Electrophysiological Techniques in Kidney Micropuncture

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Electrophysiological techniques have been widely used for the investigation of membrane properties in excitable and inexcitable tissues. The application of such methods to epithelia has been extensive, particularly for structures that can be mounted as an isolated tissue sheet in special chambers designed to control the bathing solutions. With respect to the study of the renal epithelium, electrical methods combine classical micropuncture approaches with intracellular microelectrode techniques. The anatomy of the tubular epithelium imposes a number of special problems. First, access to both boundaries of the tubular cell layer demands the use of special perfusion techniques. Second, the maintenance of an optimal stability of the tubular wall is a matter of serious concern. Finally, the use of microelectrodes raises problems of exact localization of the electrode tip within the tubular epithelium.

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2 If one considers Ringer's or Tyrode solution to be a 0.1 or 0.14 M NaCl solution, and such fluid is brought into contact with another uni-univalent electrolyte at the same concentration C and having one ion in common, the following equation applies providing both electrolytes can be considered completely dissociated:

\[ E_L = \frac{RT}{F} \ln \frac{\Lambda^1}{\Lambda^2} \]

where \( E_L \) is the standard liquid junction potential, \( RT/F \) at 25° equals 25.677 \( 10^{-3} \) V, and \( \Lambda^1, \Lambda^2 \) are either the limiting equivalent conductances \( \Lambda \) for salt 1 and 2, or more correctly the equivalent conductances \( \Lambda \) at the given concentration \( C \).

Using for NaCl, Choline Cl, and Na propionate the following values of \( \Lambda_c \): 126.45, 115, 85.9 ohm\(^{-1}\) cm\(^2\) equiv\(^{-1}\), respectively, one calculates at 25° for a typical junction where a cation substitution is performed in the bath, e.g., a junction NaCl: Choline Cl \( E_L = 2.4 \ 10^{-3} \) V.

For a typical anion substitution, e.g., the junction NaCl: Na propionate one obtains \( E_L = 9.9 \ 10^{-3} \) V.

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The first problem, i.e., accessibility to both epithelial surfaces, luminal and peritubular cell boundaries, bears on two different considerations. First, adequate methods of visualization must be used to expose the nephron for electrical measurements. Second, the luminal and peritubular fluid compartments should be controlled in such a way that its composition can be modified. Kidney preparations that offer good anatomical accessibility for transepithelial electrical measurements are essentially those which have been used successfully in micropuncture(1). For the visualization of single cells, because of their large size, kidney preparations of amphibian species such as *Necturus maculosus*(2) or *Amphiuma* offer special advantage. An abundance of distal tubules in the ventral surface of the kidney distinguishes the *Amphiuma* kidney(3). Since several electrical parameters are critically dependent on the chemical concentration gradient across the tubular epithelium, the perfusion of both the lumen and the peritubular blood capillaries with solutions of known ionic composition is of considerable interest. Concerning amphibian kidney preparations their dual blood supply makes possible the separate control of the luminal and peritubular fluid in doubly perfused kidneys where separate solutions perfuse the aortal and portal circulation(1,2). Superfusion of the kidney surface with solutions of composition similar to that in the capillaries assures an external area of adequate size to make a reliable electrical contact with a large reference electrode. In the mammalian kidney, the development of peritubular capillary perfusion techniques has made possible extensive control of the extratubular fluid composition(4). Again, a small fluid layer covering the kidney constitutes a surface reference bath. These techniques are satisfactory for cortical segments of the nephron since the generation of liquid junction potentials at the interface of bath to interstitial fluid can be avoided. For studies on renal papillary structures the reference bath should ideally be the interstitial fluid or the fluid within the vasa recta at the level along the medullary axis where electrical measurements are made. Alternatively it is desirable to correct the measured potential difference for the asymmetry potentials arising at the interface between fluid on the papillary surface and the extracellular fluid within the papilla if the composition of this fluid is known(5). It should be noted that the actual ionic concentrations at the level of the peritubular membrane, i.e., within the basal labyrinth can still be different from the capillary or interstitial composition if a Donnan distribution is approached across the basement membrane. In general, extensive control of the luminal fluid composition is achieved by means of continuous microperfusion or stationary microperfusion techniques (see papers on microperfusion from this workshop). Continuous perfusion techniques at free flow pressure offer an advantage if it is desirable to maintain transepithelial concentration differences which are different from equilibrium values.

Stability of the electrical measurements is an obvious prerequisite for accurate determinations of potential or resistance values. Mechanically, the kidney surface of amphibian kidneys is usually very stable but mammalian kidneys, particularly large kidneys such as dog kidneys, are quite unstable since respiratory and pulsatile movements interfere with reliable determinations of potential differences.
Thus, in the dog kidney in vivo, pulsatile displacements of the kidney are often of the order of several tubular diameters. Microelectrodes do not offer enough mechanical resistance to immobilize a small area around the pipette and frequently serious damage to the epithelium results from movements of the kidney surface. Whereas means of immobilization are fairly effective against respiratory movements, several methods of immobilization such as the use of agar gels on the kidney surface, slight compression with a ring(6), or the use of a double cup(7) have proven to be unsatisfactory in our laboratory to adequately control pulsatile movements. The development of an isobaric autoperfused dog kidney preparation afforded a solution to this problem. As shown in Fig. 1, the isolated dog kidney is perfused in a system that completely abolishes the pulse pressure in the arterial circuit. Autoperfusion is preferred over a pump-perfused system because it assures a better survival of the kidney preparation. Arterial blood passes from the carotid artery through a depulsator at 37°, the latter being connected to a damping reservoir maintaining a constant perfusion pressure. The venous blood returns by gravity to the jugular vein of the animal. Pulsatile movements at the surface of the kidney were completely abolished. The isolated kidney is imbedded in agar, leaving only a small area of about 1 cm² accessible for micropuncture. Tyrode solution is continuously dripped into a small well made up by the surrounding Tyrode–agar gel, which serves as a convenient return lead. Assessment

![Diagram of the method used for mechanical stabilization of the dog kidney by means of isobaric autoperfusion. Arterial blood passes from the carotid through a depulsator at 37° connected to a damping reservoir maintaining a constant perfusion pressure. The isolated kidney is held in a cup and imbedded in agar leaving only a small area accessible for micropuncture (taken from Ref. 6).]
of the renal function of such preparations with respect to overall hemodynamic and excretory parameters as well as with respect to proximal tubular function indicates that for 3 hours the performance of these preparations remains well within normal limits(6). Excellent mechanical stability is achieved in this autoperfused kidney preparation and minimizes the possibility of mechanical damage and leaks at the impalement site. Concomitantly the stability of electrical potential measurements is greatly improved(8).

The problem of electrical stability of the recording system itself is determined mainly by the type of electrode arrangement used for potential and resistance measurements. Essentially, the precautionary measures are identical to those of similar electrode systems in other tissues. Briefly, any extraneous electrical asymmetry should be avoided or appropriately canceled in the circuit. Figure 2 illustrates the arrangement of different electrode and diffusion potentials in series with the actual membrane or transepithelial potential to be determined at the interphases 6 and 7. One distinguishes in Fig. 2 at level 1 and 2, or 10 and 11 electrode potentials and at all other levels, except possibly across the cell membrane itself, diffusion potentials or concentration cells with transference.

(1) Each measurement involves at both ends a pair of electrode potentials arising at the transition between the solid and aqueous phase, i.e., at the junction between a metal electrode and any solution brought into contact with either saturated KCl or 3 M KCl solution of either the reference bridge or the fluid-filled microelectrode. If care is taken to arrange these metal–liquid junctions symmetrically, potential differences 1–2 cancel 10–11 and good stability can be expected at that interphase over several hours.

(2) Liquid junction potentials may arise at the level where a concentrated (e.g., 3 M KCl) solution makes contact with relatively diluted biological fluids of a different composition: (a) At the side of the large reference electrode, either at level 3 or level 4 (Fig. 2), depending whether the bridge is filled with the concentrated KCl solution or with Ringer’s. The experimental potential difference at that junction point might not agree closely with the theoretical value for a KCl bridge. Indeed, in static junctions of the type shown in Fig. 2, one solution is immobilized with agar and the two solutions at the interface are not sufficiently stirred. The profile of concentration at level 4 does not stay constant with time and changes in junction potential could occur. It has been suggested to use either

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|   |   |   |   |   |   |   |   |   |   |   |
|---|---|---|---|---|---|---|---|---|---|---|
| Ag | AgCl | Hg | Hg2Cl2 | 3 M KCl | 3 M KCl-agar | Ringer-agar | Bath | Peritubular Interstitial | Epithelium Cell membrane | Lumen | 3 M KCl | 3 M KCl | KCI | AgCl | Ag |
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Fig. 2. Schematic representation of the chain of electrode, diffusion and membrane potentials during measurements of transepithelial or transmembrane potential (for explanation see text).
salt dilution or biionic potentials as a junction for that purpose. However, actually only small junction potentials are found so that junction 3 cancels 9, and 4 should cancel 8 (see below). Thus, except for long-term potential measurements which are usually not performed in kidney tubules the stability of KCl reference electrodes can be considered adequate, when kept positioned in the same bath solution. To the contrary, special precautions are mandatory when the surface solution in which the reference bridge is located, is changed during the course of electrical measurements. Changes in ionic strength of the solution are particularly critical. Therefore a common procedure is to use at junction 4 (Fig. 2) a biionic static junction consisting of two solutions of salts having either the anion or the cation in common. Such junctions are more independent of the junction concentration profile and thus of time. In substitutions of single ions in the bath it is satisfactory to use a Ringer’s-agar bridge instead of a KCl agar bridge, and to compute the standard liquid junction potential for each single ion substitution. Indeed the potential at junction 4 is now certainly different from that expected at junction 8. An intermediate solution, whenever it is required to work within a bath of a very small volume (see Fig. 5), is to use a rather large 3 M KCl microelectrode as a reference where the junction 4 can be considered to be flowing. The procedure then consists in comparing the difference in junction potential at 4 between the condition of either a Ringer’s-agar macrobridge or a 3 M KCl microelectrode as reference. The discrepancy in asymmetry should be reasonably close to the standard junction potential (see footnote) between Ringer’s and the bath solution. (b) Contrary to the general rules outlined above for reference electrodes, the liquid junction potential at the tip of a Ling–Gerard microelectrode (junction 8) does not behave as a KCl bridge and often generates an asymmetry referred to as the tip potential. The tip potential is not constant in solutions of different ionic composition. This leads to a nearly insoluble problem for cellular impalements since it is neither possible to know exactly the ionic composition of the cell nor to control it artificially. The suggestion is made to select electrodes with low tip potential when tested in a solution similar to the cytoplasm, since it is likely that low tip potentials (<5 mV) change less during cellular impalements. Concerning transepithelial impalements no problems should arise if the intraluminal and peritubular fluids are nearly identical, as in the proximal tubule, or if both fluid phases are made similar by simultaneous luminal and peritubular perfusion with identical fluids. In all other instances in which a solution of different composition is used in the lumen a possible change of the magnitude and constancy of the tip potential should be checked when the electrode tip is moved from one solution to the other. A special case of inconstant behavior of the tip potential during tubular impalements independent of changes in the external solution has been ascribed to reversible contamination of the tip with foreign material (e.g., tubular debris during impalement). This is discussed further in this workshop.

(3) Liquid junction potentials may be present between different biological fluids separating the electrodes from the epithelium (junction 5, Fig. 2). This can be the case if the reference electrode is brought into contact with a reference bath
that is of an entirely different composition than the fluid within peritubular capillaries or interstitium. This difficulty arises during electrical measurements on renal papillary structures. Most appropriately the potential difference should then be determined between the renal interstitium (vasa recta) and the tubular structures of the medulla. In principle, the same criticism is valid concerning any measurements of peritubular membrane potentials or transepithelial potentials since the ionic composition within the basal labyrinth or within the interspaces may be different from that of the extracellular fluid with which the reference electrode makes contact. Accordingly, such uncertainty cannot be entirely solved by the simple correction of plasma electrolyte values for the full Donnan effect of plasma proteins or by assuming ionic concentrations determined from ultrafiltration of plasma through artificial membranes.

Precise localization of the microelectrode tip within the renal epithelium is another important problem. Since different electrical parameters are expected to obtain depending on either intracellular and intraluminal recording sites, and a microelectrode tip of about 1 \( \mu \) size cannot be seen under the light microscope several means of tip localization have been used. Only the gross position of Ling–Gerard microelectrodes within several microns can be assessed directly, making use of the refraction of light on the wider parts of the microelectrode shank. For proper localization of the tip itself one should resort to additional, albeit indirect means.

1) The most obvious method of localization is the use of the potential profile itself once it has been established(2,12). Providing the potential steps across the epithelium are quite discrete and appreciably different from zero a continuous recording of the electrical potential difference between microelectrode tip and surface of the kidney is performed as the microelectrode is advanced along the radius of the tubule (see Fig. 7). At first the peritubular membrane potential is recorded between extracellular fluid and cell. Subsequently, when the tip of the microelectrode breaks into the lumen, the reference electrode remaining on the surface of the kidney, the total transepithelial potential is observed. Intracellular and intraluminal localization can thus be distinguished provided that the cellular measurement is sufficiently stable, a condition only satisfied in large amphibian tubule cells. The mere correlation between mechanical advancement of the microelectrode tip and the geometry of the different structures or other mechanical maneuvers(13) is inadequate in the absence of the recording of discrete potential steps. Due to the elastic properties of connective tissue surrounding the tubule it is sometimes possible that the electrode tip displaces the tissue over a distance of several tubular diameters without any actual cellular or luminal impalement. An exception is of course the visualization of the shank of a large electrode within the tubular lumen.

2) Another type of profile obtains during the continuous recording of the input resistance as the microelectrode tip advances(8,12). Considering the size of single cells as compared to the geometry of the tubule larger input resistance values are usually obtained from cellular than from intraluminal localizations. A ratio of two or three of cellular over transepithelial input resistance exists.
This may be due to two factors: (i) If the specific resistance of cellular and transepithelial membrane material was the same, identical current inputs into a small cell structure would lead to a higher current density and therefore higher voltage deflections. (ii) The presence of large shunt pathways across the proximal tubular epithelium at least, can potentially lower the specific resistance of the epithelium below that of single cell membranes. Such measurements can be done (see below) either by means of a single microelectrode in a bridge circuit(12), or utilizing a similar arrangement allowing for current injection and potential recording through a single barrel. Such measurement can also be obtained by using double-barreled electrodes in which one barrel is used for current injection and the other for voltage recording (see below)(8,14). Both methods are limited by errors due to changes of the electrode resistance in series with the tubular structure during impalement. In Necturus kidney the total values of input resistance are sufficiently high so that they can still be adequately distinguished from possible changes in electrode properties(15). The much lower values of the transverse resistance across the mammalian, particularly the proximal tubule(8,14), make the distinction between tissue resistance profile and possible electrode artifacts difficult.

(3) A method involving the use of the tubular resistance profile without actually determining the absolute value of the input resistance is often practical during multiple impalements of the tubular lumen with more than one microelectrode. An appropriate example, for instance, is the measurement of cable properties in tubular structures (Fig. 4, top). Separation of the current and voltage barrel by several micra is sufficient to avoid the problems of variable electrode resistance or coupling resistance. With one electrode already placed in the lumen and current flowing between the tip of that electrode and the surface it is possible to assess the location of additional microelectrodes by the amplitude of the electronic potential spreading along the tubule and picked up by each of these electrodes subsequent to current application via the first electrode(8,14,15). The degree of coupling between the two electrodes will be better if both are located within the lumen of the tubule. A combination of this localization procedure with that mentioned under no. 4 has tested the adequacy of this method(8).

(4) A very reliable method for the intraluminal localization of the microelectrode tip is the observation of changes in the magnitude of the transepithelial potential difference subsequent to luminal microperfusion with test solutions of altered Na+, K+, Cl- concentrations (Fig. 3, top)(8). It is easy to demonstrate that, depending on the position of the microelectrode within a cell or within the lumen, different potential changes would be expected. For instance, if both the entire epithelium and individual cell membranes are less permeable to choline than to sodium, perfusion of the lumen with choline–Ringer’s (zero Na) would render the lumen more positive, if the transepithelial potential difference is treated as a biionic potential. On the other hand, the peritubular membrane potential should remain unaffected by the luminal perfusion, unless intracellular concentration changes occur or some small amounts of the luminal perfusion solution leak to the surface. If the electrode tip were located within the tubule cell,
Fig. 3. Methods for the study of the transepithelial potential difference and the transepithelial permeability characteristics. Top, luminal microperfusion for localization of the tip of the microelectrode and applying changes in luminal composition. Bottom, peritubular capillary and luminal microperfusion for imposing ionic concentration or osmotic gradients across the epithelium (taken from Ref. 8).

one would predict a hyperpolarization of the peritubular membrane potential, i.e., the electrode would become more negative because choline ions are less effective than sodium ions in shunting the transmembrane potential at this site.

(5) A similar experimental arrangement using intraluminal microperusions could be performed in which changes in input resistance are used as an indication of the tip localization. Given the insensitivity of the input resistance measurements, solutions of very different resistivity should be used. The method has also been used such that continuous measurements of input resistance are carried out while an intraluminal oil droplet of high resistivity is passing along the site of impalement(16). A sudden increase in the recorded resistance value signals the intraluminal position of the electrode tip. Since thin tubular fluid layers can be present in the brush border of the luminal membrane, the tip of the electrode may not always be surrounded by the oil droplet. Moreover an intracellular microelectrode might also detect the passage of the oil droplet as a small increase in input resistance, if the luminal cell membrane suddenly is covered with a nonconducting fluid. Accordingly, the results of this localization procedure have to be evaluated with caution.

(6) Deposition of substances from the tip of the electrode is quite a reliable method of localization and can take three different forms.

(a) Fluid ejection from the tip of the electrode under high pressures(17). The fluid is colored and remains confined as a small spot of dye when injected into a single cell, while diffuse coloration of the tubule lumen results from intratubular localization. The disadvantage of the method is that the dye can hardly
be considered physiological and that the same cell cannot necessarily be used further in the experiment. Also displacement of the electrode tip due to the high ejection pressure is relatively frequent. In the case of a luminal localization the dye can easily be washed away by the ultrafiltrate or by microperfusion but fairly large amounts of dye need to be ejected from the tip for adequate visualization.

(b) Iontophoretic deposition of a dye such as, e.g., lithium carmine has been used for intracellular localization(18). This is quite a reliable method but necessitates fixation of the tissue and subsequent microscopic work-up. Also, it does not allow for an immediate choice regarding the use of a particular tubular impalement site.

(c) A more reversible technique is provided by electrophoretic injection of ions, either ion species to which the membrane is permeable or ions which can be pumped out. This ensures the ability of the cell to recover its initial condition. Such a method exploits the occurrence of transient potential changes after ions have been injected into the cell. Its application to single proximal tubule cells of Necturus is described in another paper of this workshop(19).

(7) Finally, the tip of the microelectrode can be coupled to a sensor with respect to the ionic activity differences between the different compartments of the epithelium. Combining a Ling–Gerard microelectrode with an ion-selective barrel enables one to test the localization of the microelectrode tip according to the activity of specific ions. Electrodes selective to ions which are most strikingly different in the intracellular and extracellular medium, e.g., K+ are most appropriate. The manufacture of K-sensitive microelectrodes has been described in this symposium(20,21). Intracellular activity determinations have been performed in distal tubule cells in combination with determinations of membrane potential(20). In the proximal tubule with larger differences between luminal and cellular K activities, this method of localization would even be more promising.

Contrary to Ling–Gerard microelectrodes, larger electrodes can usually be positioned by means of direct visualization. Such is the case with relatively large sharpened micropipettes inserted across the tubular wall(5,22). Large pipettes can also be inserted axially into single isolated tubules as shown in this workshop(23,24). Appropriate means of insulation around the pipette are necessary. Finally, axial metal electrodes can also be inserted under microscopic observation in split-drops in proximal tubules of Necturus. Adequate electrical insulation is then obtained from the two oil-blocks on either side of the split-drop(25).

In the following a brief review is given of the different types of electrical measurements that can be performed. They can be conveniently divided into measurements across the total epithelial cell layer (transepithelial measurements), and into measurements performed on individual tubular cell membranes, i.e., measurements across the peritubular and luminal cell membrane.

(1) Transepithelial potential measurements

The transepithelial potential difference is measured as illustrated in the top section of Fig. 3. Measurements are made between a single microelectrode impaling the lumen and a reference electrode on the surface of the kidney. A
double-barreled microperfusion pipette can also be introduced into the lumen in order to perfuse the latter with either control Ringer's or various test solutions. In this manner localization procedure no. 4 is performed and simultaneously the effect of different ionic composition in the lumen on the potential difference can be tested. Combination of the luminal perfusion with peritubular capillary perfusion (4) as shown in the lower part of Fig. 5 makes possible the complete control of the fluid environment on the two sides of the epithelium. The following experiments have been carried out: (a) Proximal transepithelial potential changes have been determined under different transepithelial salt gradients by replacing in either lumen or peritubular compartment both the cation and anion of various salts for which the epithelium is permeable. The results of such study are then treated as an ordinary liquid junction potential assuming a single-diffusion barrier. This provides a measurement of the relative mobility of cations versus the anion across the epithelium (8). (b) Potential changes can also be observed when single ions of the control solution are substituted by less permeant species. Relative permeability coefficients of the substituting ion with respect to the substituted ions can be calculated from the observed potential displacements (8). (c) Potential changes subsequent to transepithelial osmotic gradients have also been determined by this technique, establishing the existence of streaming potentials across the mammalian proximal tubule (8).

(2) Transepithelial resistance measurements

Two different techniques have been applied to assess the total transepithelial conductance. The first one illustrated in Fig. 4 (top) is usually referred to as cable analysis (8,14,15). This method considers the wall of the tubule as the insulating

![Fig. 4. Method for the study of the transepithelial resistance. Top, arrangement of microelectrodes for determination of the length constant in cable analysis combined with microperfusion for localization of the microelectrodes. Bottom, measurement of the relative conductance by means of transepithelial input resistance measurements and control of the chemical gradient in both luminal and peritubular compartments (taken from Ref. 8).]
layer of an infinite cable surrounded by a single uniform core constituted by the tubular fluid. A double-barreled infusion pipette is inserted to achieve appropriate changes in the composition of the tubular fluid. Several microelectrodes, one double-barreled and additional single-barreled electrodes, are inserted at some distance apart into the same tubule. With microperfusion of the tubule with, e.g., a choline–Ringer’s solution the intraluminal portion of all electrodes is checked as manifested by simultaneous potential changes recorded at each site. Current signals are subsequently passed through one of the barrels of the double-barreled electrode at distance zero. The voltage wave spreading along the tubule is recorded by a single-barreled electrode \( V_1 \). This electrode is then withdrawn and reinserted somewhat closer or further away to the current injection site. An alternative consists in leaving different single-barreled electrodes \( V_1 \) and \( V_2 \) in place and record all deflections at the same time. The first method had the disadvantage that damage to the wall brought by the first impalement of \( V_1 \) could affect the second reading with \( V_1 \) at the distance 2. The sequence of impalement should therefore be adequately randomized. The second method is technically more difficult since its success depends on the maintenance in place of four individual pipettes in a rather short length of tubule. Also, it is difficult to avoid that the insertion of one pipette induces movement of the preceding pipette and possibly damage of the tubular wall. Since for a given current injection changes of the absolute voltage at the injection site can vary with time (due possibly to progressive damage of the tubular wall at the site of impalement of the double-barreled micropipette), the measurements of voltage deflection at both \( V_1 \) and \( V_2 \) should be obtained while for a constant current injection, identical voltage deflections are measured at \( V_w \), i.e., the injection site. At least two electronic voltage deflections should be recorded along the same tubule for the determination of the length constant of the tubule. By means of multiple impalements, linearity of the plot of the voltage deflection against distance has been shown\(^\text{14,15}\).

It should be noted that retropulation of the voltage deflections found at various distances along the tubule to zero distance on a plot of the \( \log dV \) against tubular length does not consistently show an extrapolated value similar to the observed voltage deflection at distance zero, even after appropriate correction for the coupling resistance of the electrode. The origin of this deviation has been discussed elsewhere\(^\text{15}\). Since the actual deviation is poorly predictable the slope of \( V \) as a function of distance cannot be predicted from only two impalements, i.e., one at distance zero, and the other at \( V_1 \). From the length constant of the cable (derived from the voltage attenuation to \( 1/e \)) the radius of the tubule and the resistivity of the solution within the tubule, it is possible to calculate the specific transverse resistance of the wall \( (R_w) \) expressed in ohms cm\(^2\).

Another approach is the determination of the input resistance by means of one double-barreled microelectrode. Since current not only flows across the epithelium, but also across a fraction of the electrode resistance in series with both the voltage and current barrel, a part of the voltage deflection is due to the so-called coupling resistance. This component should be kept minimal for the given current used by the choice of appropriate microelectrodes. The voltage
deflection due to this artifact must be subtracted from the total value. Also, constancy of the coupling resistance during impalement is an obvious prerequisite for accurate resistance measurements but no proof exists that this condition is fulfilled. It can only be inferred from a good correspondence between the coupling resistance before and after impalement and from a satisfactory agreement among different transepithelial measurements with different microelectrodes. The input resistance, expressed as the ratio of the voltage deflection over the injected current, does not allow a correct calculation of the specific resistance of the wall $R_m$ except when no deviation from linearity exists at tubular distance zero. However, as a first approximation $R_m$ is proportional to the square of $R_{\text{input}}$. Use of this relationship has been made in order to assess the relative conductance changes during alteration of the ionic composition of fluids in either lumen or peritubular capillaries (Fig. 4, bottom). This was accomplished by the simultaneous perfusion, with a double-barreled pipette, of the lumen and by means of a single barrel, in the peritubular capillaries. Thus, changes in transepithelial input resistance have been assessed on the same tubular segment when a particular ion species was omitted on both sides of the membrane and replaced by a less permeant one(8). From these data it is possible to compute the ratio of the transepithelial conductance, e.g., in the presence and absence of chloride ions. Accordingly, such measurements can be used for the estimate of the partial ionic conductance of the tubular epithelium to a specific ion. In a similar way relative conductances have been evaluated during the imposition of osmotic gradients(8).

3) Peritubular membrane potential and cellular input resistance measurements

Figure 5 illustrates a technique for the determination of the peritubular membrane potential. The surface of the kidney is superfused by means of a double-barreled pipette, and adequate control of the ionic environment is obtained in the area surrounding the microelectrode. In control conditions the reference electrode can be constituted by the usual Ringer’s–agar bridge in contact with the surface at some distance from the impalement site. Electrical measurements of cell potentials during fast changes in ionic concentration in the peritubular fluid compartment require a different technique(12,26). When the ion concentration was changed on the surface in order to avoid artifactual voltage transients due to the different time for diffusion of the test solution to the impaled cell membrane and to the reference electrode, a second microelectrode is used and placed in the immediate neighborhood of the impaled cell. In this manner the recorded potential changes during rapid ion substitutions reflect the true time course of both the membrane potential change and the diffusion delay due to unstirred layers between bath and peritubular membrane. Membrane potential changes during various ionic substitutions have been used for the assessment of the permeability properties of the peritubular membrane(12,26). In Fig. 5 is also shown the method for determination of the peritubular input resistance, which more properly should be considered an estimate of the overall cellular input resistance considering the presence of a low-resistance paracellular shunt pathway between cells(27,28). This method can be performed by means of a double-barreled electrode(15,27) where the voltage deflections are measured subsequent
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FIG. 5. Methods for the study of the peritubular cell membrane. Top, measurement of the peritubular membrane potential with method of superfusion for fast changes of the peritubular ionic composition. Bottom, measurement of the peritubular (or cellular) input resistance by means of either a single microelectrode and a bridge, or by means of a double-barreled microelectrode.

to current application and $R_{\text{input}}$ is expressed as the ratio $dV/dI$. Alternatively, a high impedance bridge makes possible, as shown in Fig. 5, the same measurement with a single electrode(12), where the current $dI$ across the membrane is the bridge current and $dV$ are the membrane potential deflections. It is important to note that any change in the microelectrode resistance during impalement will appear as a resistance change in series with the membrane resistance and measured as such. Hence, the electrode resistance should remain constant before and after impalement.

The peritubular input resistance is not suitable for an accurate calculation of the specific resistance of the peritubular membrane since the kidney cells cannot be considered as separate from adjacent cells(28) and current flow is not limited to the peritubular surface. A low-resistance path between tubule cells provides a route of current flow between peritubular and luminal membrane along intercellular interspaces(12,27,28). These factors make it impossible to accurately assess the area across which current flow takes place. Assuming no changes in the degree of coupling between neighboring cells, the $R_{\text{input}}$ of the peritubular membrane can be used as a measurement to assess the relative changes in conductance when the peritubular ionic fluid composition is altered(12). As such these measurements provide a confirmation of permeability characteristics deduced from membrane potential changes.

(4) LUMINAL MEMBRANE POTENTIAL MEASUREMENTS

In order to determine accurately the luminal membrane potential, at least two microelectrodes are necessary—one impaling the lumen and the other impaling
the cell. Figure 6 illustrates the arrangement of the electrodes for simultaneous measurements of $V_1$, the peritubular membrane potential, $V_2$, the luminal membrane potential, and $V_3$, the transepithelial membrane potential. A double set of double-barreled pipettes controls the composition of either the luminal or peritubular fluid. During the changes in the chemical concentration gradient across the luminal membrane it is necessary at any time to record with $V_2$ simultaneously either $V_1$ or $V_3$. Indeed, with two cellular membranes in series but shorted by a paracellular low-resistance pathway it has been shown that apparent changes in $V_2$ are possibly artifactual if during the same time the transepithelial potential would have changed, e.g., due to progressive leaks around the intraluminal electrode. Accordingly, a complete short-circuit of the epithelium would reduce the luminal membrane potential measurement to a measurement of the peritubular membrane potential. A continuous recording of both the luminal and peritubular membrane potential is shown in Fig. 7, where the top tracing is the potential difference between the microelectrode positioned in the cell, $M_1$, and the microelectrode placed in the lumen, $M_2$. The lower tracing is the potential difference between $M_1$ and ground, i.e., the surface of the kidney. Figure 7 also illustrates the sequence of possible impalements of the different electrodes, the reference or ground electrode not being shown. The successive potential steps are recorded as either electrode is moved across the epithelium. From the discussion it is clear that changes in the luminal membrane potential during changes of ionic gradients provide us with a tool for assessing the permeability characteristics of that membrane(12, 29).

(5) Individual cell membrane resistance measurements

As mentioned earlier, input resistance measurements on single cells are inadequate for the assessment of the specific resistance of the individual cell borders. A first approximation of the relative resistance of the peritubular versus the luminal membrane can be obtained from estimates of the ratio of the voltage deflection across either membrane, when current is passed across the entire epithelium(27). For calculation of the absolute specific resistance, an approach as illustrated in Fig. 8 can be attempted. Here a double-barreled electrode is in-

![Fig. 6. Method for the study of the luminal membrane potential, with simultaneous recording of $V_1$ = peritubular membrane potential, $V_2$ = luminal membrane and $V_3$ = transepithelial potential difference.](image-url)
Fig. 7. Simultaneous recording of the luminal and peritubular membrane potential. Top, cross section of a proximal tubule and sequence of impalement of microelectrodes $M_1$ and $M_2$. The third reference electrode on the surface of the kidney is not shown. Middle, $M_1$-$M_2$: Differential input of the potential difference between microelectrode $M_1$ and $M_2$. First, the transepithelial potential is noted with impalement of $M_2$, showing a transient spike due to the fast advancement of the microelectrode through a cell, indicating the higher value of the peritubular membrane potential. The subsequent impalement of $M_1$ yields a potential difference equal to the luminal membrane potential. Bottom, potential difference of $M_1$ with respect to reference surface electrode measures the peritubular membrane potential of the same cell of which the luminal potential is shown above. (taken from Ref. 12)

Fig. 8. Method for the determination of the specific resistance of the individual cell membranes by means of the double-core cable analysis. Top, determination of the intraepithelial "length constant" when the lumen is filled with Ringer's. Bottom, identical procedure when the lumen is filled with oil yields a longer intraepithelial "length constant."
sersed in the tubular cell layer, allowing for injection of current between the two cellular membranes. Another single-barreled microelectrode $V_1$ detects the electronic potential invading the neighboring cells, provided a sufficient degree of cell-to-cell coupling exists. The electrode is then withdrawn and reinserted at a different distance $V_2$ from the current injection electrode. The amplitude of the potential deflections decreases with distance and an intraepithelial "length constant" can be estimated from the plot of the logarithm of the voltage deflections against tubular length. A second measurement, illustrated in the lower part of Fig. 8 repeats the procedure but in a condition in which the conductance of the luminal membrane is minimal and does not contribute to the overall conductance. This is accomplished when the lumen of the tubule is entirely filled by a nonconducting core of oil. A second, different intraepithelial length constant is found. By means of a special double-core cable theory, these two sets of data can be used to estimate the specific resistance of the two tubular cell membranes separately(28).

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