J. Membrane Biol. 64, 45–53 (1982)

The Journal of
Membrane Biology

Miniature Ag-AgCl Electrode for Voltage Clamping of the *Ambystoma* Collecting Duct

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Summary. We have developed a miniature silver-silver chloride electrode. The outer diameter of the electrodes averaged 22 μm and the input resistance 8.8 kΩ. Since the core of the electrode is a glass fiber, the problem of the extreme malleability of a small diameter silver fiber is circumvented. The properties of the electrode permit us to insert it into short (600 μm) fragments of the amphibian collecting duct while they are being perfused *in vitro*. The passage of currents in the range of 0 to 6×10^{-8} amperes allowed us to voltage clamp the nephron fragment between +20 and −20 mV. The current-voltage plots are linear over this range. Two lines of evidence suggest that the voltage clamp is homogeneous. First, the voltage measured at the perfusion end during a voltage-clamp experiment of the tubule is not significantly different from that measured at the collecting end. Secondly, the specific resistance of collecting ducts estimated from the "core conductor analysis" is $3.3 \pm 0.8 \times 10^4$ Ω cm, a value not significantly different from that computed from the current-voltage plots as determined with the Ag-AgCl electrode, $3.0 \pm 0.5 \times 10^4$ Ω cm. This method permits precise control of both the ionic and electrical gradients across fragments of the amphibian collecting duct.

Key words miniature Ag-AgCl electrode · voltage clamp · amphibian collecting duct · ion transport · short-circuit current · transepithelial resistance

Introduction

The Koefoed-Johnsen and Ussing model [11] for active sodium transport across the frog skin allows for the passive entry of sodium at the apical border of the epithelium and its active extrusion at the basolateral border. The formulation of this model resulted from the availability of three types of experimental data: 1) measurements of both electrical and chemical gradients across the epithelium [18], 2) control of chemical and radioisotopic contents of the bathing media, and 3) manipulation of transepithelial electrical gradients [19]. Unlike the frog skin the microscopic dimensions of the nephron preclude the use of some of the techniques used to investigate ion transport. A major step in the application of such methodology to the renal tubule was the development of a technique for *in vitro* perfusion of isolated nephron fragments by Burg et al. [3]. This technique permits the investigator to measure the transepithelial potential of the renal tubule and at the same time precisely control the ionic composition of the fluid on both sides of the tubule. This advantage permitted Bourdeau and Burg [2] to chemically voltage clamp the rabbit thick ascending limb by controlling the sodium chloride gradient across the epithelium and to study the effects of voltage on calcium transport. While such a technique offers useful information about the mechanism of distribution of calcium ion, it may not be suitable for obtaining data on the movement of sodium and chloride since it requires manipulating the concentrations of the ions under investigation.

Several laboratories have attempted to voltage clamp the *in vivo* renal tubule using glass microelectrodes. The earliest such attempt using the split drop technique was that of Eigler [5] who passed current through a microelectrode such that the spontaneous voltage across the *Necturus* proximal tubule was reduced to zero (short-circuit current). A similar attempt was made by Windhager and Giebisch [20] who performed split droplet short-circuit experiments in the proximal tubule of the rat. More recently Garcia-Filho et al. [6] have used *in vivo* methods to study the mechanism of potassium transport across the rat distal tubule. These investigators used free-flow techniques instead of the split-drop method, and attempted only to voltage clamp the nephron at the site of fluid collection. The problem with the use of microelectrodes, however, lies in the fact that a uniform voltage clamp of a cylindrical structure such as a renal tubule with a point source of current is impossible.

An attempt to uniformly voltage clamp a renal tubule with an axial electrode was made in the proximal nephron of the *Necturus* by Spring and Paganelli [14, 15]. Using the split-drop technique to measure the Na^+ and fluid fluxes, they short-circuited the

0022-2631/82/0064-0045 $01.80

transepithelial voltage with an intraluminal platinized tungsten wire electrode. While quite ingenuous the methodology has failed to gain widespread usage. In addition to the demanding nature of the method there are other limitations. First, only nephrons on the surface of the kidney can be studied. Second, the use of split-drop methodology may permit changes in the chemical contents of the luminal fluid with time. Finally, there is the potential problem of generation of H^+ ions in a closed system.

The use of Ag-AgCl electrodes for voltage clamping nephron segments has been prevented by the extreme malleability of fine silver wire. At least three laboratories have circumvented this problem in the development of miniature Ag-AgCl electrodes. Marmont [12] described a 100 μm diameter axial Ag-AgCl electrode consisting of a silver-plated platinum wire. Such an electrode is too large to insert into a nephron segment. Augustus and Bijman [1] used a preshaped glass capillary pipette as the core for an axial Ag-AgCl electrode. The glass micropipettes were plated with a thin layer of metallic silver by placing them in the chemical silvering solution. Additional silver was then electroplated onto the electrode tip. The chief advantage of such an arrangement was that the glass core allowed the electrode to be flexible but not malleable thus overcoming one of the principle disadvantages of small silver wire electrodes. The diameter of this electrode was also 100 μm, too large to be inserted into the lumen of a renal tubule. More recently, Hegel and Boulpaep [8] have reported the use of a small diameter (15 μm) axial Ag-AgCl electrode which could be inserted into the lumen of the *in vivo* proximal tubule of the *Necturus*.

This paper describes a miniature glass core Ag-AgCl electrode small enough to be inserted axially into the lumen of an amphibian distal nephron segment *in vitro* and capable of passing sufficient current to permit uniform clamping of the nephron fragment, under freeflow conditions.

Materials and Methods

Construction of Electrodes

Fine glass fibers averaging 9.0 ± 0.25 μm in diameter (Pyrex Brand Wool Filtering Fiber, Cat. # 3950, Corning Glass Works, Corning, N.Y.) were thinly dispersed on the surface of a large petri dish. The fibers were then chemically coated with a thin layer of metallic silver [13]. The process is similar to that originally used to plate silver onto glass in the production of mirrors. The silvering solution was prepared fresh for each group of fibers by mixing 10 ml of 10% aqueous $AgNO_3$, 10 ml of 10% NaOH, 4 ml of concentrated NH_4OH and 150 ml of glass-distilled water in a beaker. Upon mixing the above constituents a silver oxide precipitate is initially formed. After 1–2 min of agitation with a glass rod, this precipitate dissolves. Once the silvering solution has completely cleared, it is mixed with 50 ml of glucose solution (100 mg/ml) and immediately poured over the glass fibers so that they are completely immersed. Glucose acts to reduce the silver salts to metallic silver which adheres to the surface of the glass fibers. The fibers are allowed to remain undisturbed in this solution for a period of 5 min after which the reaction is terminated by dilution with large volumes of distilled water. The silvered fibers are removed from the petri dish by floating them onto a piece of filter paper. They are then exhaustively washed with additional distilled water and placed in a dust-free cabinet to dry. Microscopic examination of these fibers at 800× shows them to be uniformly coated with a mirrorlike layer of silver metal (1.3 μm thick) which is firmly adherent to the surface of the glass. The mean diameter of the fiber following chemical silvering was 11.5 ± 0.51 μm.

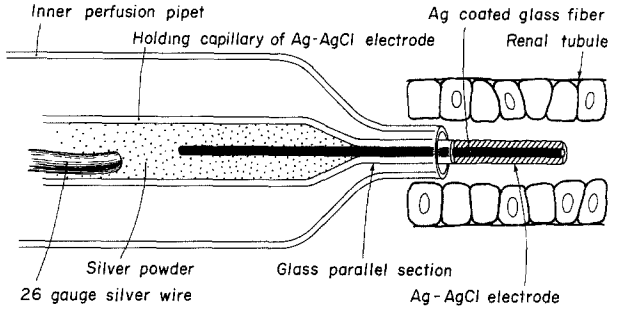

Fig. 1. Diagrammatic representation of the axial Ag-AgCl electrode. The relative position of the electrode to the inner perfusion pipette and the renal tubule fragment is depicted. Sylgard was used to form a seal between the silver-coated glass fiber and the glass parallel section (*see* text for details)

Following preparation of the silver fibers, the electrodes (Fig. 1) are constructed. A silvered fiber usually 3–5 cm in length is aspirated into a glass capillary which has been prepared from 0.047 OD × 0.040 ID custom glass tubing (Drummond Scientific, Broomall, Penna.) using a microforge (Stoelting Co., Chicago, Ill.). These capillaries had a parallel section approximately 500 μm in length, whose inside diameter averaged 14–17 μm. This diameter was just sufficient to permit the insertion of the silvered fiber. Approximately 1–1.5 cm of the fiber is aspirated beyond the parallel section and into the barrel of the capillary. When the fiber has been properly positioned within the parallel section, Sylgard 184 Silicone Elastomer (Dow Corning Corp., Midland, Mich.) mixed with catalyst was touched to the interface between the silver fiber and the tip of the glass capillary. The resin is then solidified by applying mild heat with the loop of the microforge. When applied in this manner, the Sylgard acts as an insulator, holds the fiber within the capillary and prevents any solution from entering the parallel section.

When the resin has completely solidified, approximately 0.2 g of metallic silver powder (2.0–3.5 μm, Alfa Product, Danven, Mass.) is poured into the open barrel of the capillary. The powder is carefully pressed into the tip of the capillary with a thin wire probe until it makes contact with the end of the silvered fiber. A 26-gauge silver wire (Medwire, Mt. Vernon, N.Y.) is then introduced through the open end of the capillary and pressed into the silver powder for a distance of several centimeters. The silver wire is fastened securely into the end of the capillary with melted wax. Thus an electrical contact is made between the silver wire and the silvered glass fiber with little risk of damaging the fiber or disturbing the Sylgard seal.

Once electrical contact has been established with the electrode tip, additional silver metal can be electroplated onto the silver fiber. This is accomplished by using the electrode as a cathode and a silver wire as an anode and passing a current of 10 μamp

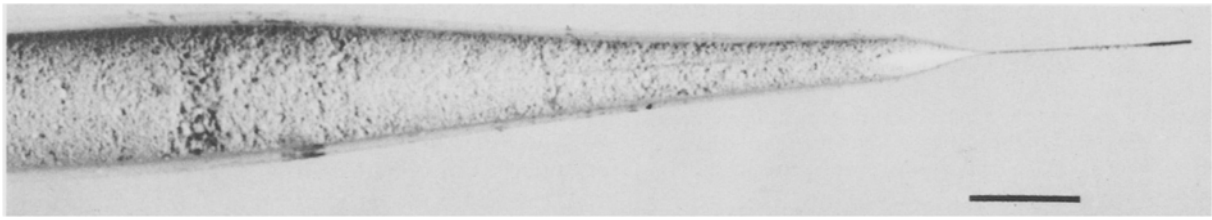

Fig. 2. Photograph of an Ag-AgCl electrode used to voltage clamp an *Ambystoma* collecting duct. The bar indicates a distance of 1 mm. The dark portion of the electrode tip is the Ag-AgCl electrode

for 3 min while it is immersed in a commercial silver-plating solution (Norchem Supply Corp., Syracuse, N.Y.). Following this process, each electrode was examined microscopically at 800× to assure that the silver plate was uniformly distributed along the length of the tip. The silver electrode was then converted to Ag-AgCl in 0.15 M KCl solution by using the electrode as anode and a silver wire as cathode and passing 4 μamp for 2 min. The average diameter of the electrode tip following the electroplating and chloriding process was 22.2 ± 2.1 μm with the narrowest electrode having a tip diameter of 14.2 μm. Figure 2 is a photograph of such an electrode. The input resistance of each electrode was measured in 0.15 M NaCl using a Keithley Electrometer (Model # 602, Keithley Instruments Inc., Cleveland, Ohio) and a large Ag-AgCl wire as the opposite pole.

General Methods

Male *Ambystoma tigrinum* were obtained from a commercial supplier (NASCO, Ft. Atkinson, Wisconsin) and maintained in a fasting state in fresh tap water at 7 °C. The animals were double pithed and the kidney immediately removed and placed in amphibian saline solution [16]. This medium contained the following constituents in mM: NaCl 90, $NaHCO_3$ 25, KCl 3, KH_2PO_4 0.5, $MgSO_4$ 0.6, $CaCl_2$ 1.0 and dextrose 5.5. The total osmolality was 225 mOsm/Kg H_2O as measured with an Osmette Precision Osmometer (Precision Systems, Sudbury, Mass.). The solution was maintained at pH 7.7 by constant bubbling with 3% CO_2, 97% O_2 gas.

Isolated segments of collecting duct were dissected from the kidney and transferred to a perfusion chamber containing amphibian saline at room temperature. The tubules were perfused with an identical solution using concentrically aligned glass pipettes. The technique of isolated *in vitro* perfusion has been described previously [3, 16, 17] but several important modifications were made during the study: (a) As depicted in Fig. 1 the innermost component at the perfusion end consisted of the axial Ag-AgCl microelectrode described above. The electrode was inserted through the inner perfusion pipette (constructed from 0.084 OD × 0.064 ID glass) into the lumen of the collecting tubule, thus permitting the passage of current along the entire length of the tubule. The holding pipette (not shown in Fig. 1) was constructed from 0.190 OD × 0.150 ID glass tubing. (b) The development of a special perfusion chamber permitted the electrode to be inserted and accurately centered within the tubule lumen under direct visualization in two planes. The entire lateral wall of a standard perfusion chamber was replaced with a glass window which allowed the use of an additional microscope (Gartner Scientific Corp., Chicago, Ill.) for observing the tubule, electrode, and pipettes in the vertical plane. This, together with the view of the horizontal plane normally afforded through the inverted microscope, allowed the electrode to be advanced and retracted while visualized in two planes. This modification proved to be an advantage in preventing inadvertent damage to the tubule during insertion of the electrode. (c) A third modification involved the cannulation of both the perfusion and collection ends of the tubule. The "double cannulation method" originally developed by Grantham et al. [7] uses collecting pipettes similar to those used at the perfusion end. This arrangement permitted the spontaneous transepithelial voltage and current-induced voltage deflections to be recorded simultaneously at both ends of the tubule.

The electrical system used in this study consisted of two entirely separate circuits and was patterned after that used by Ussing and Zerahn [19]. One circuit was used for passing current through the axial Ag-AgCl voltage-clamp electrode and consisted of a current source capable of passing DC currents of either polarity in the range from 1×10^{-10} to 1×10^{-6} amperes and a Keithley electrometer which was used to monitor the current output. These were connected in a series with the short-circuit electrode and a second Ag-AgCl electrode which was placed in the bath and extended longitudinally along the floor of the perfusion chamber, parallel to the perfused tubule and the axial short-circuit electrode.

Transepithelial potentials were recorded simultaneously from both the inner perfusion and inner collecting pipettes as described by Helman et al. [10]. The potentials at each end of the tubule were channeled through separate WPI single-channel amplifiers (WPI Microprobe System Model # M4A at the perfusion end and WPI Microprobe Amplifier Model # 725 at the collection end: New Haven, Conn). The amplifier outputs were displayed on a Tektronix dual-channel storage oscilloscope (Model # 5403, Tektronix Inc., Beaverton, Oregon). Prior to cannulation of the tubule, the electrical resistance of the inner perfusion pipette was nulled by a bridge circuit within the Microprobe System.

In the course of a typical experiment, segments of collecting duct were cut to a length appropriate to that of the short-circuit electrode. These tubules were then double cannulated and perfused while spontaneous voltage differences between the lumen and bath were recorded from both the perfusion and collection ends. When it appeared that the voltage was stable, 1.5×10^{-8} ampere current pulses of 100 msec duration were injected through the inner perfusion pipette via the Microprobe System and the resulting voltage deflections recorded at both ends of the tubule. These measurements were used to calculate the transepithelial specific resistance by the core conductor method of Helman et al. [9, 10].

Following completion of this portion of the experiment, the axial Ag-AgCl voltage-clamp electrode was advanced into the lumen of the tubule. The electrode was kept centered within the lumen by manipulating both the perfusion and collection ends of the system in all three planes. Great care had to be taken to avoid damage to the tubule during electrode insertion. The voltage-clamp electrode was advanced until all of the electrically active portion was beyond the tip of the inner perfusion pipette. The inner collecting pipette was then advanced through the lumen of the collecting end of the tubule until it almost made contact with the distal tip of the voltage-clamp electrode. The short-circuit current was then measured by passing a current through the axial Ag-AgCl electrode which was just sufficient to reduce the trans-

epithelial potential at both ends of the tubule to zero. In addition to short circuiting the spontaneous potential to zero, each tubule was voltage clamped at 10-mV steps between +20 and −20 mV by passing the appropriate current through the axial electrode. In this fashion, a current-voltage relationship for each tubule was obtained. The slope of the current-voltage plot represents the input resistance of the tubule. The transepithelial specific resistance can then be calculated from the equation:

$$R = V/I \times L \quad (1)$$

where V/I = slope of the current-voltage plot and L is the length of tubule.

The specific resistance (R^*) calculated in this manner can be compared with that calculated by the core conductor method [9].

By measuring the short-circuit current of each tubule, it is possible to calculate the net active ionic transport across the epithelium with the following equation:

$$J_{net}(\text{pM/mm} \cdot \text{min}) = \frac{\text{SCC (amperes)} \times 60}{L(\text{mm}) \times F} \quad (2)$$

where F is Faraday's constant, and L is the length of the tubule.

In other collecting ducts 25 to 50 μCi/ml of $^{22}Na^+$ was added to the perfusate and the lumen-to-bath flux of radioisotope measured. The height of the perfusion reservoir in these experiments was maintained at approximately 15 cm. This resulted in an average fluid collection rate of 206 nl/min. At this flow rate it is presumed that sodium transport does not cause measurable changes in chemical or radioisotopic contents of the perfusate. The details of the methods and calculation of transport rates have been reported elsewhere [17]. Aliquots of collected fluid and bath, along with the appropriate standards, were analyzed on a Beckman Model 8500 liquid scintillation counter.

Data are reported either for individual tubules or as means ± standard error of the mean (number of tubules).

Results

A group of 10 electrodes was assembled as described above. The lengths and outside diameters of the Ag-AgCl tips were measured with an ocular micrometer. In addition, the input resistance was measured in 0.115 M NaCl. These data along with the calculated specific resistance of each electrode are presented in Table 1. The input resistance averaged 8.8 kΩ. This value can be compared with the data of Augustus and Bijman [1] whose input resistance for 15 mm electrodes was slightly over 100 Ω. Marmont [12] developed a miniature Ag-AgCl electrode for use in the perfused squid axon with a resistance of 27 Ω for an electrode 13 mm in length. The relatively high input resistance of our electrode is probably related to its small diameter (22 μm in this study versus 100 μm in the others), and relatively short length. Since our electrode is to be used only to pass current, not to record voltage, we do not consider the relatively high resistance a problem.

Table 1. Dimensions and resistance of miniature Ag-AgCl electrodes

Electrode	Length (cm)	Outside diameter (μm)	R_{Input} (KΩ)
1	0.388	36.6	7.5
2	0.470	19.8	2.0
3	0.270	21.2	3.3
4	0.163	15.6	16.0
5	0.023	19.8	15.0
6	0.392	14.2	2.5
7	0.188	22.6	11.0
8	0.166	26.9	7.5
9	0.161	25.5	9.2
10	0.214	19.8	14.0
Mean	0.244	22.2	8.8
SEM	±0.043	±2.0	±1.6

On a series of three electrodes trimmed to a length of 500 μm, currents (5.5×10^{-7} amp) of both positive and negative polarities were passed for periods of 10 min. In each case the electrode resistance remained stable throughout the 10-min interval. We believe that this stability indicates that there is sufficient AgCl on the electrode to short-circuit an amphibian distal nephron segment for at least 10 min. This is based on the fact that the short-circuit of an amphibian late distal tubule as calculated from the transport rate [16] should be on the order of 10^{-7} amp. The maximum current passing capability of these electrodes was not determined.

In our opinion the only way to establish that the electrode is capable of voltage clamping a renal tubule is to demonstrate that the insertion of such an electrode and the passage of current would result in a stable, homogeneous voltage clamp which does not change the electrical properties of the epithelium. Since it is anticipated that short segments of the amphibian nephron are more likely to be functionally homogeneous than those several mm in length we chose to work with tubule segments 400 to 1200 μm in length. Therefore, some of the electrodes in Table 1 were trimmed to the desired length by cutting with a scalpel blade under a dissection microscope.

The amphibian collecting duct was chosen for preliminary evaluation of the electrode for several reasons. First, it was felt that the comparatively large inside diameter of the collecting duct of the male amphibian [4] would offer the best chance of successfully inserting the short-circuit electrode and obtaining a homogeneous voltage clamp. In addition, the orientation of the potential difference suggested that the amphibian collecting duct [16] was at least qualitatively similar to that of the mammal. Thus, one might expect this segment of the amphibian nephron to actively reabsorb Na^+.

While Stoner [16] has investigated NaCl transport in the diluting segment and late distal tubule of these amphibia, little is known about the electrophysiologic characteristics of the *Ambystoma* distal nephron. The collecting duct has been reported to have a lumen

negative spontaneous voltage and a relatively low osmotic water permeability [16].

Consequently it was necessary to obtain some basic information about these nephron segments at the outset of this investigation. The mean spontaneous transepithelial voltage, length constant and specific resistance (calculated by core conductor method) are shown in Table 2 along with the rate of $^{22}Na^+$ efflux (flux from lumen to bath). The spontaneous voltage was found to average -8.9 mV, lumen negative. Ouabain (2×10^{-4} M) was placed in the bath of two collecting ducts whose control spontaneous voltages were -17.5 and -15 mV. Within 5 min the transepithelial voltage fell to -2.0 and -3.0 mV, respectively. Nine of the collecting ducts were cannulated at both ends to permit measurement of the spontaneous voltage at two different points [7]. The distance between the points where voltage was measured averaged 574 ± 81 μm. The mean voltage of these tubules at the perfusion end was -6.8 ± 3.4 mV, while that at the collecting end averaged 1.1 ± 0.5 mV more negative. The collecting end voltage was not significantly different from that measured at the perfusion end of the tubule. These data suggest that at least with respect to short nephron segments we are dealing with a homogeneous nephron segment.

The specific resistance (Table 2) of the collecting ducts averaged 0.31×10^5 Ω cm. This corresponds to 626 Ω cm^2, a value similar to that of the rabbit cortical collecting tubule at room temperature [10]. Such a high value for tubular resistance is generally regarded as an indication that the epithelium is relatively impermeable to ions.

In eight tubules the efflux (lumen to bath) of radioisotopic $^{22}Na^+$ was measured. The spontaneous voltage of these eight tubules averaged -10.9 ± 1.4 mV. The efflux rate was 25.9 pM mm^{-1} min^{-1}. This unidirectional flux is similar to the rate of net transport reported for the late distal convoluted tubule of this species [16]. The ouabain-sensitive lumen negative voltage, high rate of $^{22}Na^+$ efflux and comparatively low ionic conductance (high specific resistance) all suggest this nephron segment may be involved in active sodium reabsorption.

Following determinations of specific resistance, the axial Ag-AgCl voltage-clamp electrode was advanced into the lumen of each of five collecting tubules. The short-circuit current was measured by passing a current through the axial electrode that was just sufficient to reduce the transepithelial potential at the perfusion end of the tubule to zero. A dual channel oscilloscope tracing from a representative short-circuit experiment is shown in Fig. 3. The current necessary to hold the voltage at zero was constant over the time course of the voltage clamp. Upon the cessation of current passage the voltage returned to the control value.

Table 2. Transport properties of *Ambystoma* collecting duct

Transepithelial voltage (mV)	$-8.9\ \pm1.9$	(17)
Specific resistance (Ω·cm)	$0.31\pm0.05\times10^5$	(9)
λ (μm)	$836\ \pm184$	(9)
^{22}Na flux (lumen to bath) (pM/mm min^{-1})	$25.9\ \pm4.9$	(8)

The specific resistance listed corresponds to a resistance of 626 Ω cm^2. The number in parentheses denotes the number of tubules studied.

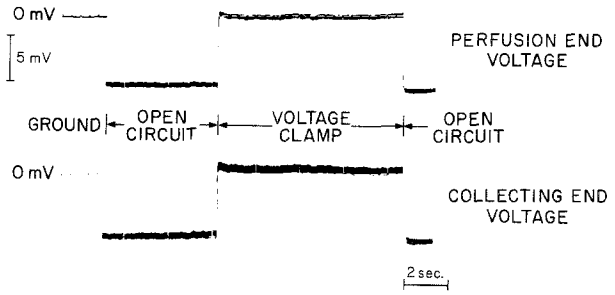

Fig. 3. Representative oscilloscope tracing of voltage-clamp experiment in *Ambystoma* collecting duct. The upper and lower tracings represent the transepithelial voltages recorded simultaneously at the inner perfusion pipette and inner collecting pipette, respectively. During the interval labeled ground, both the amplifiers are shorted to ground thus making the baseline zero potential. Open circuit represents the spontaneous transepithelial voltage across the epithelium at the perfusion (-7.0 mV) and collection (-6.8 mV) ends of the tubule. At the beginning of the voltage clamp, a current of 6.5×10^{-9} A is passed through the axial electrode. This current results in a stable voltage clamp to zero mV at both the perfusion and collection ends of the tubule

In addition to measuring the short-circuit current, each tubule was clamped at 10-mV intervals between $+20$ and -20 mV. Figure 4 shows the current-voltage relationship of a representative voltage-clamp experiment in the collecting duct. It is presented to demonstrate that the relationship is linear over the range studied. Such linearity was observed in each of the other tubules as well. In this particular experiment the spontaneous transepithelial potential at the perfusion and collection ends of the tubule was identical. In addition, the potential at both ends of the tubule remained equal (within 1 mV) as the voltage clamp was varied over the 40-mV range. It should be noted that the currents used in this plot have not been factored for surface area. Hence the slope of

the current-voltage relationship in this figure is equal to the input resistance of the tubule.

A current-voltage plot incorporating data from all five tubules is presented in Fig. 5. In this case, current was divided by the calculated surface area.

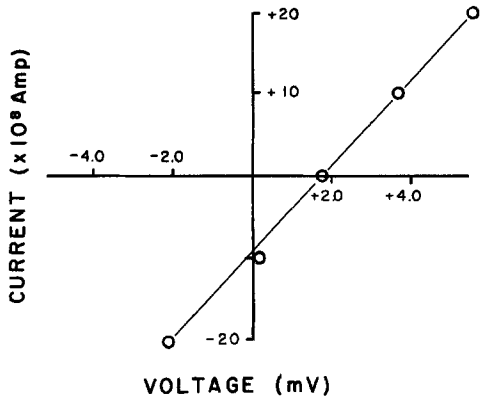

Fig. 4. Current-voltage plot of a representative *Ambystoma* collecting duct. Current (abcissa) is in units of amperes. Note raw current data is presented

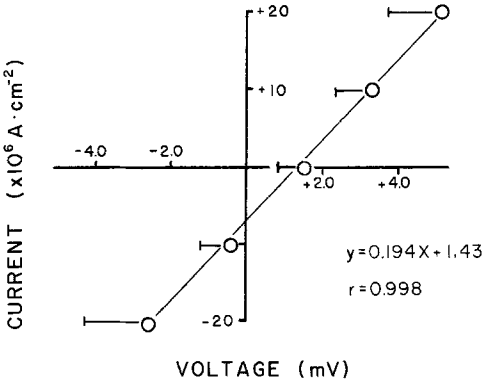

Fig. 5. Current-voltage relationship for five *Ambystoma* collecting ducts. Line drawn by linear regression analysis. Bars represent one standard error of the mean. Current (abcissa) is presented in units of A/cm^2.

The line was derived by regression analysis and the horizontal bars represent one standard error of the mean. During current passage, the short-circuit current remained stable and no voltage transients were observed.

In view of the linear nature of the current-voltage relationship, it is possible to calculate the transepithelial specific resistance (R^*) by Eq. (1). These data along with specific resistance values calculated by the core conductor method (R_t) are presented in Table 3. Paired analysis using students t-test shows no significant difference between the transepithelial specific resistance as determined from the current-voltage relationship (R^*) and that calculated by the core conductor method.

Given the short-circuit current which averaged 1.5×10^{-6} A/cm^2, it is also possible to calculate the net ionic transport rate across the epithelium (*see* Materials and Methods). Calculated net ionic transport rates for each tubule are shown in Table 3. This value agrees well with the rate of ^{22}Na efflux (lumen to bath flux) measured in eight open-circuit tubules (Table 2). While the backflux of Na^+ was not determined, it seems reasonable to assume it is quite low in light of the relatively high value obtained for specific resistance. Since the ^{22}Na efflux rate and the net ion transport rate as calculated from the short-circuit current are similar values it is tempting to speculate that in this nephron segment the active transport of sodium largely accounts for the movement of charge under short-circuit conditions. It should be noted that these fluxes need not be equal, since distal nephron segments may transport other ion species. In addition, in other epithelia [18, 19] the open-circuit voltage is believed to reduce the active transport of sodium. However, the relatively low spontaneous voltage in this epithelium (−8.9 mV) represents a substantially smaller electrical driving force than that normally observed across the frog skin [18].

Table 3. Electrical characteristics of *Ambystoma* collecting duct

Tubule	Luminal diameter (μm)			Specific resistance (10^5 Ω cm)			Calculated transport rate
	optical	electrical	Δ	R_t	R^*	Δ	(pM mm^{-1} min^{-1})
1	106.6	104.7	− 1.9	0.57	0.42	−0.15	3.4
2	33.3	48.7	+15.4	0.43	0.13	−0.30	17.7
3	43.3	44.5	+ 2.2	0.20	0.38	0.18	22.8
4	36.6	27.5	− 9.1	0.20	0.26	0.06	9.0
5	103.2	88.6	−14.6	0.25	0.32	0.07	63.3
Mean	64.6	62.8	1.6	0.33	0.30	−0.03	23.3
SEM	± 16.6	± 14.5	± 5.1	±0.08	±0.05	±0.08	±10.8

Optical luminal diameters were measured with an ocular micrometer. Electrical diameters were calculated using the equations of Halman [9]. R_t is the specific resistance calculated by core conductor analysis and R^* is the specific resistance calculated from Eq. (1). *See* Materials and Methods for the calculation of transport rate.

It is possible that the failure to use Sylgard pipettes may have led to leaks at the ends of the tubule. Since such leaks would cause major errors in both the electrical analysis and the homogeneity of the voltage clamp, we evaluated this possibility. The electrical diameter of each tubule was calculated from the core resistance (R_c) and the membrane resistance (R_t) and compared to the optical diameter as measured with an eyepiece micrometer [9]. These data are also presented in Table 3. The calculated electrical diameter of the five collecting ducts averaged 62.8 ± 14.5 μm while the optical diameter of these same tubules averaged 64.6 ± 16.6 μm. The fact that the difference between the electrical and optical diameters was only 1.8 ± 5.1 μm suggests that the electrical analysis of tubular resistance is not confounded by large leaks.

In an effort to demonstrate further the uniformity of the voltage clamp along the length of the tubule, the perfusion end voltage was plotted against that at the collecting end while each of the collecting ducts was clamped at various potentials between +20 and −20 mV. This relationship is shown in Fig. 6. The potential at the collection end of a voltage-clamped tubule is not significantly different from that at the perfusion end over the range studied. While these values are not significantly different from the line of identity, there appears to be a bias such that the values are all about 1.5 mV more negative at the collecting end regardless of the polarity of the clamps. This disparity is in very good agreement with the difference between the two spontaneous voltages. If the voltage clamps were not uniform, we would have expected the line to intercept at zero and rotate around that point rather than reflect a consistent polarity bias. At this time it is impossible to be certain whether the bias is due to physiologic differences at the two ends of the tubule (hererogeneity of the tubule fragment) or some small consistent leak at the perfusion end. In either case, within reasonable limits, the spontaneous voltage and clamp voltages are relatively uniform down the length of the tubules.

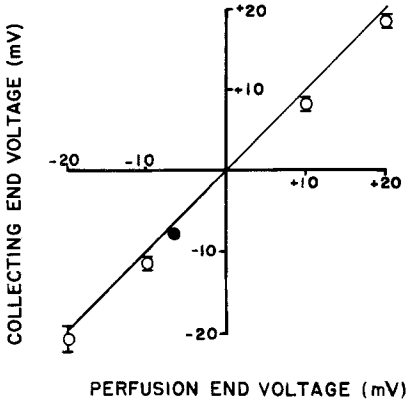

Fig. 6. Relationship between voltage measured at the perfusion (abcissa) and collecting ends (ordinate) of voltage-clamped *Ambystoma* collecting ducts. The line of identity is provided. Open circles are data from voltage-clamp experiments. Closed circle is the spontaneous voltage measured prior to voltage clamping. The bars represent 1 SEM

Discussion

The present study involves the development of a miniature, axial Ag-AgCl electrode which can be inserted into the lumen of an isolated perfused amphibian collecting duct and is capable of passing currents sufficient to short circuit the tubule. We feel that the design of this electrode offers several advantages. A considerable degree of mechanical flexibility results from the use of a glass core for the electrode tip. Unlike silver wire electrodes, silver-plated glass fibers will withstand considerable distortion and still return to their original shape. This quality has proven quite valuable during the process of centering the electrode within the inner perfusion pipette. Secondly, the use of a glass capillary to hold the silver fiber provides excellent insulation for all but the exposed Ag-AgCl tip. Finally, the compatibility of this electrode with the *in vitro* microperfusion technique allows both the electrical and chemical gradients across the epithelium to be carefully controlled.

In using an axial Ag-AgCl electrode capable of short-circuiting a renal tubule, several criteria had to be met. The first of these was that the tubule be homogeneous. In order to minimize variability of function along the tubule length, short segments were used. The fact that the mean potential difference between the perfusion and collection ends was only 1.1 ± 0.5 mV indicates that the electrical variability was $< 15\%$ in the tubules studied.

A related question concerns the uniformity of the voltage clamp provided by the electrode. Figure 6 shows that when the tubule is clamped over a 40-mV range (+20 to −20 mV) the potential at the collecting end is not significantly different from that at the perfusion end. Thus we feel that within reasonable limits the spontaneous voltage and clamp voltage are relatively uniform down the length of the tubule.

An essential feature of any short-circuit electrode is stability. The Ag-AgCl electrode described in this study is capable of passing current of 5.5×10^{-7} A for at least 10 min. Throughout this period, the amount of current passed as well as the resistance of the electrode remains stable. Since the mean short-circuit current for the tubules studied was 1.6×10^{-8} A, the current-passing capability of this electrode is at least an order of magnitude greater than

that needed to short circuit the *Ambystoma* collecting tubule. The stability of the electrode and voltage clamp under short-circuit conditions is demonstrated on the oscilloscope tracing in Fig. 3. It can be seen the initiation and termination of the voltage clamp result in a square wave form with no evidence of voltage transients.

A further indication that the short-circuit electrode is functioning in an appropriate manner is the fact that the mean net ionic transport rate calculated from the short-circuit current (23.3 ± 10.8 pM/mm min) is not significantly different from the mean lumen-to-bath transport rate as determined by Na^+ flux measurements.

One potential criticism of this electrode is the possibility of Ag metal poisoning of the tubule. While such a possibility cannot be completely ruled out, there are several reasons why we do not believe this to be the case. The first of these is the fact that the use of the *in vitro* microperfusion technique coupled with the relatively large lumen diameter of these segments enabled us to use very high fluid flow rates. Thus any silver metal which might result from the presence of the short-circuit electrode could be rapidly diluted and washed out of the tubule lumen. Second, the transepithelial potential measured at both ends of the tubule following the end of the short-circuit period are stable and unchanged from those observed during the initial control period. If indeed the tubular epithelium was in some way adversely affected by the presence of the Ag-AgCl electrode within the lumen, one might expect to see a decrease in the transepithelial potential. Thirdly, the transepithelial resistance measured with the short-circuit electrode is not significantly different from that obtained by the core conductor method. If the presence of the electrode was in some way damaging to the tubule, one might expect to see changes in both the transepithelial resistance and the short-circuit current with time. Such changes were not observed in this study. Lastly, the mean net ion transport rate calculated from the short-circuit current is not significantly different from that determined by sodium flux measurements. In view of these observations we conclude that silver poisoning due to the use of this electrode probably does not present a significant problem under the conditions of this study.

The methodology used in this study to short circuit the renal tubule differs in a number of respects from that described by Spring and Paganelli [15]. The use of *in vitro* microperfusion techniques allows the short-circuit and voltage-clamp experiments to be carried out under free-flow conditions thus eliminating the possibility of H^+ ion or nonreabsorbable solute accumulating in the lumen. Double cannulation of the nephron segment permits some assessment of tubule homogeneity as well as the uniformity of the voltage clamp. Modifications of this technique may also permit the ionic composition of the fluid on both sides of the epithelium to be changed during the experiment. Hence it is possible that in the future, ion substitution experiments as well as bidirectional radioisotope fluxes may be carried out under short-circuit conditions. An additional advantage of this system arises from the fact that the *in vitro* microperfusion technique is not limited to the investigation of surface nephron segments. Thus other amphibian nephron segments may be studied with the methods presently described.

The authors are grateful for the expert technical assistance of Marilyn Dixon and for the many helpful suggestions of Drs. Irwin Weiner and Ernest Natke Jr. This study was supported by NIAMDD Grant # 5R01-AM-1952103 and Biomedical Research Support Grant # 2S07RR05402-20.

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Received 17 March 1981; revised 23 June 1981