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Some Properties of KCl-Filled Microelectrodes: Correlation of Potassium "Leakage" with Tip Resistance

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Summary. This study was undertaken in order to determine directly the rates of K leakage (J_K) out of the tips of microelectrodes into a solution of 100 mM KCl (approximating the K concentration of the cell interior) and to relate these rates to the concentration of the filling solution and the tip resistance. The values of J_K for electrodes filled with 3 M KCl having resistances of 16 and 30 MΩ (when measured in 3 M KCl) were 10 and 5.5 fmol/sec, respectively. When the same electrodes were filled with 0.5 M KCl, the resistances (measured in 0.5 M KCl) increased to 62 and 115 MΩ, respectively, and J_K fell to 1.8 and 1.0 fmol/sec, respectively. These values are in reasonable agreement with what would be expected from theoretical considerations if leakage of KCl were the result of diffusion plus convective flow due to the hydrostatic pressure of the filling solution.

We conclude that K leakage out of microelectrodes filled with 3 M KCl is unnecessarily high; leakage can be reduced fivefold by filling electrodes with 0.5 M KCl without incurring significant increases in tip or diffusion potentials or unmanageable tip resistances.

Finally, the lowest rate of K leakage observed (1 fmol/sec) is still very considerable for the case of animal cells with an intracellular volume of approximately 1 pl and a K content of approximately 100 fmol. The finding of stable intracellular potentials, often for many minutes, in some tissues suggests that K which enters the cell rapidly diffuses into neighboring cells via high conductance intercellular communications.

Key words: Microelectrodes, K leakage, membrane potentials, tip resistance

Not many years after the introduction of KCl-filled microelectrodes for the determination of intracellular potentials (Ling & Gerard, 1949), a number of investigators considered the problem of KCl leakage from electrodes filled with 3 M KCl into a much more dilute cytoplasm (e.g., Nastuk & Hodgkin, 1950; Coombs, Eccles & Fatt, 1955). However, in more recent years, this problem appears to have been dismissed or ignored and many investigators have employed so-called "conventional" microelectrodes to measure transmembrane electrical potential difference in small cells without expressed concern. Recently, Nelson, Ehrenfeld and Lindemann (1978) directly observed swelling of frog skin epithelial cells following impalement with 3 M KCl-filled microelectrodes and speculated that this could be due to leakage of KCl from the tip; their results re-raise serious questions regarding the use of such electrodes to impale small animal cells.

However, although Nastuk and Hodgkin (1950) and others (e.g., Coombs et al., 1955; Geisler, Lightfoot, Schmidt & Sy, 1972) have calculated expected rates of KCl loss from theoretical considerations, we are aware of only one study in which K leakage was measured directly under limited conditions (Isenberg, 1979). The present study was undertaken to examine this important question in greater detail and under more varied conditions.

Materials and Methods

Microelectrodes were drawn from fiber-filled borosilicate glass tubing having an inner diameter of 0.68 mm and an outer diameter of 1.2 mm (W.P.I. #1B120F-4). The glass was cleaned by immersion for 4 hr in nitric acid followed by overnight rinsing with distilled water. Two 2-stage pullers were employed: a Narishige (Model PN-3) and a Brown and Flaming (Sutter Instruments, Model P-77); the latter features a gas-jet cooling system that permits the drawing of very fine tips without increasing the tip length. The pulling parameters were adjusted so that electrode shape (ascertained by light microscopic examination) and resistance remained essentially

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Table 1. Effects of filling solutions and bathing solutions on electrode resistance

Electrode type	Filling solution M KCl	Bathing solution				
		0.1 M KCl	0.5 M KCl	3 M KCl	*C-R*	*W-R*
LR	0.5	–	64±2	31±1	111±8	101±7
	3.0	–	28±1	16±1	41±2	39±2
HR	0.1	–	–	71±4	–	–
	0.5	–	115±3	54±2	203±5	187±5
	3.0	70±4	56±2	33±1	70±3	65±3

Tip resistance is in MΩ; each value is the average of determinations on 18 electrodes.

constant, and so that the electrodes drawn using the Brown and Flaming puller always had a higher ("intrinsic") resistance than the electrodes drawn using the Narishige puller when measured using the same filling and bathing solutions; for convenience, we will refer to the former as *HR* (high resistance) and the latter as *LR* (low resistance) electrodes.

Electrode resistance (R_e) was measured using an electrode impedance meter operating at a fixed AC frequency of 130 Hz (Sutter Instruments, Model PV-10M). The readings were taken approximately 30 sec after dipping the electrode tip into the bathing solution. When different bathing solutions were used, the order of dipping was randomized with a final control determined using the first solution.

In order to measure K leakage, the electrodes were back-filled with approximately 3 µl of a solution of either 0.5 or 3 M KCl containing ^{42}K (100 µCi/mg, New England Nuclear Corp.) by direct injection through a fine needle attached to a syringe. The electrode was then partly backfilled with a 2–3 cm column of paraffin oil to prevent evaporation which is otherwise enhanced by the capillary action of the inner fiber. Six electrodes were inserted into the cap of a mini-counting vial that contained six holes and an additional hole for pressure equilibration. The tips were then dipped into a large reservoir of 100 mM KCl to wash away any initial outflow of K. The six electrodes were then inserted into a mini-vial that contained 1 ml of 100 mM KCl; with the cap firmly seated, the tips of the electrodes were immersed approximately 2 mm. After 1 hr, the electrodes were removed and ^{42}K in the bathing solution was assayed using a liquid scintillation counter (Tracor Analytic, Mark III). The lowest counting rate was four times greater than background, and all samples were counted to a variance less than 1%. Immediately after removing the electrodes from the mini-vial, they were backfilled with either 0.5 or 3 M KCl in such a way that the oil was flushed out, and R_e was determined as described above.

The rate of K leakage, J_K, was calculated by dividing the ^{42}K content of the bathing solution by the specific activity of ^{42}K in the filling solution. The result, expressed in fmol/sec, is a *unidirectional* efflux. However, as will be discussed below, J_K does not differ markedly from the *net* leakage under these conditions.

In order to determine tip and diffusion potentials, the microelectrodes were connected to the input stage of a high impedance electrometer (W.P.I. Model F23B). A calomel half-cell connected to the bathing solution via a KCl-agar bridge served as the reference electrode. Tip potentials are defined as the diffusion potential observed after breaking the tip of the electrode *minus* the potential difference measured with the intact electrode. The *relative* diffusion potential is defined as zero when the bathing solution was a Ringer's solution.

Two types of Ringer's solutions were used: (A) A solution commonly used with warm-blooded animals which we refer to as *W-R* and which contained (mM): Na, 140; Cl, 124; HCO_3, 21; K, 5.4; HPO_4, 2.4; H_2PO_4, 0.6; Mg, 1.2; Ca, 1.2; and glucose, 10.

(B) An electrolyte solution commonly employed in studies of amphibian epithelia which is referred to as *C-R* and contained (mM): Na, 100; Cl, 105; K, 2.5, HPO_4, 1.1; H_2PO_4, 0.3, Mg, 1.2; Ca, 1.2; and glucose, 10.

Unless otherwise indicated, all experiments were carried out at 22 °C.

Results are expressed as the mean±standard error; significance of differences were assessed using the unpaired Student *t* test and a value of $P<0.05$ was considered significant.

Results

Electrode Resistance (R_e)

Although it is self-evident and has long been recognized that R_e depends on both the filling solution and the bathing solution (*cf.* Coombs et al., 1955), values of R_e are often given in the literature without identifying the bathing solution. The effects of these solutions on R_e of *HR* and *LR* electrodes are given in Table 1. When the filling and bathing solutions are the same, the "intrinsic" resistance of the *HR* electrodes was approximately twice that of the *LR* electrodes, and this relation is roughly true under any set of comparable conditions. Further, we see that the filling solution and the bathing solution contribute equally to the final R_e. That is, an electrode filled with solution A and dipped into solution B has the same R_e as one filled with solution B and dipped into solution A. This is to be expected if the steady-state R_e is determined by the steady-state composition of the fluid in the tip after interdiffusion has taken place (Firth & DeFelice, 1971).

Figure 1 is a plot of the resistance of *LR* electrodes against that of the *HR* electrodes under identical filling and bathing conditions. Clearly, the data can be well described by a linear relation with a slope of 1.9 which reflects the ratio of the intrinsic resistance of the *HR* electrodes to that of the *LR* electrodes. This indicates that, although the shape and tip diameters of the two types of electrodes differed, the *relative* changes in resistance resulting from switching filling and bathing solutions are not affected. It is, therefore, possible to generalize the relative resistance changes resulting from differences in filling and bathing solutions for all electrodes. This is given in Table 2 where the value of R_e when the electrode is filled and bathed with 3 M KCl is defined as unity.

Also shown in Table 2 are the relative resistances when the bathing solution was *W-R* at 22 °C and *W-R* at 37 °C; the ratios of these values (1.2) are

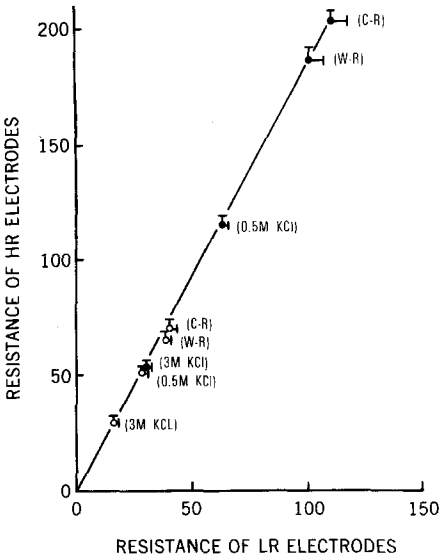

Fig. 1. Relation between R_e of *HR* and *LR* electrodes when filled with either 0.5 M KCl (●) or 3 M KCl (○) and dipped into various bathing media (given in parentheses). The line has a slope of 1.9

Table 2. Relative electrode resistances

Bathing solution	Filling solution	
	3 M KCl	0.5 M KCl
3 M KCl	1.0	1.8
0.5 M KCl	1.8	3.7
C-R	2.4	6.5
W-R (23 °C)	2.2	5.9
W-R (37 °C)	1.8	4.7

Relative resistance of electrode filled with 3 M KCl and bathed with 3 M KCl is defined as unity.

in reasonable agreement with those expected on theoretical grounds.[1]

Potassium Leakage (J_K)

The results of the experiments designed to evaluate ^{42}K leakage from the electrodes into the bathing solution are given in Table 3. There are several points worthy of note:

First, the values of R_e for *HR* and *LR* electrodes with different filling and bathing solutions in this series of experiments are in excellent agreement with those given in Table 1. The "intrinsic" resistance of the *HR* electrodes is approximately twice that of the *LR* electrodes and the relation illustrated in Fig. 1 is obeyed.

[1] The ratio of the viscosity of water at 37 °C to that at 23 °C is 1.3.

Table 3. Potassium leakage from microelectrodes

Electrode type	Filling solution	Resistance in 3 M KCl	Resistance in 0.5 M KCl	J_K	n/m
LR	3 M KCl	15.5±0.8	30.0±1.5	10.2±0.7	4/24
	0.5 M KCl	30.9±0.9	62.1±2.1	1.8±0.2	5/30
	r	0.50	0.48	5.7	
HR	3 M KCl	30.2±1.8	58.3±2.9	5.5±0.4	6/36
	0.5 M KCl	55.4±2.4	112.6±4.7	1.0±0.2	4/24
	r	0.54	0.51	5.5	

Values of resistance in MΩ; J_K is in fmol/sec; n is the number of experiments, and m is the number of electrodes used. r is the ratio of the value of electrodes filled with 3 M KCl to those filled with 0.5 M KCl.

Table 4. Resistance of electrodes under conditions of efflux measurements

Filling solution	Bathing solution		
	Identical to filling solution	0.1 M KCl	0.0002 M KCl
3 M KCl	30.1±1.4	70.3±3.4	225±11
0.5 M KCl	119 ±6	179 ±8	

Each value represents the results of determinations on 11 *HR* electrodes.

Second, the rate of ^{42}K leakage, J_K (in fmol/sec), from electrodes with the *same* filling solution is approximately two times greater for the case of *LR* electrodes than *HR* electrodes and is, thus, inversely related to the ratio of "intrinsic" resistances.

Third, for both types of electrodes, the rate of leakage when the electrode is filled with 3 M KCl is approximately 5.6 times that observed when the electrodes are filled with 0.5 M KCl; this value is in reasonable agreement with the ratio of KCl activities of 5.3 (Robinson & Stokes, 1959).

Finally, Table 4 gives the resistances of a series of *HR* electrodes under the conditions of the efflux measurements. It should be noted that the values of R_e when the filling and bathing solutions were 3 or 0.5 M KCl are in excellent agreement with those given in Table 3; the latter were determined after 1 hr immersion in 0.1 M KCl, whereas the former were determined immediately after filling. Clearly, there is no significant "aging effect" during the efflux measurement. The significance of these data will be discussed below.

Tip and Diffusion Potentials

As shown in Table 5, the tip potentials of the *HR* electrodes are low and, equally important, do not

Table 5. Tip and diffusion potentials of *HR* electrodes

Bathing solution	Filling solution			
	Tip potential (mV)		Diffusion potential (mV)	
	0.5 M KCl	3 M KCl	0.5 M KCl	3 M KCl
W-R	-3.0 ± 0.3	-3.6 ± 0.5	0	0
0.1 M KCl	-1.6 ± 0.3	-2.2 ± 0.4	-0.4 ± 0.1	-0.2 ± 0.1
Δ	$+1.4\pm0.2$	$+1.4\pm0.2$		

Results of 22 experiments using *HR* electrodes.

change very much when the electrode is moved from a bathing solution consisting of *W-R* to a solution of 0.1 M KCl. In addition, tip and diffusion potentials of 3 and 0.5 M KCl filled *HR* electrodes do not differ significantly. It is reasonable to infer that the same conclusions would apply to *LR* electrodes.

Discussion

These studies were undertaken in order to gain further insight into the behavior of glass microelectrodes commonly employed for measurements of intracellular electrical potentials with particular emphasis on assessing the extent of K(Cl) leakage from the tip; as pointed out most recently by Nelson et al. (1978), this leakage could present a serious problem when relatively low resistance electrodes filled with 3 M KCl are employed to impale small animal cells. The results, summarized in Table 3 indicate that K leakage from *LR* electrodes filled with 3 M KCl and having a resistance of 15 MΩ when dipped in 3 M KCl is tenfold greater than leakage from *HR* electrodes filled with 0.5 M KCl whose R_e measured in 0.5 M KCl is approximately 115 MΩ.

In the only other published study dealing with the direct determination of K leakage from microelectrodes[2], Isenberg (1979) measured the *net* K loss from electrodes filled with 3 M KCl and having resistances between 1 and 12 MΩ when dipped in 3 M KCl. The technique involved immersing the tip of the microelectrode into a small droplet of 0.1 mM KCl under oil and measuring the rate of increase of the K concentration in the droplet with a K-selective microelectrode. The lowest J_K reported by this investigator was 2 fmol/sec for electrodes with a tip resistance of 12 MΩ. This value is five times lower than our results with comparable microelectrodes.[3]

A possible explanation for this discrepancy, which may also provide further insight into the nature of K leakage from the tip, emerges from the following considerations:

Hodgkin (1951) has shown that in the absence of electrochemical potential differences, the conductance of a barrier is given by

$$G=(\mathcal{F}^2/RT)\Sigma z_i^2 J_i \qquad (1)$$

where $\mathcal{F}$ is the Faraday constant, R is the gas constant, T is the absolute temperature, z_i is the valence of the ionic species i and J_i is the unidirectional flux of i across the barrier. As pointed out by Coombs et al. (1955), this equation can be rearranged to give the unidirectional fluxes of K and Cl from a microelectrode when the filling and bathing solutions are the same, assuming that the mobilities of K and Cl do not differ significantly; *viz.*

$$J_K=J_{Cl}=RT\,G_e/2\mathcal{F}^2=129\ \text{nmol/(S}\cdot\text{sec)} \qquad (2)$$

where G_e is the electrode conductance under symmetrical conditions.

The values for J_K from *LR* and *HR* electrodes are plotted against G_e determined when the filling and the bathing solutions were the same in Fig. 2. The line is that predicted by Eq. (2) and appears to provide a reasonable fit to the data.

However, as pointed out by Coombs et al. (1955), Eq. (2) predicts the *maximum*, upper limit of efflux which is observed when the electrode is dipped into a solution identical to the filling solution; J_K should be considerably smaller when the electrode is dipped into a more dilute solution because of dilution of KCl within the microelectrode close to the orifice. Indeed, if J_K is inversely related to R_e, as suggested by the data in Table 3, the data in Table 4 suggest that the values for J_K observed with the *HR* electrodes are approximately twice what would be expected for a strictly diffusional flow. Further, the low values

[2] Helman, Nagel and Fisher (1979) attempted to measure K leakage from electrodes filled with 3 M KCl but reported, "In most electrodes, the rate of K leakage was so low that an estimate of leakage rate could not be obtained despite the high level of ^{42}K activity in the pipettes." These electrodes had resistances between 15–40 MΩ when dipped into the equivalent of our *C-R* solution (S. Helman, *personal communication*). According to Table 2, they would have resistances between 6–17 MΩ if they were tested in 3 M KCl, and leakage should have been readily detected even from a single electrode. We have no explanation for this discrepancy.

[3] It should be emphasized that Isenberg determined *net* leakage whereas we determined *unidirectional* efflux, which must be greater than net efflux. However, when electrodes are filled with 3 M KCl and dipped into 0.1 M KCl, the difference between unidirectional efflux and net efflux (i.e., the unidirectional influx) must be negligible. When electrodes are filled with 0.5 M KCl and dipped into 0.1 M KCl, the difference will be larger but, at worst, the unidirectional efflux is not likely to exceed the net efflux by more than 50%. Such a discrepancy would not significantly alter our conclusions.

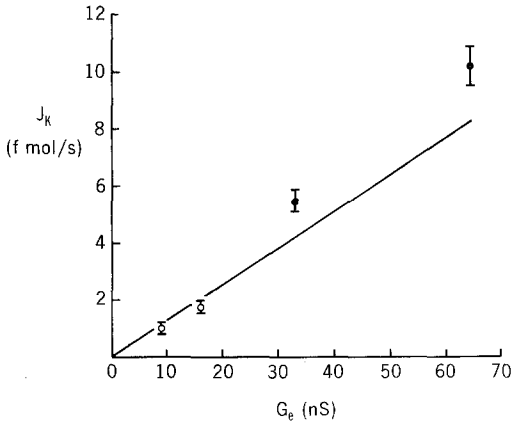

Fig. 2. Relation between J_K into 100 mM KCl and G_e determined when the filling and bathing solutions were the same. The line shown is the relation predicted by Eq. (2)

for J_K observed by Isenberg, one-fifth the upper limit predicted by Eq. (2), may be due to the fact that his 3 M KCl electrodes were immersed in a solution initially containing 0.0001 M KCl which rose during the determination to 0.0002 M; since the fluid within the electrode is unstirred, dilution at the tip could have been responsible for the low values observed. In any event, the 0.1 M KCl bathing solution used in our experiments more closely mimics cell composition (with respect to K) so that the values of J_K reported in Table 3 may be a more realistic reflection of what is to be expected following cell impalement.

As pointed out by Krjnevic, Mitchell and Szerb (1963), and stressed by Isenberg (1979), the presence of a hydrostatic pressure within the microelectrode can add considerably to K leakage above and beyond that due to diffusion. Clearly, every effort must be made to minimize any possibility of pressure development resulting from the electrode-holding device.[4] But, as Krjnevic et al. (1963) have noted, the pressure arising *solely* from the fluid column in the electrode is appreciable. These investigators calculated that for a 7-cm electrode filled with 3 M acetylcholine chloride and having a tip opening with a diameter of approximately 0.125 μm, half the total outflux can be attributed to the hydrostatic pressure. The finding in the present study that the unidirectional efflux is approximately two times that which would be expected from diffusion alone may be attributable to this pressure effect.[5]

Finally, the choice of electrode-type and filling solution is dictated by three considerations: (i) to maintain R_e within limits compatible with the associated electronic equipment; (ii) to minimize possible changes in tip-potential when the electrode is passed from the external solution into the cell; and (iii) to minimize leakage of the filling solution out of the electrode tip. The data given in Tables 3 and 5 indicate that electrodes filled with 3 M KCl that have a R_e of 15 MΩ when bathed with 3 M KCl (commonly employed) have a leakage rate 10 times greater than electrodes with a higher "intrinsic" resistance which, when filled with 0.5 M KCl, have a R_e of approximately 115 MΩ. Further, the tip-potentials and changes in tip-potential of 0.5 M KCl-filled *HR* electrodes do not differ significantly from *HR* electrodes filled with 3 M KCl.[6] Consequently, there is certainly no obvious advantage to using "conventional" 3 M KCl-filled microelectrodes and, indeed, the rate of leakage from such electrodes observed in these studies appears to be unacceptably high. For example, a cuboidal cell measuring 10 μm in each direction has a volume of 1 pl and, assuming an intracellular K concentration of about 0.1 M, the K content is about 100 fmol. If such a cell were impaled with a *LR* electrode filled with 3 M KCl, the amount of K that would leak into the cell after only 5 sec would be one-half the original content; and, using *HR* electrodes filled with 0.5 M KCl, the leakage into the cell after 5 sec would be approximately 5% of the original content. The latter is certainly more acceptable than the former!

Clearly, these findings would seem to preclude the use of relatively low resistance microelectrodes filled with 3 M KCl for prolonged impalement of small, single cells. However, a number of investigators have reported stable intracellular potentials for many minutes after impaling a "single" epithelial cell. The most probable explanation for these findings is that

[4] The electrode holder employed in all of our studies is the Ag/AgCl pellet-type supplied by W.P.I. In earlier studies, we noted that insertion of the microelectrode into the sleeve through the tight-fitting rubber gasket generated a significant hydrostatic pressure as evidenced by the formation of a small droplet of the filling solution at the tip of the electrode clearly visible with a dissecting microscope under 180× magnification. We have remedied this problem by drilling a small hole into the sleeve of these holders.

[5] In order to illustrate the effect of hydrostatic pressure of the column of filling solution, let us assume that the tip of the microelectrode can be simply viewed as a right cylinder with a length of 1.0 μm an inner diameter of 0.1 μm. If there is a 5-cm column of filling solution above this tip, the hydrostatic pressure will be approximately 5×10^3 dyne/cm^2. Assuming a viscosity of 0.01 dyne·sec/cm^2 (i.e., 1 centipoise), the volume flow given by Poiseuilles' law would be approximately 1 fl/sec. If the filling solution is 3 M KCl, J_K due to this hydrostatic pressure would be 3 fmol/sec and, if the filling solution is 0.5 M KCl, J_K would be 0.5 fmol/sec.

[6] Palmer and Civan (1977) have calculated the changes in "tip potential" of microelectrodes filled with 3 and 0.5 M KCl upon penetration of a cell where the intracellular K and Cl activities are approximately 100 and 40 mM, respectively, predicted by the Henderson equation; these are +2 and +4 mV, respectively. The difference (2 mV) is well within the spontaneous variations encountered in such studies.

because of junctional complexes that permit low resistance cell-to-cell coupling, the epithelial cell layer behaves as a syncytium. Indeed, using Frömter's (1972) data on *Necturus* gallbladder, one can deduce that the conductance of the coupling pathways between one cell and its neighbors is approximately three orders of magnitude greater than the combined conductanes of the limiting cell membranes and about three orders of magnitude greater than the conductance of the electrode tip. Thus, in epithelia with well developed cell-to-cell communication KCl released from the electrode tip is probably not confined to the impaled cell but may be rapidly distributed to neighboring cells. However, it should be clear from these results and those reported by Nelson et al. (1978) (where swelling of frog skin epithelial cells after impalement with KCl-filled microelectrodes was directly observed) that considerable caution must be observed when KCl-filled microelectrodes are employed to impale small cells. These results also suggest that the use of "conventional" 3 M KCl-filled microelectrodes with relatively low resistance to impale isolated cells (e.g., ascites tumor cells) is precluded.

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