Progress in Microelectrode Techniques for Kidney Tubules

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Although microelectrode measurements have caused a good deal of confusion in the investigation of renal electrolyte transport (for literature see Ref. 4), we cannot do without them. Knowledge of the electrical potential steps, which the ions have to pass during their reabsorption, is indispensable for understanding the transport mechanisms involved. Hence the development of more reliable methods to measure the electrical properties of kidney tubules was an important as well as challenging problem. This paper deals with some of the advances effected in our laboratory in the past 5 years.

The properties of microelectrodes that present the major problems are: 1. the invisibility of the electrode tip, 2. the instability of the tip potential, and 3. the instability of the tip resistance. Since a brief description of resistance measurement problems has been published elsewhere recently(14), only the first two points will be discussed here.

Simple microscopic observation alone can never tell whether the invisible microelectrode tip has penetrated the invisible cell membrane. In order to decide whether the tip sticks within a cell or whether it has reached the tubular lumen a number of localization methods have been developed.

A. Localization Methods

1. Correlation between potential difference and electrode movement (potential profile). A very simple method is to pierce the middle of a tubule with an electrode and record the potential difference continuously. If a potential profile is obtained as shown in Fig. 1, the problem is already solved, since the distinction between single cell membrane potential differences and transepithelial potential difference is self-evident and unequivocal in this case. Unfortunately, this technique does not work in all tissues. Figure 1 was obtained from experiments on nephridia of the earthworm, Lumbricus terrestris(2). Attempts to reproduce such profiles in rat proximal tubules have been far less successful, probably because
the rat tubules and their cells are much smaller and because the tubular wall is more flaccid. In the same type of experiment in the rat kidney instead of discrete potential plateaus usually only transients are observed during which the potential difference jumps to negative values of 10–80 mV and then declines more or less rapidly towards zero.

In most of the earlier literature it was thought that a potential value which remained constant, when the electrode was moved more than 1 cell diameter, could arise only from the tubular lumen. This conclusion is subject to errors, however, since the tubular wall is not stiff and together with the electrode tip can well be displaced over such a distance. In order to provide definite proof of the intraluminal location the electrode should be advanced much farther (we have reached up to 5 luminal diameters(4)) and visible distortions of the tubular wall should be prevented.

Another useful modification of the potential profile method, which we have successfully employed in both proximal and distal tubules of the rat kidney, is to push the electrode through the tubule and record the transepithelial potential difference during retraction of the electrode, when the tip enters the lumen from the peritubular space underneath. Since the two cells in the upper and lower tubular wall, are totally damaged and have lost their potential difference, only a transepithelial potential difference will be recorded on the way back. Damage of the two cells in the upper and lower wall does not appear to affect the transepithelial potential difference to an appreciable extent.

2. Correlation between potential and resistance measurements. Since cell membranes generally exhibit high electrical resistances and since the tubular cells and the tubular lumen have a completely different geometry, it can be anticipated that the effective resistances for current flow from a cell into the interstitium and

![Diagram](image)

**Fig. 1.** Original trace record of a potential profile from a nephridium of the earthworm, *Lumbricus terrestris*. Abscissa: time in seconds, ordinate: potential difference in millivolts. In this experiment the electrode pierced the tubule as shown in the upper half of the figure and the potential difference was continuously recorded.
for current flow from lumen to interstitium differ. In this case it should be possible to distinguish between cellular and luminal potential levels on the basis of simultaneous resistance measurements. Although this approach is straightforward, it has not been of much practical importance until now, because of technical problems with the resistance measurements. For example, at present there is no reliable method for measuring the effective resistance of a tubular cell or luminal lumen with a circuit involving only a single-barreled microelectrode. This is a consequence of the instability of the electrode resistance(9,11). Even with double-barreled microelectrodes, where the situation is a little more favorable, the constancy of the so-called coupling resistance may still be insufficient to allow proper determinations of the tissue resistances(9). On the other hand, when puncturing the tubule with two separate microelectrodes, one for passing the current and one for measuring the potential difference, the correlation between resistance and potential data becomes a very powerful tool(6). As shown in Fig. 2, this method allows us to follow directly the process of penetration of an electrode tip from one compartment into the other.

3. Ejection of electrode fluid. By applying several atmospheres of pressure small amounts of fluid can be ejected from the tip of 3M KCl-filled microelectrodes of up to 10 MΩ resistance. Coloured fluid will either stain a tubular cell or flow into the tubular lumen(7). Both aspects can be clearly distinguished under the microscope, thus permitting a separation of luminal and cellular measurements. In rat proximal tubules the 3 M KCl solution can be seen to fill the tubular lumen even without the addition of a dye(4). Although the microscopic aspects are unambiguous, the interpretation of potential changes associated with the fluid ejection presented a problem initially. In rat proximal tubules we had observed that the negative potential values of around 20 mV would disappear irreversibly when the electrode fluid flowed into the tubular lumen(4). A careful investigation of this phenomenon revealed that in these instances the electrode had not reached the tubular lumen prior to the fluid ejection, but was still located in the intracellular compartment. The outflowing KCl then ruptured the luminal cell membrane, and helped the electrode enter the tubular lumen, without producing a significant transepithelial leak (Fig. 2). In addition to this irreversible potential change a number of reversible potential changes can be observed during fluid ejection: 1. Depending on its composition, the ejected electrode fluid can change the fluid composition in the neighbourhood of the puncture site and thus generate membrane diffusion potentials or streaming potentials across the tubular wall or across single cell membranes. 2. Ejection of electrode fluid can generate streaming potentials across the electrode tip, the magnitude of which increases with decreasing ionic strength of the filling solution. 3. Ejection of electrode fluid can change the electrode tip potentials (see Fig. 6).

4. Luminal ion substitution. Since membrane potentials arise from concentration gradients for ions or nonelectrolytes, from pressure gradients, and from active transport, they should respond to changing ion concentrations in the external compartments. This response can be used as a further means for distinguishing between cellular and luminal potential measurements and can also help
to differentiate between good and leaky impalements in tubules, that normally
do not generate high transepithelial potential differences. Figure 3a shows the
results of a series of experiments in which rat proximal tubules were punctured
with one microelectrode and the potential difference was recorded against the
peritubular space during alternate perfusions of the tubular lumen with isotonic
NaCl or choline chloride solutions. The abscissa depicts the potential values
which were observed after the tubular puncture during perfusion with NaCl
solution just prior to switching the perfusion over to choline chloride. Since no
special precautions were taken during the impalement, these values could com-
prise both cellular and luminal measurements, depending upon how far the

![Diagram of tubule punctured with microelectrode](image)

**Fig. 2.** Original trace record from a rat proximal tubule demonstrating the penetration of an
electrode into the tubular lumen during ejection of KCl solution from the electrode tip. Abscis-
sa: time in seconds (line 1). Before the trace record was started a microelectrode (A) had been
inserted into the tubular lumen to pass square-wave constant-current pulses across the tubular
wall. The current pulses are monitored as the voltage drop across electrode A in line 2 in volts.
The current pulses flowed across the luminal and peritubular membrane into the interstitium
and generated small potential changes across both cell membranes as shown in the upper part
of the figure. In this situation the same tubular loop was punctured with microelectrode B and
the potential difference between microelectrode B and interstitium was continuously recorded in
line 4 in millivolts. After the puncture a dc potential difference of approximately −50 mV was
observed and the height of the coupling pulses increased somewhat above the bath level, indic-
ating that the electrode had passed the outer cell membrane. Upon fluid ejection (mark E) from
electrode B the dc potential broke down to zero irreversibly and the coupling pulses increased
further indicating that electrode B had now penetrated the luminal cell membrane. Note that
the potential breakdown occurs at the very moment that the concentrated KCl solution appears
in the lumen as evidenced by a drop of the resistance of electrode A (line 2).
electrode happened to penetrate into the tubule in a given impalement. The values scatter from zero to -80 mV and are not different from similar observations under free-flow conditions. The ordinate depicts the potential change which occurred when the luminal perfusion was switched from sodium chloride to choline chloride. This change was reversible in all instances. Depending on their response to the luminal choline chloride perfusion the potential values can be divided into two groups: 1. The data of the first group gather around the abscissa. These data were practically unaffected by the choline chloride perfusion although in some cases a small potential change was observed either in positive or in negative direction. 2. The data of the second group gather around the ordinate. This group is comprised of experiments in which, during luminal NaCl perfusion, the potential difference was around zero mV and perfusion with choline chloride produced a potential change of around +20 to +40 mV. Since it was possible in some experiments by simply advancing the electrode to proceed from a group 1 to a group 2 behaviour (Fig. 3b), the observations suggest that group 1 can be identified with cellular measurements and group 2 with luminal measure-

![Graph A](image)

![Graph B](image)

**Fig. 3a and b.** Response of the potential difference observed in random proximal tubular punctures to perfusion of the tubular lumen with choline chloride solution. Abscissa: potential difference measured prior to switching the luminal perfusion from NaCl to choline chloride in millivolts. Ordinate: change of the potential difference observed upon luminal perfusion with choline chloride. Figure 3b depicts the results from individual impalements (marked with different symbols) in which the luminal perfusion could be changed repeatedly, while the potential values on the abscissa either declined spontaneously or because of a stepwise advance of the electrode. Note that the response to choline chloride can be used to separate the data into two groups comprising either luminal or cellular measurements and that there are also some intermediate values, which suggest that the penetration of the electrode from the cell into the lumen is a gradual process. (For further details see text)
ments. Accordingly, this method indicates in agreement with our previous findings(4,5) that the single cell membrane potential is negative by up to 80 mV in rat proximal tubules, whereas the transepithelial potential difference is near zero in this nephron segment.

It is interesting to note that the transition from group 1 to group 2 is not sharp. In some instances a positive response to choline chloride can be observed, when the negative potentials on the abscissa are still in the range of −30 mV. This does not mean that these values arose from the tubular lumen. If they were transepithelial measurements, which break down because of a transepithelial leak, the magnitude of the choline chloride-induced potential change should also decline to reach the free liquid junction value of +4 mV (choline chloride solution positive), when the negative potentials disappear completely. (This behaviour has been observed in distal tubules (Fig 4), when the transepithelial potential difference is gradually destroyed.) In Fig 3b, however, the choline response increases with decreasing negative potential values and reaches its maximum of +20 to +40 mV when the negative values approach zero. This observation must indicate that the penetration of the electrode through the brushborder is a gradual process during which a transitional state is passed until the tip, finally, reaches the lumen and records values near zero during NaCl perfusion and values of +20 to +40 mV during choline chloride perfusion. Further evidence for a gradual penetr-ation of the electrode tip into the lumen was formerly obtained with localization method 2 (see Ref. 6). During the transitional state the electrode may have access to the cellular and luminal compartment simultaneously and may record intermediate potential values, which depend on the relative resistances between the tip and each fluid compartment.

The existence of a transitional state requires some precaution in using the choline response as a means to localize the electrode, since a small positive po-

![Image](image_url)

**Fig. 4.** Response of the transepithelial potential difference of distal tubules to luminal perfusion with choline chloride solution during progressive destruction of the tubular wall. The data are plotted as in Fig. 3. Identical symbols denote data from the same tubule. The tubular wall was damaged by repetitive crude impalements with the microelectrode. Note that response to choline chloride perfusion diminishes proportionally to the decline of the negative potential differences. This behaviour conforms to the gradual development of a transepithelial leak.
tential change after choline chloride perfusion could be mistaken for evidence of a proper intraluminal position of the electrode tip. Hence we have decided to accept only those measurements as true transepithelial measurements, in which the potential difference reaches more than +25 mV during luminal perfusion with isotonic choline chloride solution(5). This criterion also rejects leaky impalements, since the choline chloride-induced positive potential differences break down toward the free liquid junction potential, when the tubular wall is damaged (compare Fig 5).

5. Other techniques. Additional methods used to distinguish between luminal and cellular measurements are the oil droplet technique(3) and the iontophoretic deposition of dyes from the electrode tip(15). The first approach which does not yield unambiguous results, is based upon the observation that the electrode resistance rises when the tip is covered with insulating material. After the tubular puncture the tubule is filled with oil and the electrode resistance monitored; an increase is taken to indicate a luminal measurement. This conclusion is not definitive, however. Rather than rising because the tip is covered with oil, the electrode resistance can also rise, when the tip is still situated within a cell and membrane material is pressed against it, as the tubule widens under the oil column(8).

Experience with iontophoretic dye injection into kidney tubules is not yet sufficient to allow pertinent conclusions to be formed as to the capability of this method. In our view it would seem questionable, whether it is able to distinguish between true transepithelial measurements and the transitional states which have been described above.

![Graph](image)

**Fig. 5.** Effect of progressive damage of the tubular wall on the transepithelial potential difference and on the effective resistance of rat proximal tubules perfused with choline chloride solution. Abscissa: transepithelial potential difference in millivolts. Ordinate: effective resistance (= input resistance) of single tubules in kΩ. Identical symbols denote data from the same tubule. The effective resistance was measured with two single-barreled microelectrodes which were inserted into the same tubular loop, one for passing current pulses and one for measuring the potential difference. The scatter of the resistance values may be partly due to varying distance between the tips of the current and voltage electrode within the tubular lumen. It can be seen that the choline-induced positive potential difference and the effective resistance decline proportionally when the tubular wall is damaged by repetitive forceful punctures with a micro-pipet.
B. Methods to Reduce Tip Potential Artifacts

The glass microelectrodes are filled with 3 M KCl solution to reduce liquid junction potentials that occur between the electrode fluid and the fluid surrounding the electrode tip. This principle is commonly used in the saturated KCl bridges. Although it can never eliminate liquid junction potentials completely, since the transference numbers of K+ and Cl− are not absolutely identical and the activity of the saturated KCl solution is not infinite, the remaining potentials are generally so small that they can be neglected. However, if the liquid junction is formed within the tip of a glass microelectrode of less than 0.5 μm outer diameter, the situation is no longer that simple. This is due to the presence of negative fixed charges at the glass surface. In the presence of the fixed charges the KCl concentration gradient gives rise to a potential difference between electrode fluid and immersion fluid—the so-called tip potential—probably in the same way as a membrane potential is generated across an ion-exchange membrane in the presence of a salt gradient. The polarity of the tip potential is always such that the capillary lumen is negative. The problem with the tip potential is that it is not constant. It changes with the Na+ and K+ concentrations of the immersion fluid (1) and it can also change reversibly or irreversibly during puncture.

To our knowledge all attempts to eliminate tip potentials completely have been unsuccessful(11). Common laboratory experience suggests that one factor which increases the tip potentials is dirt. In our laboratory the following procedure, which yields a satisfactory yield of low tip potential (≤ 5 mV) and high resistance (20–100 MΩ) electrodes has been adopted. The glass capillary tubing (Pyrex, Corning) is washed with a mild detergent, rinsed thoroughly with distilled water, and boiled for up to 3 hr in distilled water. Then the capillaries are quickly dried with acetone and pulled. After pulling they are filled with methanol according to the technique of Tasaki et al.(13) and the methanol is replaced overnight with the desired electrolyte solution. In our experience tip potentials are higher when the electrodes are directly filled in hot KCl solution.

In some special situations the adverse effects of tip potentials can be further reduced by the following methods.

1. Continuous ejection of KCl solution from the electrode tip. As demonstrated by Adrian(1), the tip potential is a logarithmic function of the external sodium and potassium concentrations (compare also Fig. 6). This can cause severe problems during transepithelial potential measurements when the tubular lumen is perfused with hypotonic salt solutions. To overcome these difficulties we have taken advantage of the observations that the tip potential vanishes almost completely during ejection of 3M KCl solution from the electrode tip. This effect is shown in Fig. 6. It is found regardless of the ion concentrations of the immersion fluid. The abolition of the tip potential during fluid ejection seems to be caused by the displacement of the KCl concentration gradient from the site of the fixed charges within the electrode tip into the outside solution. This displacement separates the two causes necessary for the generation of the tip potential. After this observation we have routinely used a continuous ejection of
Fig. 6. Tip potential of a microelectrode filled with 3 M KCl solution as a function of the external salt concentration. The effect of ejection of fluid from the electrode tip. Abscissa: Na\(^+\) + K\(^+\) concentration of the immersion fluid in mmoles/liter. Ordinate: tip potential in millivolts. The filled circles were measured before fluid ejection, the triangles during fluid ejection, and the open circles after fluid ejection. The abolition of the tip potential during fluid ejection was also observed when the immersion fluid was stirred.

KCl solution from the electrode tip in some of our experiments(5). The application of this technique is restricted to transepithelial measurements in which the tubular lumen is artificially perfused at high perfusion rates, since a significant contamination of the luminal perfusate with the outflowing KCl solution has to be avoided. At luminal perfusion rates of 0.1–0.5 \(\mu\)liter/min no adverse effects of the outflowing KCl solution were observed, provided that the ejection of electrode fluid was reduced to the minimum rate required to just abolish the tip potential of a given electrode in the kidney bath.

2. **Use of isotonic bicarbonate Ringer's solution as electrode fluid.** Determination of the transepithelial potential difference of proximal tubules under physiologic conditions presents a peculiar problem because the values are so close to zero that the reliability of the microelectrode measurement becomes questionable. This is especially true with KCl electrodes. There are several observations which suggest the presence of small systematic errors in such measurements. For example, the data from proximal tubules under free flow conditions which we published in 1966(4) show an asymmetric distribution within the range of +7 to −7 mV with a mean value of −0.5 mV. Since it would seem highly likely that a small potential difference, if it existed, would have the same polarity in all tubules, a considerable number of the 92 observations must have been masked by errors. Such errors could arise from reversible changes of the tip potential. The tip potential could increase for example during the impalement, through contamination of the tip with protein material, and the increase could be reversed upon withdrawal of the electrode into the bathing solution when the material was stripped from the tip again. Corresponding reversible changes of the electrode resistance are well known to occur in almost every impalement(9). Under favourable con-
ditions one can also observe such tip potential changes directly. When measuring the potential profiles, as shown in Fig. 1, the electrode was pierced through the nephridium until it entered the coelom cavity again underneath. In this position the tip potentials had often increased and the increase disappeared after the electrode was withdrawn from the nephridium. Furthermore, we have observed in rat proximal tubules that potential differences of $-1$ to $-3$ mV which are recorded with KCl-filled electrodes during luminal perfusion with plasma-like Ringer's solution do neither vanish upon poisoning with cyanide nor upon destruction of the tubular wall with chloroform. This observation strongly suggests that the small negative values may arise across the capillary tip rather than across the tubular wall.

In order to overcome these problems and to obtain more reliable potential measurements in the near-zero range, we have decided to eliminate one of the causes responsible for the generation of the tip potentials, by filling the electrodes with the same fluid which they encounter in the tubular lumen. In this case a contamination of the electrode tip with protein material during the impalement should no longer matter, since there is no ion concentration gradient to generate a tip potential. [If the composition of the luminal (= electrode fluid) and peritubular fluid is different, appropriate corrections have to be applied for the liquid junction potential, which arises between electrode fluid and external solution before and after the puncture, and this potential has to be checked frequently during the experiment in order to confirm that it is indeed equal to the liquid junction potential measured under macroscopic conditions.] Using this principle we have been able to reduce the scatter of the transepithelial measurements remarkably. During luminal and peritubular perfusion with identical bicarbonate Ringer's solution the transepithelial potential difference of rat proximal tubules was $+0.3$, SD $\pm 0.3$ mV, with the 28 single observations ranging from $-0.1$ to $+0.6$ mV. Under free-flow conditions the tubular lumen was found to be consistently positive, the mean value of 52 observations was $1.8$, SD $\pm 0.35$ mV, and the range was $+1.0$ to $+3.1$ mV. Figure 7 shows that the positive potential difference observed under free-flow conditions disappeared upon poisoning the tubule with cyanide, probably as a result of a dissipation of the transepithelial concentration difference for Cl and HCO$_3^-$, and reappeared again after the cyanide solution was washed away.

CONCLUSIONS

The following conclusions can be drawn from the above discussion. 1. With regard to localization techniques, no single method can be considered ideal or suitable under all experimental conditions; each has its own advantages and limitations. Since the emphasis of future electrophysiologic work on kidney tubules will certainly shift from the more basic understanding of the methods and their interference with the measured values, to a more routine application, a simple and unambiguous test will be needed. For this purpose we would recommend the measurement in each impalement of the control potential differences under free-
flow conditions, before the introduction of the experimental parameters. This would correspond to the general practice of muscle and neurophysiologists. Regarding the proximal tubule of the rat kidney this approach would mean that the electrode should be advanced into the tubular lumen before the free flow of tubular urine is interrupted and that in this situation the first potential reading should be obtained. If it is in the order of +2 mV, and if the electrode resistance has not changed, it can be inferred that the tip is located intraluminally, that it is not blocked by membrane material, and that there is no appreciable leak across the tubular wall, since fortunately all electrode artifacts and all extraluminal positions of the electrode would shift the reading to more negative values and since the positive values would break down to zero when the tubular wall were injured. Whenever this test cannot be performed, we would recommend the use of the luminal choline chloride perfusion and acceptance of only those measurements in which the potential difference reaches more than 25 mV, lumen positive. It should be emphasized, however, that in order to use the choline response in a quantitative way an appropriate perfusion technique has to be applied which will prevent contamination of the perfusate with glomerular filtrate(5). Furthermore the adjacent blood capillaries should be perfused with Ringers solution(5,14) in order to reduce unstirred layers in the peritubular space.
2. In attempting to prevent tip potential artifacts the choice of methods is smaller, and the techniques described above are confined to certain perfusion conditions. Thus, the continuous KCl ejection can be used only in the presence of high-speed luminal perfusions and the technique of filling the electrodes with luminal fluid cannot be applied when the composition of the luminal fluid changes.

Since we have shown above that the luminal perfusion with choline chloride solution can be used as an objective criterion for detecting whether or not a given impalement is affected by transepithelial leaks, we would predict that the future development will be to avoid the Ling-Gerard type microelectrodes, with all their problems, and to use instead sharpened micropipets with a larger tip diameter. Such electrodes of 3–7 \( \mu m \) outer diameter do permit proper transepithelial potential and resistance measurements, as we have discovered in rat distal tubules\(^{(4)} \) and which has been observed also in hamster collecting ducts\(^{(10,12)} \).

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