen remains isopotential with the bath or that the shunt resistance R_s (in Fig. 8) is assumed to be small. *See* discussion for cable-to-cable interactions for the case of $\Delta V_{te}^x \neq 0$.

The above four assumptions taken together exclude from the mathematical treatment the distributed network of the lateral intercellular space which was described by Boulpaep and Sackin [5].

References

1. Anagnostopoulos, T., Teulon, J., Edelman, A. 1980. Conductive properties of the proximal tubule in *Necturus* kidney. *J. Gen. Physiol.* **75**:553–587
2. Anagnostopoulos, T., Velu, E. 1974. Electrical resistance of cell membrane in *Necturus* kidney. *Pfluegers Arch.* **346**:327–339
3. Boulpaep, E.L. 1967. Ion permeability of the peritubular and luminal membrane of the renal tubular cell. *In:* Transport und Funktion intracellularer Electrolyte. F. Krück, editor, pp. 98–107. Urban and Schwarzenberg, München
4. Boulpaep, E.L. 1972. Permeability changes of the proximal tubule of *Necturus* during saline loading. *Am. J. Physiol.* **222**:517–531
5. Boulpaep, E.L., Sackin, H. 1980. Electrical analysis of intraepithelial barriers. *In:* Current Topics in Membranes and Transport. E.L. Boulpaep, editor. Academic Press, New York Vol. 13, p. 169–197.
6. Boulpaep, E.L., Seely, J.F. 1971. Electrophysiology of proximal and distal tubules in the autoperfused dog kidney. *Am. J. Physiol.* **221**:1084–1096
7. Cemerikic, D., Giebisch, G. 1981. Intracellular Na^+ activity measurements in *Necturus* kidney proximal tubule cells. Abstracts of VIIIth International Congress of Nephrology. p. 71
8. Forster, J., Steels, P.S., Boulpaep, E.L. 1980. Organic substrate effects on and heterogeneity of *Necturus* proximal tubule function. *Kidney Int.* **17**:479–490
9. Frömter, E., Müller, C.W., Wick, T. 1970. Permeability properties of the proximal tubular epithelium of rat kidney studied with electrophysiological methods. *In:* Electrophysiology of Epithelial Cells. G. Giebisch, editor. pp. 119–148. F.K. Schattauer Verlag, New York
10. Giebisch, G. 1961. Measurements of electrical potential differences on single nephrons of the perfused *Necturus* kidney. *J. Gen. Physiol.* **44**:659–678
11. Grandchamp, A., Boulpaep, E.L. 1974. Pressure control of sodium reabsorption and intercellular backflux across proximal convoluted tubule. *J. Clin. Invest.* **54**:69–82
12. Guggino, W.B., Boulpaep, E.L., Giebisch, G. 1982. Electrical properties of chloride transport across the *Necturus* proximal tubule. *J. Membrane Biol.* **65**:185–196
13. Hegel, U., Frömter, E., Wick, T. 1967. Der elektrische Wandwiderstand des proximales Konvolutes der Rattenniere. *Pfluegers Arch.* **294**:274–290
14. Hoshi, T., Kawahara, K., Yokoyama, R., Suenaga, K. 1980. Changes in membrane resistances of renal proximal tubule induced by cotransport of sodium and organic solute. *Union Physiol. Sci.* **14**:479
15. Hoshi, T., Sakai, F. 1967. A comparison of the electrical resistances of the surface cell membrane and cellular wall in the proximal tubule of the newt kidney. *Jpn. J. Physiol.* **17**:627–637
16. Kempton, R.T. 1937. The dimensions of the renal tubules of *Necturus maculosis*. *J. Morphol.* **61**:51–58
17. Kimura, G., Spring, K.R. 1979. Luminal Na^+ entry into *Necturus* proximal tubule cells. *Am. J. Physiol.* **236**:*F*295–*F*301
18. Loewenstein, W.R., Socolar, S.J., Higashino, S., Kanno, Y., Davidson, N. 1965. Intercellular communication: Renal, urinary bladder, sensory, and salivary gland cells. *Science* **149**:295–298
19. Lutz, M.D., Cardinal, J., Burg, M.B. 1973. Electrical resistance of renal proximal tubule perfused *in vitro*. *Am. J. Physiol.* **225**:729–734
20. Sackin, H., Boron, W., Boulpaep, E.L. 1980. Intracellular sodium activity in *Ambystoma* renal proximal tubule. (*Abstr.*) *Kidney Int.* **19**:148*A*
21. Schwarzmann, G., Wiegandt, H., Rose, B., Zimmerman, A., Ben-Haim, B., Loewenstein, W.R. 1981. Diameter of the cell to cell junctional membrane channels as probed with neutral molecules. *Science* **213**:551–553
22. Windhager, E.E., Boulpaep, E.L., Giebisch, G. 1966. Electrophysiological studies on single nephrons. *In:* Proceedings of the Third International Congress of Nephrology, Washington D.C. G.E. Schreiner, S. Karger, editor. Vol. 1, pp. 35–47. Basel — New York

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