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Microelectrode Study of K^+ Accumulation by Tight Epithelia: I. Baseline Values of Split Frog Skin and Toad Urinary Bladder

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Summary. Toad bladder and split frog skin were impaled with fine-tipped single- and double-barrelled K^+-selective microelectrodes. In order to circumvent membrane damage induced by impaling toad bladder, a null point method was developed, involving elevations of mucosal potassium concentration. The results suggest that intracellular potassium activity of short-circuited toad bladder is approximately 82 mM, twice as large as earlier estimates. Far more stable and rigorously defined intracellular measurements were recorded from short-circuited split frog skins. The intracellular positions of the micropipette and microelectrode tips were verified by transient hyperpolarizations of the membrane potential with mucosal amiloride or by transient depolarizations with serosal barium or strophanthidin. Simultaneous impalement of distant cells with separate micropipettes demonstrated that both the baseline membrane potentials and the responses to depolarizing agents were similar, further documenting that frog skin is a functional syncytium. Measurements with double-barrelled microelectrodes and simultaneous single-barrelled microelectrodes and reference micropipettes suggest that the intracellular potassium activity is about 104 mM, lower than previously reported. Taken together with measurements of intracellular potassium concentration, this datum suggests that potassium is uniformly distributed within the epithelial cells.

Key Words syncytium · barium · strophanthidin · null point technique · subcellular compartmentalization · junction potentials

Introduction

Knowledge of the chemical activity of intracellular potassium is of value in several respects. First, as summarized elsewhere (Civan, 1978), intracellular potassium activity may play a role in regulating a number of cellular activities. Second, an apparent activity coefficient (γ_K^{app}) can be calculated by dividing the potassium activity by the average intracellular potassium concentration. Comparison of γ_K^{app} with the true activity coefficient for potassium (γ_K) in the extracellular fluid can provide information concerning the possible significance of subcellular ionic compartmentalization (e.g., Palmer, Century & Civan, 1978). Third, knowledge of the intracellular and serosal activities of potassium permits calculation of the reversal potential for potassium across the basolateral membrane. The reversal potential can serve as a useful biological standard in measuring the absolute membrane potential under certain experimental conditions (Brown & Brown, 1973). In addition, some investigators have found it experimentally useful to reduce the reversal potential to zero (Fuchs, Hviid Larsen & Lindemann, 1977; Palmer, Edelman & Lindemann, 1980).

Despite the interest in such measurements, published estimates of intracellular potassium activity have varied over a very wide range. Early studies of amphibian urinary bladder suggested that the intracellular potassium activity (a_K^c) was 40 to 50 mM (Kimura et al., 1977; Kimura & Fujimoto, 1977; DeLong & Civan, 1978). In contrast, Nagel, Garcia-Diaz and Armstrong (1981) have measured a_K^c to be 132 ± 10 mM in frog skin. In preliminary communications, we have reported estimates of cellular potassium activity of toad bladder which lie between these two sets of values (DeLong & Civan, 1978, 1979). That conclusion is supported by further information reported in this manuscript obtained with split frog skin. During the course of making these measurements, we have come to make use of transient applications of strophanthidin or of barium routinely. In the measurements of toad bladder, application of a null point approach proved very helpful. These techniques may also be of value to other investigators in helping to establish the reliability of intracellular electrometric measurements.

Materials and Methods

Preparations and Solutions

Female specimens of the toad *Bufo marinus* were obtained from the Dominican Republic (National Reagents, Inc., Bridgeport, Conn.) and maintained on moist sphagnum moss at room tem-

perature. Frogs (*Rana pipiens pipiens*) were purchased from West Jersey Biological Supply (Wenonah, N.J.) and Connecticut Valley Biological (South Hampton, Mass.) and kept at room temperature with access to water. Both toads and frogs were periodically force-fed mealworms. After the animals were doubly pithed, the urinary bladders were excised from the toads, and the abdominal skins from the frogs. Before the frog skins were mounted, the underlying corium was removed, using the technique of Fisher, Erlij and Helman (1980). After gently scraping the inner surfaces of the skins, these surfaces were exposed to collagenase (0.75 mg ml^{-1}; CLS II, Worthington, Millipore, Freehold, N.J.) for 1 to 2 h.

Toad bladders were mounted mucosal side up and split frog skins were mounted inner surface up between the two halves of a Lucite chamber (DeLong & Civan, 1978). Toad bladders were supported by a Millipore filter and underlying stainless steel screen; split frog skins were supported by a finer mesh stainless steel screen alone. Tissue areas of 1.9 cm^2 were exposed for experimental study. The serosal solution was aerated. In most of the experiments, the serosal and mucosal media were circulated separately across the tissue surfaces and then discarded. In three early experiments (Table 3*B*), the mucosal medium was not circulated, but rather replaced periodically.

The hydrostatic pressure of the serosal medium bathing the tissues was reduced by approximately 5 to 10 torr in order to help immobilize the tissue for intracellular recording.

In all of the experiments to be described, the serosal or inner medium consisted of a standard Ringer's solution (*R*) (Table 1) whose pH was 7.6 to 7.8 and osmolality 215 to 225 mOsm. The mucosal or outer surface was bathed with a number of modified Ringer's solutions of similar pH and osmolality, but containing a range of potassium concentrations from 1.4 to 80.1 mM (Table 1).

Amiloride was generously provided by Dr. George M. Fanelli, Jr. (Merck Institute for Therapeutic Research, West Point, Pa.). Strophanthidin (Grade I) was obtained from Sigma Chemical Co. (St. Louis, Mo.).

Micropipettes and Microelectrodes

Single-barrelled micropipettes and microelectrodes were drawn from omega-dot borosilicate capillary glass tubing having an outer diameter of 1.5 mm and an inner diameter of 0.75 mm (Glass Company of America, Millville, N.J.). Double-barrelled microelectrodes were constructed from theta glass tubing having 1.5 mm outer diameter, 0.2 mm wall thickness and 0.4 mm septum thickness (Glass Company of America). The micropipettes and microelectrodes were drawn with a Brown-Flaming micropipette puller (Model #P-77, Sutter Instrument Co., San Francisco, Calif.).

Longer, finer tips were constructed in impaling toad bladder than in puncturing frog skin. When filled with 3 M KCl solution, single-barrelled micropipettes used for toad bladder displayed resistances of up to 100 MΩ, when measured in the standard Ringer's solution; under comparable conditions, micropipettes for impaling split frog skin had resistances of approximately 40 MΩ.

The potential sensed by the reference barrels of fine double-barrelled microelectrodes depended upon the ionic composition of the test solution (Walker, 1976: Acker, 1978: DeLong & Civan, 1979). Equimolar replacement of KCl for NaCl was without effect, while large changes in potential were observed in comparing simple aqueous solutions containing 10 and 100 mM KCl. Thus, the effect seemed to be a function of ionic strength. However, very little, if any, change was found upon increasing the ionic strength beyond that of Ringer's solution *R*. The intracellular ionic strength should be higher than that of Ringer's since: (i) osmotic equilibrium is thought to hold across the basolateral membrane, and (ii) polyanions are constrained to the intracellular compartment. Therefore, the measured membrane potential should be little affected by the ionic strength-dependence of the reference micropipette. However, to ensure that such was the case, the composition-dependence was suppressed by filling the reference barrels of the double-barrelled microelectrodes with 1 M magnesium acetate solution (Acker, 1978; DeLong & Civan, 1979). As discussed in the Results, account was taken of the junction potentials established between the reference filling solution and test medium.

Several different techniques were used to coat the inner glass surface of the K^+-selective microelectrodes. Single-barrelled microelectrodes for impaling toad bladder were exposed to dimethyldichlorosilane or trimethylchlorosilane vapor before baking at 200 °C for 2 h. The K^+-sensitive barrel of double-barrelled microelectrodes for studying toad bladder was coated by introducing a drop of one-third trimethylchlorosilane and two-thirds xylene before baking. For the subsequent impalements of frog skin, the basic technique of Garcia-Diaz and Armstrong (1980) was followed. The inner glass surface of the K^+-selective barrel was coated by first dipping the microelectrode for approximately 15 s in a 0.2% solution of silicone (DC 1107, Dow Corning, Midland, Mich.) in acetone, and then heating for about 60 min on a hot plate. The reference barrels of these double-barrelled microelectrodes were protected by being filled with deionized water before the dipping procedure. After the coating was completed by any of these techniques, a small volume of potassium-selective liquid resin (#477317, Corning Glass, Corning, N.Y.) was placed in the back of the barrel. After the tip filled spontaneously to a length of 1 to 2 mm, the barrel was backfilled with a reference solution of 0.5 M KCl.

The voltage outputs from both reference and potassium-selective barrels were sensed with chlorided silver wires.

The voltage output (E_K) of the potassium-sensitive barrel was fitted by the empirical equation:

$$E_K = E_o + S \log(a_K + k a_{Na}) + \psi \tag{1}$$

where E_o, S and K are constants, a_K and a_{Na} are the chemical activities of K^+ and Na^+, respectively and ψ is the electrical potential. In the 29 experiments to be described, S and k were 59.0 ± 0.4 mV and 0.030 ± 0.002, respectively. (Throughout the current manuscript, data are reported as the mean $\pm$SEM, with the numbers of measurements entered in parentheses.) In view of the high selectivity for K^+ over Na^+ (low value of k), it was unnecessary to correct the measured values of E_K within the cell for the contribution of intracellular Na^+. Therefore, the intracellular potassium activity (a_K^c) can be calculated from Eq. (1) as:

$$a_K^c = (a_K + k a_{Na})_e \, 10^{(\Delta E_K^c - \Delta\psi_c)/S} \tag{2}$$

where the subscripts or superscripts "c" and "e" refer to the intracellular and extracellular phases, respectively, and where:

$$\Delta E_K^c \equiv E_K^c - E_K^e \tag{3}$$

$$\Delta\psi_c \equiv \psi_c - \psi_e. \tag{4}$$

All data to be reported were obtained under short-circuited conditions. Therefore, using the above convention, membrane potentials measured either by basolateral impalements from the serosal medium or by apical punctures from the mucosal medium should be of identical magnitude and sign.

Both split frog skins and toad bladders were impaled by mounting the microelectrodes and micropipettes on modified Huxley micromanipulators (Huxley, 1961). Minimal criteria for acceptability of penetrations included: (i) rapid changes in volt-

age on entering and leaving the cell, (ii) constancy of the voltages measured by the K^+-selective microelectrode and reference micropipette in the external Ringer's solution before and after impalement to within a few millivolts; usually the changes in potential were less than 3 mV, and (iii) constancy of the value of S to within 3 mV, when measured before and after impalements. Additional criteria are discussed below.

Electronics

A conventional four-electrode system was used to measure and clamp the transmural potential of the preparations. Voltage was sensed and current passed through agar salt bridges in series with Ag−AgCl half-cells. The transepithelial potential ($\Delta\psi_T$) was held at 0 mV, except for periodic hyperpolarizations of 10 to 20 mV for durations of 3 to 10 s (Civan & Hoffman, 1971). Transepithelial current (I_T) was monitored by measuring the voltage drop across a known resistance in series with the tissue.

The outputs of the microelectrodes and micropipettes were introduced either into a single-input preamplifier (Model 602, Keithley Instruments, Cleveland, Ohio) or into a differential-input preamplifier Model F-223A, WP Instruments, Inc., New Haven, Conn.), operated in driven shield configurations. The output voltages of the K^+-selective (E_K) and reference (ψ) barrels, as well as I_T and often ($E_K-\psi$) were continuously displayed on two dual-pen chart recorders in the studies of split frog skin. In all experiments, E_K and ψ were displayed continuously on a storage oscilloscope.

Results

Single-Barrelled Impalements of Toad Bladder

Using the finer-tipped micropipettes, the membrane potential ($\Delta\psi_c$) was found to be more negative than in previous studies of short-circuited toad bladder (Frazier, 1962; Civan & Frazier, 1968; Reuss & Finn, 1974; DeLong & Civan, 1978). Stable values of −25 to −30 mV were occasionally recorded. At the other extreme. $\Delta\psi_c$ was also sometimes found to be only −4 mV under baseline conditions. However, most commonly, a large negative deflection was initially associated with impalement, decaying over a period of 15 to 20 s towards the value of some −4 mV reported in previous studies. For purposes of data reduction, $\Delta\psi_c$ was taken to be the potential measured 1 s after puncture, corresponding to the time at which peak deflections of ΔE_K^c were commonly observed.

As illustrated Fig. 1*b*, cellular impalement was also sometimes associated with a deflection in E_K which remained stable for 10 to 30 s. However, such stable plateaus were encountered in only 5 to 10% of the penetrations conducted with the standard Ringer's solution serving as mucosal medium. In the great majority of the impalements, ΔE_K began to decline a few seconds after reaching a maximal value.

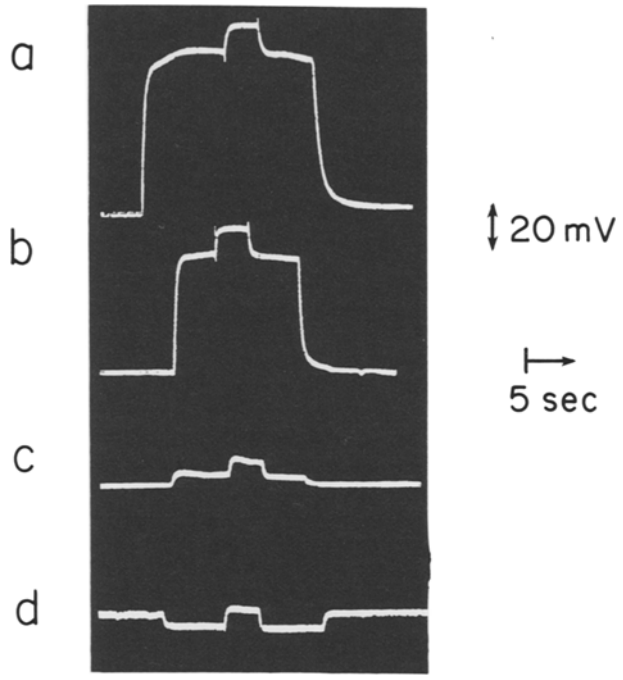

Fig. 1. Impalements of short-circuited toad bladder with a single-barrelled microelectrode as a function of mucosal potassium concentration. The records are from Exp. 2, Table 3*B*. The initial and final deflections of each trace reflect entry and exit, respectively, of the microelectrode into and out of the cell. The voltage pulse in the center of each trace constitutes the response of the apical membrane potential to periodic pulses applied across the entire preparation. The mucosal media (Table 1) bathing the bladder were (in chronological order): (*a*) 1.4*K*, (*d*) 80-*Kb*, (*c*) 41-*Ka* and (*b*) *R*. Although the traces might suggest an inverse dependence of apical fractional resistance on mucosal K^+ concentration, no such relationship was systematically observed

The instabilities of ΔE_K^c and $\Delta\psi_c$ indicated that impalements of toad bladder are commonly associated with significant membrane damage, as suggested by several previous reports (Lindemann, 1975; Higgins, Gebler & Frömter, 1977; Sudou & Hoshi, 1977). In order to deal with this technical difficulty, a null point technique was developed. The rationale for this approach is that impalement-induced damage should not lead to redistribution of potassium, so long as the electrochemical force driving K^+ is the same inside and outside the cell. Accordingly, impalements were performed with mucosal solutions containing a wide range of potassium concentrations (Table 1). This technique is applicable to impalements of toad bladder from the mucosal medium because K^+ (Kallus & Vanatta, 1970; Guggenheim, Bourgoignie & Klahr, 1971; Robinson & Macknight, 1976) and Cl^- (Macknight, 1977) are reported to exchange much more slowly across the apical than across

Table 1. Compositions of Ringer's solutions (in mM)

Component	1.4 K	3.5 K	*R*	41 *K-a*	41 *K-b*	80 *K-a*	80 *K-b*
Na^+	47.7	38.4	115.1	43.0	38.4	35.0	38.4
K^+	1.4	3.5	3.5	40.7	40.7	80.0	80.1
Ca^{2+}	0.3	0.8	3.8	0.6	0.8	0.8	0.8
Cl^-	44.9	37.4	113.9	79.4	75.0	110.5	113.9
HCO_3^-	0.9	2.2	2.2	1.5	2.2	2.2	2.2
HPO_4^{2-}	1.8	1.8	1.8	1.8	1.8	1.8	1.8
$H_2PO_4^-$	0.3	0.3	0.3	0.3	0.3	0.3	0.3
Sucrose	146.7	146.7	0	73.3	73.3	0	0

Table 2. Calculated intracellular potassium activity (mM) as a function of mucosal medium bathing short-circuited toad bladder

Solution	Experiment		
	1	2	3
1.4 *K*	58±1.6 (11)	47±2.5 (10)	–
3.5 *K*	–	–	48±0.9 (9)
R	54±1.2 (12)	52±1.9 (14)	64±1.5 (13)
41 *K-a*	72±1.0 (9)	69±1.2 (8)	–
41 *K-b*	–	–	72±1.2 (12)
80 *K-b*	72±1.5 (10)	71±2.0 (8)	72±2.1 (10)

Measurements were obtained by separate impalements with single-barrelled microelectrodes and micropipettes. The reference micropipette was filled with 3 M KCl solution. No corrections have been included to take account of the theoretical junction potentials.

the basolateral membrane. For this reason, altering the mucosal K^+ and Cl^- concentrations are unlikely to affect the intracellular K^+ activity seriously. On the other hand, the mucosal Na^+ concentration was maintained relatively constant.

Increasing the concentration of potassium (c_K^m) in the mucosal medium significantly improved the stability of the intracellular recordings; some 25 to 40% of the impalements now exhibited prolonged voltage plateaus. Figure 1 presents the time courses of E_K before, during and after impalements of the same tissue in the presence of four different mucosal solutions. When c_K^m was 1.5 to 3.5 mM, ΔE_K^c was strongly positive (Fig. 1 *a, b*). With c_K^m = 40.7 mM, ΔE_K^c was close to zero. When c_K^m was increased to 80.1 mM, ΔE_K^c actually reversed sign.

The values of a_K^c calculated with Eq. (2) for each of the mucosal media are presented in Table 2. No correction has been applied to take account of the junction potential between the 3 M KCl solution of the exploring micropipette and the medium surrounding the tip. The calculated values of a_K^c are low when c_K^m = 1.5 to 3.5 mM. However, the estimated value of a_K^c does not change further upon increasing mucosal K^+ concentration from 40.7 to 80.1 mM.

The results obtained with Ringer's solution 80 K-b are presented in extended form in Table 3 *B*. Here, the mean values of ΔE_K^c and $\Delta \psi_c$ are tabulated, as well as the estimates of a_K^c directly

Table 3. Measurements of intracellular potassium activity (a_K^c) in short-circuited toad bladder[a]

Microelectrodes	Exp.	ΔE_K^c (mV)	$\Delta \psi_c$ (mV)	a_K^c (mM)		
				Directly calculated	Normalized to 3 M KCl	Corrected for junction potentials
A. Double-barrelled (1 M Mg-Acetate reference solution)	1 (14)	−3±0.4	−2±0.5	65±0.9	74.	81
	2 (18)	0±0.5	−5±0.3	72±1.5	83.	92
	3 (23)	−2±0.2	−4±0.3	65±0.7	74	81
	4 A (10)[b]	−2±0.4	−1±0.3	63±1.4	71	78
	4 B (16)[c]	−1±0.1	0±0.0	65±0.3	73	80
	5 A (10)[b]	−2±0.4	−1±0.3	62±0.7	71	77
	5 B (14)[c]	−1±0.4	−1±0.2	67±0.8	76	83
	Mean±SEM (7)	−2±0.4	−2±0.7	66±1.2	75±1.6	82±1.8
B. Separate single-barrelled (3 M KCl reference solution)	1	−6±0.5 (10)	−7±0.8 (7)	72±1.5		78
	2	−5±0.8 (8)	−5±1.7 (4)	71±2.0		77
	3	−4±0.6 (10)	−10±0.7 (11)	72±2.1		79
	Mean±SEM (3)	−5±0.6	−7±1.5	72±0.3		78±0.5

[a] Measured in the presence of a mucosal K^+ concentration of 80.1 mM.
[b, c] Measured before ([b]) and after ([c]) transient periods of K^+ depletion.

calculated from Eq. (2). In the final column, the estimates of a_K^c are modified by taking the junction potentials (V_{jcn}) of the reference micropipettes into account. The correction has been performed by applying the Henderson equation (MacInnes, 1961):

$$V_{jcn} = -(RT/F) \frac{\sum_i u_i(a_i'' - a_i')}{\sum_i z_i u_i(a_i'' - a_i')} \ln \left\{ \frac{\sum_i z_i u_i a_i''}{\sum_i z_i u_i a_i'} \right\} \quad (5)$$

where V_{jcn} is the potential of the reference solution ($''$) relative to that of the test solution ($'$), R is the perfect gas constant, T is absolute temperature, F is the Faraday constant, and u_i, a_i and z_i are the mobility, chemical activity and valence, respectively, of ion "i". Ions other than Na^+, K^+ and Cl^- are omitted from the summations. Predictions based upon this approach have been found to be in good agreement with the differences in junction potential measured in *Chironomus* salivary gland by intracellular micropipettes filled with different reference solutions (Palmer & Civan, 1977). In the calculations, the intracellular concentrations of the Na^+ (c_{Na}^c), K^+ (c_K^c) and Cl^- (c_{Cl}^c) have been taken to be 23, 147 and 36 mM, respectively (Civan, Hall & Gupta, 1980). The activity coefficients of Na^+, K^+ and Cl^- have been taken to be 0.76 both in the mucosal Ringer's solution and the intracellular fluids. For Table 3*B*, V_{jcn} has been calculated to be 3.6 mV inside the cell and 1.3 mV outside. Therefore, the difference in junction potentials ΔV_{jcn}^c should have made the measured membrane potential 2.3 mV more positive than the true value of $\Delta\psi_c$.

Double-Barrelled Impalements of Toad Bladder

The results of Table 3*B* strongly suggest that the intracellular potassium activity of short-circuited toad bladder is approximately twice as high as values previously reported. However, these data were based on separate impalements of different cells with potassium-selective microelectrodes and reference micropipettes. In principle, diffusion of salt out of the nonselective micropipette could modify the state of the impaled cell, a point emphasized by Nelson, Ehrenfeld and Lindemann (1978); this phenomenon would not proceed in a cell separately impaled with a K^+-selective microelectrode. In such an event, direct comparison of $\Delta\psi_c$ and ΔE_K^c would be inappropriate.

This problem was addressed by using double-barrelled K^+-selective microelectrodes to impale toad bladder. The data obtained in seven determinations are presented in Table 3*A*. The size of the double-barrelled tips was necessarily larger than that of the single-barrelled micropipettes and microelectrodes. Presumably for this reason, the measured values of $\Delta\psi_c$ were smaller than in the earlier experiments of Table 3*B*. The reference barrels in these later studies were filled with 1 M magnesium acetate solution. In order to correct for the junction potential, a two-step procedure was employed. First, the measured values of $\Delta\psi_c$ have been normalized to the values which would have been measured with 3 M KCl-filled micropipettes. Second, the Henderson equation was used to correct for the junction potential likely associated with 3 M KCl micropipettes, as in the calculations for Table 3*B*. The first correction term was derived from impalement of 11 cells with double-barrelled micropipettes, whose twin barrels were filled with 1 M magnesium acetate and 3 M KCl solutions. The membrane potential measured with the magnesium acetate solution was consistently more positive by 3.4 ± 1.2 mV; this value was used in calculating the estimates of a_K^c presented in the penultimate column of Table 3*A*. The final column tabulates the values of a_K^c, calculated from application of the Henderson equation.

The measurements obtained by double-barrelled impalements of toad bladder in the presence of elevated mucosal potassium concentrations suggest that a_K^c is 82 ± 2 mM. This estimate is similar to that obtained with separate single-barrelled impalements.

Preliminary Measurements of $\Delta\psi_c$ in Split Frog Skin

In contrast to the technical difficulties associated with impaling toad bladder, satisfactory intracellular records can be consistently obtained with frog skin (Nagel, 1976*a*; Helman & Fisher, 1977). Under favorable circumstances, stable membrane potentials can sometimes be monitored for hours. It therefore seemed desirable to measure a_K^c by impaling split frog skins with double-barrelled microelectrodes.

As discussed above, calculations of a_K^c depend, in part, upon estimating the junction potential arising at the interface between the test solution and reference medium filling the micropipette. It seemed unwise to assume that the difference in junction potentials between 1 M magnesium acetate- and 3 M KCl-filled reference barrels measured in toad bladder would necessarily hold for frog skin. For this reason, a series of preliminary experiments was performed in which $\Delta\psi_c$ was mea-

Table 4. Membrane potentials (in mV) of short-circuited split frog skin measured with micropipettes containing different filling solutions

A.	Exp.	3 M KCl	0.5 M KCl	Δ
	1	-77 ± 1.6 (4)	-79 ± 0.5 (4)	-1.3
	2	-85 ± 1.5 (3)	-76 ± 1.1 (6)	9.0
	3	-80 ± 1.4 (7)	-75 ± 0.6 (10)	5.4
Mean $\pm$ SEM				4.4 ± 3.0
B.	Exp.	3 M KCl	1 M Mg-Acetate	Δ
	1	-68 ± 2.0 (7)	-66 ± 2.4 (4)	2.0
	2	-72 ± 1.3 (5)	-68 ± 1.4 (4)	4.0
	3	-77 ± 2.4 (5)	-76 ± 1.2 (4)	0.5
Mean $\pm$ SEM				2.2 ± 1.0

The number of satisfactory impalements for each measurement is entered in parentheses.

sured in separate impalements of the same tissue performed with micropipettes containing different reference solutions. The results are presented in Table 4.

As in the case of toad bladder, the membrane potential measured with 1 M magnesium acetate-filled micropipettes was slightly more positive than that measured with 3 M KCl-filled barrels. The mean value of 2.2 ± 1.0 mV (Table 4*B*) was used in the calculations of Table 5*A*, and is not significantly different from the value of 3.4 ± 1.2 mV measured in toad bladder.

As discussed below, the measurements of $\Delta\psi_c$ entered in Table 5*B* were carried out with micropipettes filled with 0.5 M KCl solution. In order to normalize these data to measurements obtained with 3 M KCl filling solutions, an additional set

Table 5. Measurements of intracellular potassium activity (a_K^c) in short-circuited split frog skin

Microelectrodes	Exp.	ΔE_K^c (mV)	$\Delta\psi_c$ (mV)	a_K^c (mM)		
				Directly calculated	Normalized to 3 M KCl	Corrected for junction potentials
A. Double-Barrelled (1 M Mg-Acetate reference solution)	1A	-6	-75	81	88	99
	1B	-10	-80	83	90	102
	1C	-7	-67	57	62	70
	2A	0	-79	118	129	145
	2B	0	-68	76	84	94
	2C	2	-70	90	98	110
	3	-8	-83	101	111	126
	4A	14	-54	59	64	72
	4B	5	-67	68	73	82
	4C	7	-66	70	76	85
	Mean $\pm$ SEM	-0.2 ± 2.4	-71 ± 2.7	80 ± 6.0	88 ± 6.6	98 ± 7.6
Microelectrodes	Exp.	ΔE_K^c (mV)	$\Delta\psi_c$ (mV)	a_K^c (mM)		
				Directly calculated	Normalized to 3 M KCl	Corrected for theoretical junction potential
B. Simultaneous single-barrelled (0.5 M KCl reference solution)	1A	-6	-69	73	87	97
	1B	-6	-73	89	106	118
	1C	-8	-66	76	90	101
	1D	-9	-68	82	97	109
	1E	-2	-68	88	105	117
	2A	-4	-73	70	84	94
	2B	-1	-76	81	97	108
	2C	-13	-85	75	89	100
	3	-11	-84	70	82	92
	Mean $\pm$ SEM (9)	-6 ± 1.3	-73 ± 2.3	78 ± 2.4	93 ± 2.9	104 ± 3.2
Total	Mean $\pm$ SEM (19)	-3 ± 1.6	-72 ± 1.8	79 ± 3.3	90 ± 3.7	101 ± 4.2

of experiments was performed, comparing the results obtained in the same tissue with micropipettes containing 0.5 and 3.0 M KCl solutions. The measured ΔV_{jcn} was 4.4 ± 3.0 mV (Table 4*A*), not very different from the theoretical value of 6.2 mV which can be calculated from Eq. (5). The experimentally determined value is used in the calculations of Table 5*B*.

Double-Barrelled Impalements of Split Frog Skin

In a series of 10 experiments, split frog skins were impaled with double-barrelled microelectrodes. In addition to the minimal criteria for acceptability presented in Materials and Methods, these successful penetrations provided stable records for approximately 1 min or longer. In two experiments (Exps. 4A and 4C of Table 5*A*), additional verification was obtained. As illustrated in Fig. 2, addition of 10^{-4} M amiloride to the mucosal medium produced a hyperpolarization of the membrane potential and a fall in ΔE_K^c. The time courses and magnitudes of the negative deflections were similar for $\Delta \psi_c$ and ΔE_K^c.

The individual and mean values of ΔE_K^c, $\Delta \psi_c$ and the estimates of a_K^c calculated directly from Eq. (2) are entered in Table 5*A*. The values of a_K^c modified by normalization to 3 M KCl and by correction for junction potential are presented in the final two columns. Based on these data, the intracellular potassium activity is estimated to be 98 ± 8 mM under baseline conditions. It will be appreciated that the data of this Table are far more satisfactory than those which could be obtained with toad bladder, for several reasons. First, stable records could be obtained over more prolonged periods of time. Second, in at least two experiments, the response to amiloride provided verification of the intracellular location of the microelectrode tip. Third, the measurements could be obtained in the presence of conventional Ringer's solutions bathing the two surfaces of the tissue. Fourth, penetrations were performed across the basolateral (rather than apical) membrane, minimizing the effect of impalement-induced damage on the membrane potential of short-circuited tight epithelia (Higgins et al., 1977).

Despite the above considerations, impalement of split frog skin with double-barrelled microelectrodes was not an entirely satisfactory approach to the problem. The greatest difficulty was posed by the infrequency of fabricating a double-barrelled microelectrode small enough to impale the membrane without unacceptable membrane damage. Even the results of successfull experiments

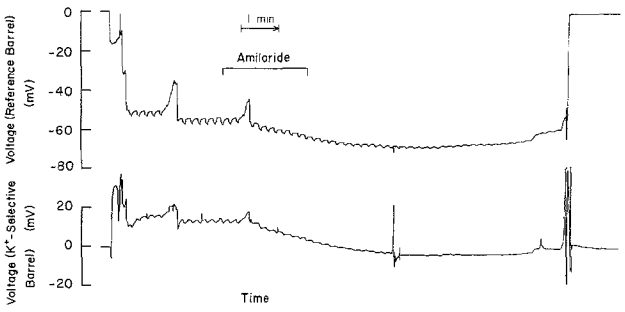

Fig. 2. Impalement of short-circuited split frog skin with a double-barrelled microelectrode. The reference barrel was filled with 1 M magnesium acetate. Upon penetrating the basolateral membrane, the membrane potential ($\Delta \psi_c$) was found to be -54 mV, while the potassium-selective barrel recorded a voltage change (ΔE_K^c) of approximately 14.5 mV (Exp. 4A, Table 5*A*). Brief perfusion of the external surface with Ringer's solution containing 10^{-4} M amiloride elicited changes in $\Delta \psi_c$ and ΔE_K^c of -14.5 and -18.5 mV, respectively. The voltage deflections are responses to alternately clamping the transepithelial potential to 0 (upper envelope of each trace) and to 10 mV (lower envelopes). The onset and offset of the amiloride effect were particularly slow because of the slow rate of mucosal superfusion during this experiment (in an effort to reduce the likelihood of dislodging the microelectrode). The fact that amiloride continued to markedly inhibit sodium entry well after nominal removal of the diuretic was documented by the observation that short-circuit current did not begin to recover until after the micropipettes were withdrawn.

Two perturbations of the voltage traces are apparent, occurring 1.3 min before and 0.6 min after the addition of amiloride. These disturbances reflected an artifact produced by the chart recorder, and not by tissue or microelectrode; this point was documented by comparing the records simultaneously monitored on the oscilloscope face and on the chart paper

(Table 5*A*) display appreciable scatter, in which there is a suggestion of an inverse dependence of a_K^c upon $|\Delta \psi_c|$. Because of these concerns, an alternative approach was developed.

Simultaneous Single-Barrelled Impalements of Split Frog Skin

This alternative technique was based upon the presumption that frog skin does constitute a functional syncytium (Nagel, 1976*b*; Rick, Dörge, von Arnim & Thurau, 1978*a*). In principle, then, simultaneous impalements of two different cells with a single-barrelled microelectrode and a micropipette should permit simultaneous measurement of ΔE_K^c and $\Delta \psi_c$.

The feasibility of this approach was first examined in several experiments illustrated by Fig. 3. Two single-barrelled reference micropipettes were introduced into different cells, more than a centimeter apart. The baseline values of $\Delta \psi_c$ measured by the two micropipettes were similar. The fractional basolateral resistances (given by the deflec-

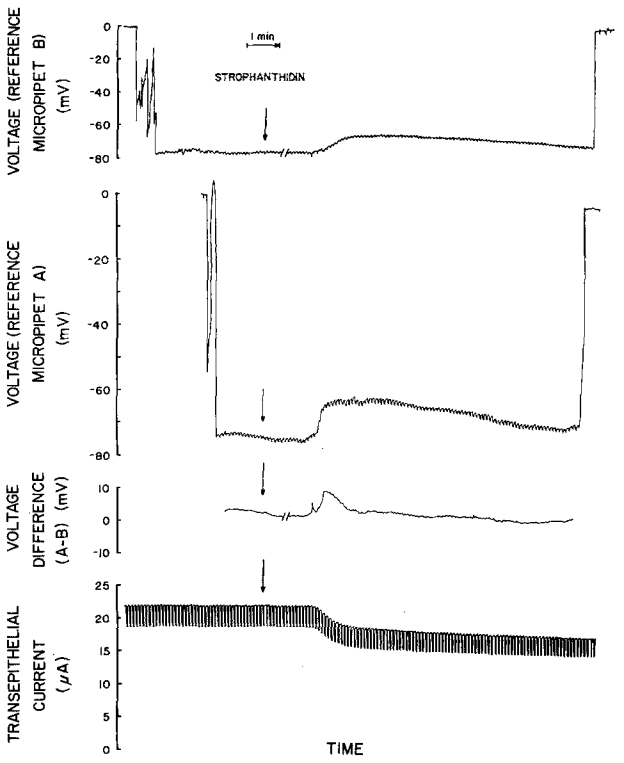

Fig. 3. Simultaneous impalements of short-circuited split frog skin with single-barrelled reference micropipettes. Each micropipette was filled with 0.5 M KCl solution. The basolateral membrane potential measured with micropipette *B* was −76 mV, while that measured with micropipette *A* was −75 mV. At the arrows, the inner surface was superfused with 5×10^{-5} M strophanthidin for 30 s. Micropipette *A*, placed closer to the superfusion inlet, recorded a depolarization earlier than did micropipette *B*. However, the difference in voltages (displayed by trace $A-B$) was only transiently perturbed by the cardiac aglycone. The maximum depolarizations sensed by micropipettes *A* and *B* were 12 and 10 mV, respectively.

At the strophanthidin concentration used, the short-circuit current began to increase (not shown in the record) 14 min after the basolateral membrane potential began to return to its baseline value. We regard this phenomenon as another example of the dissociation which can be elicited between transcellular and homocellular events (DeLong & Civan, 1978). At a lower strophanthidin concentration of 5×10^{-6} M, the transepithelial current and basolateral membrane potential follow similar time courses.

The interruption of the traces for micropipette *B* and for the voltage difference ($A-B$) shortly after adding strophanthidin were associated with sequentially presenting the voltage outputs on a digital display panel, and did not reflect any changes in tissue or micropipettes

tions of $\Delta\psi_c$ divided by the voltage steps applied across the entire epithelium) were also similar. In order to establish the reliability of the information fully, it was desirable to determine the response of both micropipettes to a perturbation of the basolateral membrane. The hyperpolarization elicited by mucosal amiloride illustrated in Fig. 2 would

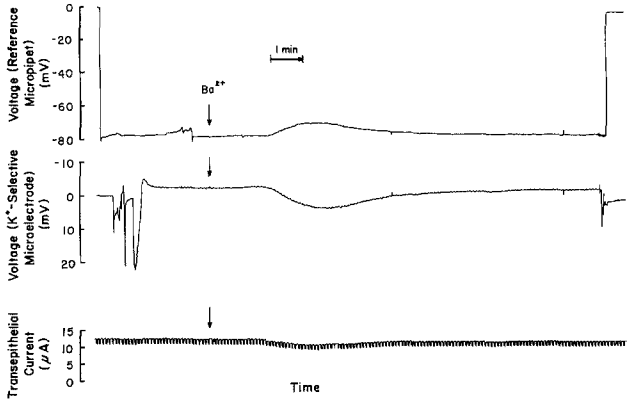

Fig. 4. Simultaneous impalements of short-circuited split frog skin with a single-barrelled K^+-selective microelectrode and a reference micropipette (filled with 0.5 M KCl) in separate cells. $\Delta\psi_c$ and ΔE_K^c were found to be −73 and −4 mV, respectively (Exp. 2A, Table 5*B*). Superfusing the inner surface with 0.5 mM Ba^{2+} for 60 s maximally depolarized $\Delta\psi_c$ and ΔE_K^c by 8 and 6 mV, respectively

have been entirely satisfactory. Unfortunately, micropipettes introduced into split frog skin from the inner medium were usually dislodged when the solution perfusing the contralateral surface was changed. However, reversible depolarizations of the basolateral membrane by transient applications of either 0.5 mM barium or 1 to 5×10^{-5} M strophanthidin provided entirely acceptable alternatives. As demonstrated by Fig. 3, the depolarizations following application of serosal strophanthidin measured by the two micropipettes were of very similar magnitude; given the different rates of access of the strophanthidin to the two cells, located some distance apart in the stream of the superfusing solution, the time courses of the depolarizations are not expected to be identical.

With the documentation of the syncytial nature of split frog skin provided by Fig. 3, an additional series of experiments was performed in order to measure a_K^c yet more precisely. In these studies, the reference micropipettes were filled with 0.5 M KCl in order to minimize diffusion of KCl into the impaled cells (Nelson, Ehrenfeld & Lindemann, 1978). The results of these experiments are illustrated in Fig. 4 and summarized in Table 5*B*. Baseline values of ΔE_K^c and $\Delta\psi_c$ were first obtained with separate simultaneous impalements with a single-barrelled microelectrode and a micropipette, respectively. With the tips resting in the cell, either Ba^{2+} or strophanthidin was added to the medium superfusing the inner surface. In addition to the requirements stated in Materials and Methods, criteria for acceptability of these data included: (i) simultaneous records of ΔE_K^c and $\Delta\psi_c$, (ii) stability

of intracellular records for at least 1 min, and (iii) transient depolarization of both ΔE_K^c and $\Delta \psi_c$ in response to serosal Ba^{2+} or strophanthidin. With the exception of Exp. 1 E, all of the measurements included in Table 5*B* fulfilled these criteria. In Exp. 1 E, the intracellular position of the K^+-selective microelectrode was documented by a transient decrease in ΔE_K^c following exposure to strophanthidin. However, here, an average value of $\Delta \psi_c$ was obtained by taking the mean of five determinations performed before and at approximately the time of measurement of ΔE_K^c. As in Tables 3 and 5*A*, values of a_K^c are tabulated both of the directly calculated estimates and of the estimates including the two-step correction for junction potential.

The mean value of a_K^c calculated from the single-barrelled impalements was 104 mM, not very different from the value of 98 mM based upon double-barrelled penetrations (Table 5). However, the standard deviation of the former data was 9.5 mM, less than half that of the latter (24.0 mM), reflecting the much reduced scatter of the simultaneous single-barrelled measurements.

Discussion

Impalements with fine-tipped micropipettes have suggested that the membrane potential ($\Delta \psi_c$) of short-circuited toad bladder is appreciably larger than that reported earlier. The frequently observed instability of the intracellular trace supports the suggestion of several investigators that impalement-induced damage can be of particular concern in studying toad bladder (Lindemann, 1975; Higgins et al., 1977; Sudou & Hoshi, 1977). This difficulty has been addressed by application of the null point technique described earlier. This approach suggests that the potassium activity (a_K^c) of amphibian urinary bladder is some 82 mM (Table 3*A*), twice as high as earlier estimates (Kimura et al., 1977; Kimura & Fujimoto, 1977; Delong & Civan, 1978). Although the null point technique has been helpful in measuring baseline a_K^c, it is unlikely to be applicable for a number of other experimental purposes (such as measuring intracellular Na^+ activity). For this reason, we have turned to another tight epithelium more favorable for electrophysiologic study.

Nagel (1976*a*) and Helman and Fisher (1977) were the first to report that large stable membrane potentials could be recorded from frog skin over prolonged periods of time. We too have been impressed by the stability of the intracellular potentials measured by penetrations from the outer (Nagel, Pope, Peterson & Civan, 1980) or, as here, from the inner medium. Frog skin possesses certain other technical advantages for study by other modalities, as well. In contrast to toad bladder, whole frog skin can be directly studied with ^{31}P nuclear magnetic resonance spectrometry without complications arising from subepithelial elements (Lin, Shporer & Civan, 1982). Frog skin is also more easily prepared for electron probe X-ray microanalysis than is toad bladder.

The single major disadvantage of frog skin is the structural complexity of its epithelium. As emphasized by Leaf (1965), toad bladder epithelium contains a single complete layer of cells (DiBona, Civan & Leaf, 1969), while the epidermis of adult frog skin consists of 5 to 9 stratified layers. However, the problems to experimental analysis posed by this epithelial stratification now appear not so formidable, since recent reports and the present results support the suggestion that the epidermis functions as a syncytium (Farquhar & Palade, 1964; Ussing & Windhager, 1964). The major points of evidence are: (i) Progressive advance of a reference micropipette permits measurement of the intracellular potential and fractional apical resistance (FR_{ap}) of cells in different layers; $\Delta \psi_c$ and FR_{ap} have been found to be similar in cellular planes below the stratum corneum (Nagel, 1976*b*). (ii) The Na^+, K^+ and Cl^- concentrations measured by electron probe X-ray microanalysis are similar for epithelial cells below the stratum corneum (Rick et al., 1978*a*). (iii) Simultaneous impalements of two distant cells document the similarity not only of the baseline membrane potential, but also of the depolarizing responses to Ba^{2+} and strophanthidin (Fig. 3).

For several reasons, isolated epidermis, rather than whole skin, was the preparation chosen for the present study. First, for the purposes of another study, we wished to characterize the baseline values of a model system responding rapidly to changes in the inner bathing medium (Fisher, Erlij & Helman, 1980). Second, splitting off the dermis usually eliminates the skin glands (Carasso, Favard, Jard & Rajerison, 1971) thought responsible for a small rate of active Cl^- transport across whole frog skin (Thompson & Mills, 1982). Third, for these baseline measurements, we wished to avoid any technical complication possibly associated with the passage of the micropipette tips through the stratum corneum.

The one problem encountered in studying split frog skin has been the technical difficulty in transiently applying amiloride to the outer medium underlying the skin without dislodging the micropipette and microelectrode tips introduced from the

inner medium. This problem was solved by transiently superfusing the inner surface with depolarizing agents, whose mechanism of action is known. Both strophanthidin and ouabain act by inhibiting the electrogenic or rheogenic Na^+, K^+ exchange pump at the basolateral membrane (Helman, Nagel & Fisher, 1979; Nagel, 1980; Nagel et al., 1980); however, in contrast to the prolonged duration of action of the cardiac glycoside, the effect of the aglycone on the basolateral membrane potential is rapidly reversible (Fig. 3). Barium seems to act primarily by reducing the passive K^+ conductance (Nagel, 1979; Nielsen, 1979), consistent with its effect on other tissues (Pacifico et al., 1969; Hermsmeyer & Sperelakis, 1970); the Na^+ pump remains active in the presence of Ba^{2+} (Nagel, 1979; Nielsen, 1979). This approach may be of assistance to other investigators in confirming the intracellular placement of micropipettes and microelectrodes.

The results of the current study suggest that the intracellular K^+ activity (a_K^c) of frog skin is 104 ± 3 mM (Table 5). This value is appreciably lower than the 132 ± 10 mM estimated by Nagel et al. (1981) in their careful study of whole frog skin. The experimental basis for this difference is unclear. The potassium concentration c_K^s of their Ringer's solution was 2.5 mM, rather than 3.5 mM; if anything, their lower value of c_K^s should have led to a lower estimate of a_K^c. One major difference between the two studies is the use of split skin here. In principle, the splitting could possibly have damaged or altered the transport state of the epithelium. However, Fisher et al. (1980) measured similar short-circuit currents before and after amiloride in split and whole skins. Certainly, the split skins of the present study were satisfactory for electrophysiologic study over periods of several hours.

Whatever the basis for the difference in results, several considerations suggest that the current data may provide a closer estimate of the true value of a_K^c in frog skin. (i) The values of $\Delta\psi_c$ and ΔE_K^c used in the calculations were measured simultaneously. (ii) Impalements across the basolateral membrane circumvented any possible artifact associated with traversing the cornified outer surface layer. While differences in $\Delta\psi_c$ measured from the two opposite surfaces cannot be very large (Fisher et al., 1980), small significant differences are still possible. (iii) The value of 132 ± 10 mM reported earlier did not include any correction for the difference in junction potential (ΔV_{jcn}) between 1.5 M KCl-filled micropipettes and the solutions within and outside the cell. Application of the Henderson equation (Eq. 5) suggests that the ΔV_{jcn} would have made their measured membrane potential 4.6 mV more positive than the true value. Presuming that their microelectrodes had a value of S similar to ours (59 mV), their estimated value of a_K^c corrected for junction potentials would be 159 mM. The intracellular potassium concentrations (c_K^c) of frog skin and toad bladder are similar (e.g., Rick et al., 1978*a*, *b*; Civan et al., 1980), amounting to approximately 147 mmol·(kg intracellular water)$^{-1}$. Thus, the apparent activity coefficient (γ_K^{app}) for intracellular K^+ would be calculated to be about one, a third larger than the true activity coefficient (γ_K) of 0.76 characterizing the extracellular K^+. For γ_K^{app} to exceed γ_K is not without precedent in other tissues (e.g., Palmer et al., 1978), but requires that we postulate the action of subcellular compartmentalization. On the other hand, the current estimate of a_K^c suggests that $\gamma_K^{app} = 0.71$ in frog skin, close to the value of γ_K, and consistent with the uniform distribution of intracellular K^+.

The comparison of the earlier and current estimates of a_K^c serves to emphasize the general difficulty in the precise measurement of K^+ activity under baseline conditions. As is evident from Eq. (2), the calculated value of a_K^c is exponentially dependent upon $(\Delta E_K^c - \Delta\psi_c)$. If this difference were only 10.9 mV too large in the earlier study (Nagel et al., 1981), the results would be in perfect agreement. Because of this exponential dependence upon $(\Delta E_K^c - \Delta\psi_c)$, precise measurement of baseline values of a_K^c is far more hazardous technically than is the monitoring of experimental changes in a_K^c.

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References

Acker, H. 1978. Measurements of potassium changes in the cat carotid body under hypoxia and hypercapnia. *Pfluegers Arch.* **375**:229–232

Brown, A.M., Brown, H.M. 1973. Light response of a giant *Aplysia* neuron. *J. Gen. Physiol.* **62**:239–254

Carasso, N., Favard, P., Jard, S., Rajerison, R.M. 1971. The isolated frog skin epithelium: I. Preparation and general structure in different physiological states. *J. Microscopie* **10**:315–330

Civan, M.M. 1978. Intracellular activities of sodium and potassium. *Am. J. Physiol.* **234**:F261–F269

Civan, M.M., Frazier, H.S. 1968. The site of the stimulating action of vasopressin on sodium transport in the toad bladder. *J. Gen. Physiol.* **51**:589–605

Civan, M.M., Hall, T.A., Gupta, B.L. 1980. Microprobe study of toad urinary bladder in absence of serosal K^+. *J. Membrane Biol.* **55**:187–202

Civan, M.M., Hoffman, R.E. 1971. Effect of aldosterone on electrical resistance of toad bladder. *Am. J. Physiol.* **220**:324–328

DeLong, J., Civan, M.M. 1978. Dissociation of cellular K^+ accumulation from net Na^+ transport by toad urinary bladder. *J. Membrane Biol.* **42**:19–43

DeLong, J., Civan, M.M. 1979. Intracellular potassium activity associated with potassium depletion from toad urinary bladder. *Colloq. Inst. Nat. Santé Rech. Med.* **85**:221–229

DeLong, J., Civan, M.M. 1980. Intracellular chemical activity of potassium in toad urinary bladder. *Curr. Top. Membr. Transp.* **13**:93–105

DiBona, D.R., Civan, M.M., Leaf, A. 1969. The anatomic site of the transepithelial permeability barriers of toad bladder. *J. Cell Biol.* **40**:1–7

Farquhar, M.G., Palade, G.E. 1964. Functional organization of amphibian skin. *Proc. Natl. Acad. Sci. USA* **51**:569–577

Fisher, R.S., Erlij, D., Helman, S.I. 1980. Intracellular voltage of isolated epithelia of frog skin: Apical and basolateral cell punctures. *J. Gen. Physiol.* **76**:447–453

Frazier, H.S. 1962. The electrical potential profile of the isolated toad bladder. *J. Gen. Physiol.* **45**:515–528

Fuchs, W., Hviid Larsen, E., Lindemann, B. 1977. Current-voltage curve of sodium channels and concentration dependence of sodium permeability in frog skin. *J. Physiol. (London)* **267**:137–166

Garcia-Diaz, J.F., Armstrong, W. Mc.D. 1980. The steady-state relationship between sodium and chloride transmembrane electrochemical potential differences in *Necturus* gallbladder. *J. Membrane Biol.* **55**:213–222

Guggenheim, S.J., Bourgoignie, J., Klahr, S. 1971. Inhibition by ammonium of sodium transport across isolated toad bladder. *Am. J. Physiol.* **220**:1651–1659

Helman, S.I., Fisher, R.S. 1977. Microelectrode studies of the active Na transport pathway of frog skin. *J. Gen. Physiol.* **69**:571–604

Helman, S.I., Nagel, W., Fisher, R.S. 1979. Ouabain on active transepithelial transport in frog skin. *J. Gen. Physiol.* **74**:105–127

Hermsmeyer, K., Sperelakis, N. 1970. Decrease in K^+ conductance and depolarization of frog cardiac muscle produced by Ba^{++}. *Am. J. Physiol.* **219**:1108–1114

Higgins, J.T., Jr., Gebler, B., Frömter, E. 1977. Electrical properties of amphibian urinary bladder epithelia. II. The cell potential profile in *Necturus maculosus*. *Pfluegers Arch.* **371**:87–97

Huxley, A.F. 1961. A micromanipulator. *J. Physiol. (London)* **157**:5P

Kallus, F.T., Vanatta, J.C. 1970. Compartmentation and exchange of potassium in the toad bladder. *Biochim. Biophys. Acta* **211**:61–64

Kimura, G., Fujimoto, M. 1977. Estimation of the physical state of potassium in frog bladder cells by ion exchanger microelectrode. *Jpn. J. Physiol.* **27**:291–303

Kimura, G., Urakabe, S., Yuasa, S., Miki, S., Takamitsu, Y., Orita, Y., Abe, H. 1977. Potassium activity and plasma membrane potentials in epithelial cells of toad bladder. *Am. J. Physiol.* **232**:F196–F200

Leaf, A. 1965. Transepithelial transport and its hormonal control in toad bladder. *Ergeb. Physiol.* **56**:215–263

Lin, L.-E., Shporer, M., Civan, M.M. 1982. ^{31}P Nuclear magnetic resonance analysis of frog skin. *Am. J. Physiol.* **243**: *C74–C80*

Lindemann, B. 1975. Impalement artifacts in microelectrode recordings of epithelial membrane potentials. *Biophys. J.* **15**:1161–1164

MacInnes, D.A. 1961. The Principles of Electrochemistry. Dover, New York

Macknight, A.D.C. 1977. The contribution of mucosal chloride to chloride in toad bladder epithelial cells. *J. Membrane Biol.* **36**:55–63

Nagel, W. 1976*a*. The intracellular electrical potential profile of the frog skin epithelium. *Pfluegers Arch.* **365**:135–143

Nagel, W. 1976*b*. Intercellular junctions of frog skin epithelial cells. *Nature (London)* **264**:469–471

Nagel, W. 1979. Inhibition of potassium conductance by barium in frog skin epithelium. *Biochim. Biophys. Acta* **552**:346–357

Nagel, W. 1980. Rheogenic sodium transport in a tight epithelium, the amphibian skin. *J. Physiol. (London)* **302**:281–295

Nagel, W., Garcia-Diaz, J.F., Armstrong, W. McD. 1981. Intracellular ionic activities in from skin. *J. Membrane Biol.* **61**:127–134

Nagel, W., Pope, M.B., Peterson, K., Civan, M.M. 1980. Electrophysiologic changes associated with potassium depletion of frog skin. *J. Membrane Biol.* **57**:235–241

Nelson, D.J., Ehrenfeld, J., Lindemann, B. 1978. Volume changes and potential artifacts of epithelial cells of frog skin following impalement with microelectrodes filled with 3 M KCl. *J. Membrane Biol.* **Special Issue**:91–119

Nielsen, R. 1979. Coupled sodium and potassium transport across isolated frog skin: Effect of ouabain, amiloride and the polyene antibiotic filipin. *J. Membrane Biol.* **51**:161–184

Pacifico, A.D., Schwartz, M., MacKrell, T.N., Spangler, S.G., Sanders, S.S., Rehm, W.S. 1969. *Am. J. Physiol.* **216**:536–541

Palmer, L.G., Century, T.J., Civan, M.M. 1978. Activity coefficients of intracellular Na^+ and K^+ during development of frog oocytes. *J. Membrane Biol.* **40**:25–38

Palmer, L.G., Civan, M.M. 1977. Distribution of Na^+, K^+ and Cl^- between nucleus and cytoplasm in *Chironomus* salivary gland cells. *J. Membrane Biol.* **33**:41–61

Palmer, L.G., Edelman, I.S., Lindemann, B. 1980. Current-voltage analysis of apical sodium transport and metabolism. *J. Membrane Biol.* **57**:59–71

Reuss, L., Finn, A.L. 1974. Passive electrical properties of toad urinary bladder epithelium: Intracellular electrical coupling and transepithelial cellular and shunt conductances. *J. Gen. Physiol.* **64**:1–25

Rick, R., Dörge, A., Arnim, E. von, Thurau, K. 1978*a*. Electron microprobe analysis of frog skin epithelium: Evidence for a syncytial sodium transport compartment. *J. Membrane Biol.* **39**:313–331

Rick, R., Dörge, A., Macknight, A.D.C., Leaf, A., Thurau, K. 1978*b*. Electron microprobe analysis of the different epithelial cells of toad urinary bladder: Electrolyte concentrations at different functional states of transepithelial sodium transport. *J. Membrane Biol.* **39**:257–271

Robinson, B.A., Macknight, A.D.C. 1976. Relationship between serosal medium potassium concentration and sodium transport in toad urinary bladder. III. Exchangeability of epithelial cellular potassium. *J. Membrane Biol.* **26**:269–286

Sudou, K., Hoshi, T. 1977. Mode of action of amiloride in toad urinary bladder: An electrophysiological study of the drug action on sodium permeability of the mucosal border. *J. Membrane Biol.* **32**:115–132

Thompson, I.G., Mills, J.W. 1982. Chloride transport in the glands of the frog skin. *Biophys. J.* **37**:279a

Ussing, H.H., Windhager, E.E. 1964. Nature of shunt path and active sodium transport path through frog skin epithelium. *Acta Physiol. Scand.* **61**:484–504

Walker, J.L. 1976. Ion selective liquid ion exchanger. *In:* Ion and Enzyme Electrodes in Biology and Medicine. M. Kessler, L.C. Clark, Jr., D.W. Lübbers, I.A. Silver, and W. Simon, editors. pp. 116–118. Urban & Schwarzenberg, Munich

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