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KCl Leakage from Microelectrodes and Its Impact on the Membrane Parameters of a Nonexcitable Cell

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Summary. Microcapillary electrodes filled with a variety of salt solutions, including 1 M KCl, have been used to measure the membrane potentials and resistances of spherical cells from the mycelial fungus *Neurospora* (cell diameters 15–25 μm, cell volumes 3–8 pl). During impalements with electrodes containing 0.3–1.0 M KCl, membrane potential and resistance decayed over a period of 3–10 min. In contrast, electrodes filled with 0.1 M KCl gave stable membrane potentials of −180 mV and membrane resistivities of 40 kΩ cm^2, values comparable to earlier results from the fungal hyphae.

Salt leakage from 1.0 M KCl-filled electrodes (tip diameters 0.2–0.3 μm, resistances 50–75 MΩ) occurred at rates of 4–5 fmol sec^{-1}, as indicated by direct intracellular measurements with ion-sensitive microelectrodes. Depending on cell size, such leakage rates could elevate cytoplasmic KCl content at initial rates of 30–170 mM min^{-1}, and actual values as high as 70 mM min^{-1} were observed. Salt leakage and changes in cytoplasmic KCl concentration were reduced five- to sevenfold when impalements were made with electrodes containing 0.1 M KCl.

The effects on cell membrane parameters of salt leakage from microelectrodes could be attributed to chloride ions. Substitution of the KCl electrolyte with half-molar K_2SO_4 or Na_2SO_4 and molar concentrations of K- and Na-MES [potassium and sodium 2-(N-morpholino)ethanesulfonate] gave stable membrane potentials in excess of −200 mV and membrane resistivities greater than 50 kΩ cm^2, while the permeant anions NO_3^- and SCN^- depressed the membrane parameters in a manner similar to that observed with 1 M KCl. Furthermore, modest elevation of cytoplasmic chloride concentration (below ca. 50 mM) affected both membrane potential and resistance in direct proportion to the concentration, and could be quantitatively described by the Constant Field Theory with a fixed membrane permeability ($P_{Cl} \sim 4 \times 10^{-8}$ cm sec^{-1}). Higher cytoplasmic chloride levels produced a collapse of the membrane resistance and drastic depolarization in a fashion requiring large changes of membrane permeability.

At least for cells with volumes of 10 pl or less, the standard practice of filling electrodes with 1 or 3 M KCl should be abandoned. Half-molar (and lower) concentrations of K_2SO_4 or Na_2SO_4 are suggested as satisfactory replacements.

Key Words *Neurospora* · microelectrode KCl leakage · intracellular K^+/Cl^- · Cl^- permeability

Introduction

Microcapillary electrodes used in the measurement of intracellular potentials are commonly filled with an aqueous solution of 3 M KCl. This practice developed originally out of a desire to minimize electrode resistance and the electrical time constant involved in measurements of the rapid action potentials in isolated muscle tissue (Ling & Gerard, 1949; Nastuk & Hodgkin, 1950). In addition to a lowered resistance, microcapillary electrodes filled with 3 M KCl rather than an isotonic salt solution (Graham & Gerard, 1946; Ling, 1948) usually exhibited tip junction potentials of only few millivolts. Thus changes in the tip potential during impalement, and hence any uncertainties with regard to the intracellular potential, were minimized (Nastuk & Hodgkin, 1950; Adrian, 1956).

One consequence of this practice was to introduce substantial quantities of salt into the tissue via the tip of the electrode. Estimates of salt leakage from 3 M KCl-filled electrodes, based on electrode resistance (Nastuk & Hodgkin, 1950; Coombs, Eccles & Fatt, 1955) or geometry (Geissler, Lightfoot, Schmidt & Sy, 1972), indicated that KCl leakage by diffusion alone could occur at a rate between 0.01 and 0.1 pmol sec^{-1} from a microcapillary electrode with a tip diameter of 0.5 μm. Direct measurements of salt leakage both from electrodes in free solution (Fromm & Schultz, 1981; Page, Kelday & Bowling 1981) and from electrodes within cells (Thomas, 1977, 1978; Isenberg, 1979) are largely in agreement with this figure. Such rates of salt loss may lead to major osmotic and electrical artifacts, concomitant with an increase in salt content of cells with volumes of several hundred picoliters or less (Nelson, Ehrenfeld & Lindemann, 1978). In the case of classically excitable tissue, leakage of salt from the electrode tip can reverse inhibitory postsynaptic potentials during the course of even short term measurements (Coombs et al., 1955).

Our own concern with the problems of salt leakage from microcapillary electrodes arose during development of small (15–25 μm-diameter,

3–8 pl-volume) spherical cells of *Neurospora* for studies of membrane transport and cell homeostasis. Spherical cells impaled with 1 M KCl-filled electrodes exhibited membrane potentials and resistances at best of −90 mV and 20 kΩ cm^2, respectively, which decayed to near zero within 2–10 min. Measurements made on the same cell or on similar cells, with electrodes containing either 0.1 or 0.2 M KCl, gave membrane potentials of −180 to −240 mV and resistances between 30 and 80 kΩ cm^2, values comparable to earlier measurements from the fungal hyphae (Hansen & Slayman, 1978). Furthermore, cells impaled by electrodes filled with the low salt solutions could be held for periods of an hour or more without any decline of either membrane parameter.

We have since examined the effects on the spherical cells of several alternative electrolyte solutions and measured the rate and extent of salt leakage into the cells. Our results indicate that the *Neurospora* plasma membrane is permeable to chloride, and that this permeability gives rise to the observed conductance increase and depolarization when the cells are impaled with electrodes filled with 1.0 M KCl.

Materials and Methods

Cell Culture

Strain P4474a (tng) of *Neurospora crassa* and P27A, a *N. intermedia* wild type (gift of Dr. David Perkins, Department of Biological Science, Stanford University, Stanford, Calif.), were examined during these studies. The strains, both of which produce giant conidia 8–12 μm in diameter, were maintained on 2% agar slants containing Vogel's minimal salts supplemented with 2% sucrose (Vogel, 1956). Slants were subcultured at 14-day intervals.

Spherical cells of the normally mycelial fungus were grown according to Bates and Wilson (1974) in Vogel's minimal salts, 1% (wt/vol) glucose and 18% (vol/vol) ethylene glycol. Two-day-old spherical cells (15–25 μm diameter) were harvested on Millipore filters (3 μm, type SM, Millipore Corp., Bedford, Mass.). The cells were washed free of the growth medium with several volumes of a buffer solution, Standard DMG (20 mM dimethylglutaric acid and 1 mM $CaCl_2$, titrated to pH 5.8 with KOH, $[K^+]=25$ mM) supplemented with 18% ethylene glycol, and resuspended in this same solution. The ethylene glycol was removed by slow dilution with Standard DMG to approximately 1.8% ethylene glycol over a period of 3 hr, after which the cells were filtered again and resuspended in Standard DMG without ethylene glycol.

All impalements were made in Standard DMG except for several experiments involving measurements of intracellular potassium and chloride activities. In these experiments Standard DMG was replaced with a comparable buffer solution (Standard MES) containing 10 mM MES titrated to pH 6.1 with $Ca(OH)_2$ and KOH ($[Ca^{++}]=1$ mM, $[K^+]=5$ mM). An ambient temperature of 20±2 °C was maintained throughout the experiments.

Suction Pipettes

Suction pipettes were manufactured from Kimax 54300 capillary tubing (Kimble Products, Owens-Illinois, Vineland, N.J.) pulled on an Industrial Science horizontal electrode puller (Industrial Science Assoc., Ridgewood, N.Y.) to give shafts of 1-cm length. Suction tips were formed by fusing the glass of each pulled capillary, at a point 30–40 μm behind the tip, to the coil of a microforge and cleanly breaking the glass. The resulting orifice was fire-polished. Tip diameters between 12 and 15 μm were suitable for holding the spherical cells. Batches of suction pipettes were silane-coated by exposure to tributylchlorosilane (Pfaltz & Bauer, Stamford, Conn.) vapors at 160 °C for 15 min (*cf.* Thomas, 1978). Silane coating prolonged the useful life of any one pipette from one or two days to a week or more. During experiments individual suction pipettes were connected via polyethylene tubing and a hand-operated four-way stopcock to a pressure line (N_2 at 14 lb/in^2), to the atmosphere, or to the house vacuum line.

Electrodes

Microcapillary electrodes were pulled from lengths of borosilicate glass (1 mm OD, 0.5 mm ID, Glasswerk Hilgenberg, Malsfeld, FRG) with an internal fiber on the Industrial Science electrode puller. Tip diameters were between 0.2 and 0.3 μm (estimate based on trial scanning electron microscope measurements), and the resistances of representative electrodes – filled with 1.0 M KCl and measured in 1.0 M KCl – were 50–75 MΩ.

Double-barrelled electrodes were produced on a horizontal Industrial Science puller on which the fixed-position spring clamp had been replaced with a small DC motor (Edmund Scientific Co., Barrington, N.H.), geared to 12 rpm, and a pin vice. The electrodes were pulled in two stages from paired lengths of borosilicate capillary tubing. First, the puller solenoid circuit was opened and the glass was heated, twisted 360°, and allowed to cool. Thereafter, the solenoid was reconnected, and a normal pull cycle was initiated. The barrels were separated behind the shank by heating the glass over a small gas flame. Double-barrelled electrodes pulled in this manner showed no evidence of electrical coupling between barrels (pulse amplitude $\leqq$10 nA, rise time $\leqq$0.1 msec). Both barrels gave resistances similar to those of the simple electrodes when comparably filled. In all cases, fresh electrodes were used for each impalement.

Ion-Sensitive Microelectrodes

Double-barrelled, ion-sensitive microelectrodes were prepared as described by Coles and Tsacopuolos (1977). Electrodes were pulled from two pieces of borosilicate glass (*see* above), only one of which contained a fiber for filling. Batches of electrodes were mounted in a small oven (volume approx. 300 cm^3) with the fiber-containing barrel of each electrode linked to a nitrogen gas line. The line was flushed with the dry gas and the electrodes were baked at 160 °C for 15 min. Thereafter, a pressure of 1–2 lb/in^2 was applied to the electrodes. A 10-μl aliquot of tributyl-chlorosilane was introduced into the oven, and after 10 min the oven was flushed with fresh air. The electrodes were baked for an additional 5 min, disconnected from the gas line, and removed from the oven. Finally, a small quantity of potassium or chloride ion exchanger (Corning 477317 and 477913, Corning Medical, Medfield, Mass.) was deposited in the silane-coated barrel of each electrode. Single-barrelled ion-sensitive electrodes were prepared in an analogous manner (Lewis & Wills, 1980).

Both the potassium- and the chloride-sensitive electrodes

were back-filled with 0.1 M KCl and allowed to sit with their tips in 0.1 M KCl for 30 min prior to testing. The electrodes were calibrated before and, occasionally, after impalements. When the electrode response was checked after a measurement, the response half-time was extended to 2–4 min, probably because of residual cytoplasm in the tip. However, in no case did the stable response differ from that measured prior to impalement. Potassium-sensitive microelectrodes were calibrated in 10^{-2}, 10^{-1} and 1.0 M KCl solutions, and selectivity to Na^+ was determined by passage either through a similar NaCl series or through mixed solutions (Lewis, Wills & Eaton, 1978). Chloride-sensitive microelectrodes were calibrated in 10^{-3}, 10^{-2}, 10^{-1} and 1.0 M KCl and selectivities to the bicarbonate, phosphate, and sulphate anions determined by passage through 10^{-3}, 10^{-2} and 10^{-1} M solutions each of $NaHCO_3$, K_2HPO_4 and K_2SO_4. The reference barrels of the double-barrelled electrodes were filled with electrolyte after calibration.

Ion-sensitive electrodes which gave near-Nernstian slopes were selected for impalements. The potassium-sensitive electrodes generally showed resistances of 50–70 GΩ and responded to changes in KCl concentration with half-times of 0.2–0.8 sec. For the chloride-sensitive electrodes the values were 100–200 GΩ and 1–2 sec, respectively. Selectivities of 30:1 (K^+/Na^+) for the potassium-sensitive electrodes, and of 15:1 (Cl^-/HCO_3^-) for the chloride-sensitive electrodes were considered acceptable. In the latter case selectivities for chloride to phosphate and sulphate were at a minimum 8:1 and 10:1, respectively. We also noted a response of the chloride ion-exchange resin to DMG (*see* Fig. 2A).

Electronics

For voltage and resistance measurements with a single intracellular microelectrode, the electrode was connected via a 1-M KCl/Ag-AgCl half cell to a WPI M701 electrometer amplifier (input impedance $>10^{12}$ Ω, WP Instruments, Inc., New Haven, Conn.) equipped with a bridge circuit for passing current. A 1-M KCl-agar bridge leading to a 1-M KCl/Ag-AgCl half-cell (matched to the electrode half-cells) served as the bath electrode and was positioned downstream in the experimental chamber (*see* below). A WPI 800 series pulse generator was connected to the amplifier to drive a bipolar pair of rectangular current pulses (nominally ±4.0 pA, each 0.5 sec in duration), at 10-sec intervals. Current was monitored at the current (voltage signal) input of the amplifier. The readings were checked, from time to time, with a virtual ground current-to-voltage converter and found to be independent of the electrode resistance and free from rectification.

For experiments in which a second, separate recording electrode (or a double-barrelled electrode) was inserted into a cell, the second electrode (or barrel) was connected to an electrometer amplifier with a pre-amplifier input impedance of 10^{13} Ω. In experiments involving ion-sensitive microelectrodes, this amplifier was replaced with an FD223 electrometer amplifier (WP Instruments, pre-amplifier input impedance, 10^{15} Ω) and the output from the M701 amplifier (membrane potential) was subtracted from that of the FD223 unit (ion-sensitive electrode signal) by means of a unity gain differential amplifier. All measurements were displayed on a Tektronix model 5113 storage oscilloscope (Tektronix, Beaverton, Ore.) and recorded on an Esterline Angus strip chart recorder (Esterline Angus Instrument Co., Indianapolis, Indiana).

Mechanical

Experiments were performed in a stainless steel version of the standard *Neurospora* chamber (Slayman, 1965*a*). Bathing solution was introduced at one end of the chamber via a 22-gauge syringe needle and removed at the other end of the chamber by aspiration. A continuous flow of Standard DMG (or Standard MES) was maintained throughout the course of each experiment and the flow rate was adjusted to approximately 5 ml min^{-1} (10 chamber volumes per min).

All operations were observed under dark field illumination at a magnification of 600× with a Reichert Zetopan microscope (Reichert, Vienna, Austria). Cell radii were measured with a calibrated ocular micrometer. Manipulations were performed with the aid of two or three Huxley micromanipulators (Custom Medical Research Equipment, Glendora, N.J.) to which the pre-amplifier probes and suction pipette could be clamped. The Huxley manipulators were positioned so that the electrode(s) and the suction pipette entered from opposite sides of the chamber and approached the lower surface of the coverslip (chamber top) at an angle of 2°.

In preparation for electrical measurements, one side of a 3 × 50 mm coverslip was coated with an aqueous solution containing 1 mg/ml polylysine (350,000 mol wt, Sigma Chemical Co., St. Louis, Mo.). Two or three drops of a suspension of spherical cells in Standard DMG were placed on the coated glass surface and the cells allowed 1–2 min to settle. The coverslip was then inverted, placed across the top of the chamber, and secured with drops of paraffin wax. The chamber was filled with Standard DMG. Cells adhering to the coated glass surface could not be washed off by solution flow, but individual cells could be picked off the surface of the coverslip with a suction pipette. Impalements were performed 50–100 μm below the surface of the coverslip by advancing an electrode into the cell or, in the case of experiments involving two separate electrodes, by advancing the suction pipette and cell onto the electrodes.

Table 1. Tip potentials of microelectrodes[a]

Electrolyte	Concentration (mol/liter)	Tip potential (mV)[d]	
		Before	After
KCl	0.1	−10±2	−8±3 (15)
	0.2	−6±3	−8±5 (15)
	0.3	−11±3	−11±4 (14)
	0.5	−7±1	−5±3 (9)
	1.0	−10±2	−12±2 (12)
NaCl	0.1	2±6	0±7 (4)
	1.2	2±6	−1±5 (5)
K_2SO_4	0.05	−9±3	−11±5 (4)
	0.5	−18±4	−17±5 (8)
Na_2SO_4	0.06	2±2	−2±4 (9)
	0.6	−12±2	−12±5 (8)
K-MES[b]	1.4	−44±6	−37±8 (7)
Na-MES[c]	1.0	−47±3	−41±6 (3)
KSCN	0.3	−15±4	−14±6 (5)
$NaNO_3$	1.0	0±1	0±1 (6)

[a] Measurement, as illustrated in Fig. 1 (inset), in Standard DMG (25 mM K^+) before and after cell impalements. Electrode half-cells, 1 M KCl/Ag-AgCl. Reference, 1 M KCl/Ag-AgCl half-cell with 1 M KCl-agar bridge.

[b] 1.4 M MES titrated to pH 7.1 with KOH.

[c] 1.0 M MES titrated to pH 7.1 with NaOH.

[d] Values given as mean ±SEM. The number of measurements is indicated in parentheses.

Once a cell had been penetrated, the vacuum on the suction pipette was released and the suction pipette was backed away from the cell. At the end of each experiment cells were removed from the microelectrodes with the suction pipette.

Electrode Tip Potentials

During experiments the electrode tip potential was taken as the recording zero. Membrane potentials were measured as the difference between the potential registered with the electrode in the cell and the recording zero (*see* Fig. 1, insert). No attempt was made to correct the membrane potential recorded for possible changes in tip potential upon impalement. While neither choice of zero – the amplifier ground or the recording zero – is entirely satisfactory in view of tip potential changes, we decided to ignore electrode tip potentials for two reasons. First, tip potentials throughout our experiments were largely independent of the electrolyte solutions filling the electrodes (*see* Table 1). Second, we observed a greater consistency in the potentials recorded simultaneously with paired electrodes (or double-barrelled electrodes) containing different electrolytes if the tip potential, rather than the amplifier ground, was taken as the recording zero (*see* Table 4 and Results).

Chemicals

Glucose, ethylene glycol and the reagent grade salts were obtained from Baker Chemical Co. (Phillipsburg, N.J.). The buffers MES and DMG were from Sigma Chemical Co.

Results

Conidia of *Neurospora* grown in 3.2 M ethylene glycol, rather than germinating to form a dense mat of fungal hyphae, swell to several times their initial diameter within 3 to 5 days (Bates & Wilson, 1974). The giant conidia of *Neurospora* strains P27A (8–12 μm) and P4474a (5–10 μm), when grown in ethylene glycol for 48 hr, will form spherical cells with radii between 10–13 and 9–11 μm, respectively. During growth in ethylene glycol the cells retain a uniform, faintly granular, and highly refractile appearance. These cells, when washed free of ethylene glycol and in the presence of a carbon source (or occasionally Standard DMG alone), quickly develop germ tubes and assume hyphal growth and morphology (*see also* Bates & Wilson, 1974).

Electrode Salt Content, Membrane Potentials and Resistances

Successful penetration of the spherical cells or of *Neurospora* hyphae with a microelectrode is characterized by a slight dimpling of the cell surface which then snaps out again as the electrode passes through the cell wall and plasma membrane. Typically, penetration of a spherical cell was accompanied by a negative jump of 10–20 mV in the recorded potential. Cytoplasm was occasionally observed to enter an electrode tip during penetration, indicating a diffusional pathway between the electrode and the cell cytoplasm.

Figure 1 shows five typical recordings from spherical cells of strain P27A obtained with electrodes containing 0.1–1.0 M KCl. Initially all five impalements are qualitatively similar, and exhibit a sigmoidal rise in membrane potential accompanied by an increase in input resistance ($R_{in} = \Delta V_m / I_{in}$, where ΔV_m is the change in membrane potential during each current pulse, I_{in}) as the electrode "seals in" (Slayman, 1965*a*). In cells impaled with electrodes containing 0.3, 0.5 and 1.0 M KCl both V_m and R_{in} peak quickly and thereafter decay at a rate which increases with the salt concentration in the electrode.

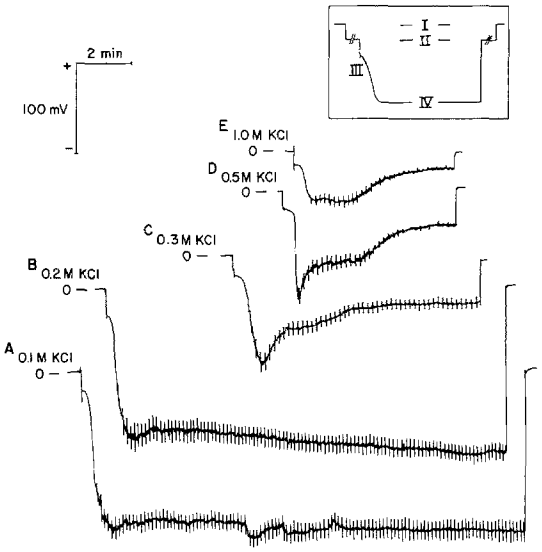

Fig. 1. Apparent membrane potentials recorded with microelectrodes containing 0.1–1.0 M KCl (*A–E*). Spherical cells (*Neurospora* strain P27A) impaled with single microelectrodes. Bipolar current pulses ($I_{in} = \pm 5$ pA) at 6-sec (*A, B*) or 10-sec (*C–E*) intervals gave rise to the small voltage deflections, ΔV, seen in each recording (*see* Material and Methods). Values of specific membrane resistance, R_m, were calculated from the voltage displacements and the surface area of each cell ($R_m = [\Delta V / I_{in}] \times 4\pi r^2$). Cell radii in *A–E*, respectively: 11.4, 12.0, 10.3, 10.8 and 11.4 μm. Each impalement is marked by a jump of −20 to −30 mV, after which the recorded potential and input resistance ($R_{in} = \Delta V / I_{in}$) rise. Note the relative stability of the recorded membrane potential and resistance in cells impaled with electrodes containing 0.1 and 0.2 M KCl (*A, B*) and the progressively rapid decay of the membrane parameters with KCl concentrations above 0.3 M. Inset: Diagram of recording sequence. *I*: Amplifier input grounded. *II*: Potential recorded with microelectrode in bathing solution before and after impalement (recording zero) as shown in traces *A–E*. *III*: Cell penetration. *IV*: Stable intracellular recording. Electrode tip potential = *II–I*. Membrane potentials were measured as the difference between the potential registered with the electrode in the cell and the recording zero (*IV–II*)

In the recordings shown in Fig. 1 the electrodes were intentionally withdrawn from the cells a few minutes after impalement. However, in contrast to measurements with 1.0 M KCl-filled electrodes, cells impaled with microelectrodes containing 0.1 M KCl could be held for at least 3 hr without diminution of either the membrane potential or resistance. Also, measurements made with 0.1 M KCl electrodes in the same cells, subsequent to withdrawal of 1.0 M KCl electrodes, showed V_m and R_m recovered over 10–20 min (not shown). In preliminary experiments, the spherical cells responded to cyanide, azide, and glucose in a manner similar to that of the fungal hyphae (Slayman, 1965b; Hansen & Slayman, 1978), indicating that the cells are physiologically normal during impalements with the 0.1 M KCl-filled electrodes.

These results suggested that salt leakage from the electrode tip was responsible for the rapid decay of V_m and R_{in} observed in impalements with electrodes containing high concentrations of KCl. We examined the effects of various alternative electrolytes on the membrane potentials and resistances recorded in spherical cells, substituting SO_4^{2-}, MES^-, NO_3^- and SCN^- for Cl^-, and Na^+ for K^+. These data are presented in Table 2. Most of the impalements tabulated were held for 15–20 min before the electrode was withdrawn, but occasional recordings were held longer. Of the anions, SO_4^{2-} and MES^- (adjusted to a pH of 7.1) gave uniformly high membrane potentials and resistances. The permeant anions NO_3^- and SCN^- (*cf.* Stroobant & Scarborough, 1979; Sze & Churchill, 1981; D. Perlin and C.W. Slayman, *in preparation*) on the other hand, gave results qualitatively similar to those of Cl^-. Measurements made with Na^+ in place of K^+ showed no obvious difference between the two cations. Consistently high potentials and resistances were obtained with either the SO_4^{2-} or MES^- salts of both cations. [Plots of V_m *vs.* R_m give straight lines with intercepts ($R=0$) of about -30 mV, suggesting that the residual membrane potentials observed with 1 M KCl-filled electrodes arise either from the ion exchange properties of the cytoplasm (Donnan potentials) or from diffusion assymetry at the tip of the electrode (tip junction potentials).]

Salt Leakage Measurement

To confirm that electrode salt leakage substantially increases intracellular KCl concentration, we monitored the intracellular activities of both potassium and chloride following impalement with electrodes

Table 2. Influence of several electrolytes on the membrane potentials (V_m) and resistances (R_m) recorded from spherical cells of *Neurospora* strain P27A[a]

Electrolyte	V_m (mV)	$V_m(t)$ (mV)	R_m (kΩ cm²)	$R_m(t)$ (kΩ cm²)	t (min)	Cell radius (μm)	n
0.05 M K_2SO_4	−246±10		105±10		16±2	11.3±0.5	4
0.5 M K_2SO_4	−253± 7		94±10		24±5	10.9±1.0	5
0.06 M Na_2SO_4	−215±20		135±22		16±3	11.4±0.8	3
0.6 M Na_2SO_4	−235± 7		104± 9		14±1	12.8±0.4	8
1.4 M K-MES	−223±10		83± 1		17±2	10.9±0.6	3
1.0 M Na-MES	−238±10		88± 9		24±2	11.4±0.6	6
0.1 M KCl	−212±12		64± 6		15±2	12.1±0.5	8
1.0 M KCl	−83± 9	−30± 4	16± 4	<0.2	5±1	11.4±0.2	8
0.1 M NaCl	−203±27		51±11		18±5	11.2±0.2	3
1.2 M NaCl	−160±26	−72±13	52± 6	14±5	7±2	10.8±0.4	3
0.3 M KSCN	−86±17	−25± 7	10± 2	<0.2	5±1	10.9±0.4	3
1.0 M $NaNO_3$	−94±26	−16±11	16± 8	<0.2	5±1	11.4±0.4	3

Linear Regression, V_m *vs.* R_m:
Intercept, −33.2 mV. Slope, −2.95. Correl. Coeff., 0.962.

[a] All impalements were made with single electrodes and membrane resistance was determined with the aid of a bridge circuit for passing current (nominally ±5 pA). Values of V_m and R_m are stable maxima, or initial maxima with the membrane parameters thereafter decaying to $V_m(t)$ and $R_m(t)$ (where indicated) by the termination of each impalement (t, time elapsed following penetration). All values are given as the mean of n impalements ±SEM. Potential and corresponding resistance values were subjected to regression analysis as indicated in the text. Substitutions of Cl^- with SO_4^{2-} and MES^- lead to impalements with high membrane potentials and resistances (input resistances typically 5–8 GΩ). The permeant anions NO_3^- and SCN^- depress R_m and V_m in a manner similar to Cl^-. No significant difference is observed between the cations Na^+ and K^+. Similar results were obtained with the P4474a strain (not shown).

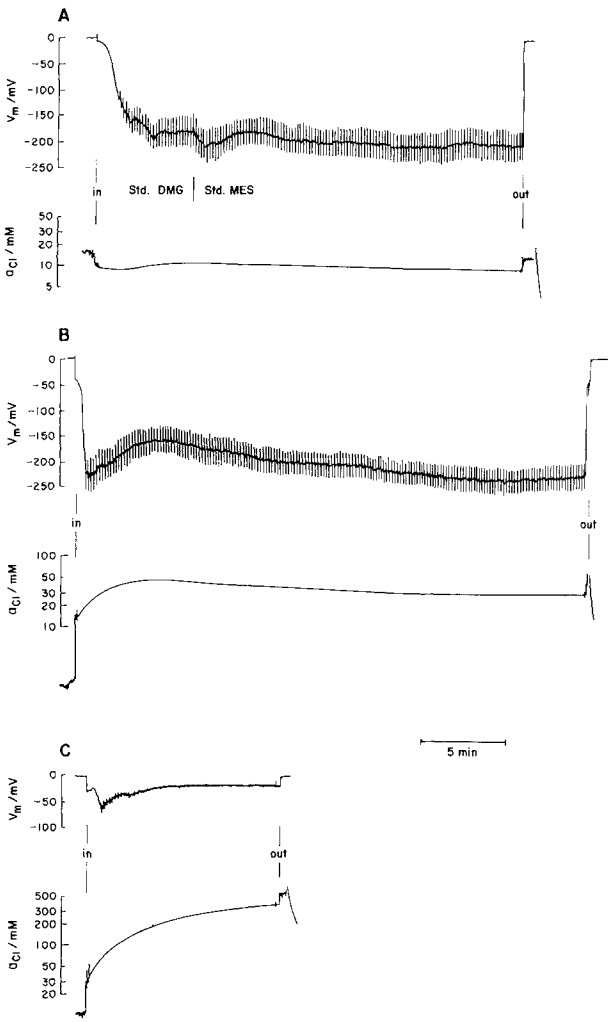

Fig. 2. Intracellular chloride activity (a_{Cl}) in spherical cells (strain P27A) impaled with microelectrodes containing various Cl^- concentrations. Salt-filled electrodes were paired with a second barrel or separate microelectrode containing the Cl^- ion exchange resin (*see* Materials and Methods). Pulse amplitude, ±4 pA. (*A*): Double-barrelled electrode. Reference electrolyte, 0.05 M K_2SO_4. Cell radius, 10.3 μm. Initial bathing solution, Standard DMG ($[Cl^-]_i$=2 mM; note the falsely high chloride signal of the ion-sensitive electrode). Stable membrane resistance, 80 kΩ cm^2. The apparent chloride activity of the cell remained close to 10 mM. At the time indicated the Standard DMG medium was replaced with Standard MES. The membrane potential and resistance oscillated slowly, probably in response to the small shift in pH and $[K^+]_o$, and eventually settled to approximately −210 mV and 95 kΩ cm^2, respectively. Substitution of the Standard DMG solution with the chloride-free medium resulted in only a small decrease, perhaps 2 mM, in a_{Cl}. (*B*): Paired single electrodes. Reference electrolyte, 0.1 M KCl. Cell radius, 11.4 μm. Bathing solution, Standard MES. Salt leakage from the electrode raised the apparent a_{Cl} from 14 mM to approximately 50 mM within 5 min and was accompanied by a depression of V_m and R_m. Thereafter, V_m recovered to −240 mV and a_{Cl} fell to 28 mM (*see also* Fig. 3). (*C*): Double-barrelled electrode. Reference electrolyte, 1.0 M KCl. Cell radius, 12.5 μm. Bathing solution, Standard MES. Both V_m and R_m decayed to near zero within a few minutes of penetration. Salt leakage from the electrode increased a_{Cl} at an initial rate of 55 mM min^{-1} to almost 0.4 M in 10 min

containing 0.05 M K_2SO_4, 0.06 M Na_2SO_4, 0.1 and 1.0 M KCl. Measurements were made with single- and double-barrelled, ion-sensitive microelectrodes, and membrane resistance was monitored by means of the bridge circuit linked to the salt-filled reference electrode (or barrel).

The results are summarized in Fig. 2 and Table 3. The a_K value reported here is in good agreement with previous measurements of the potassium content of *Neurospora* (Slayman & Slayman, 1968). Since the potassium-selective electrodes were relatively insensitive to Na^+ and the normal sodium content of *Neurospora* is low (14 mM, Slayman & Slayman, 1968), the a_K values are not corrected for the sodium cation. The measured chloride activity (10 mM), too, is consistent with the earlier report. The chloride ion exchange resin, however, is subject to interference from other anions. Both sulphate leakage from the electrodes and intracellular phosphate (10–20 mM, Lowendorf, Slayman & Slayman 1974) probably contribute to the a_{Cl} recorded. Washing with chloride-free medium did not reduce the chloride signal substantially (*see* Fig. 2*A*), suggesting that intracellular chloride is normally quite low. For practical purposes, however, the residual chloride signal can be

Table 3. Cytoplasmic K^+ and Cl^- activities in spherical cells of *Neurospora* and changes in these activities during impalements with KCl-filled microelectrodes[a]

	K^+	Cl^-
Normal intracellular activity, a_i (mM)[b]	167± 4 (6)	10± 1 (5)
a_i increment, 0.1 M KCl-filled electrode (mM)[c]	18± 2 (3)	23± 5 (5)
a_i increment, 1.0 M KCl-filled electrode (mM)[c]	308±32 (3)	297±26 (8)
Δa_i/min, 1.0 M KCl-filled electrode (mM/min)[d]	60± 8 (3)	51± 4 (9)
1.0 M KCl leakage rate (fmol sec^{-1})[e]	5.1±0.8	4.4± 0.4

[a] Measurements with single- and double-barrelled ion-sensitive electrodes from both strains, P27A and P4474a, were virtually identical. All data have been pooled in this table. Values are reported as the mean of n impalements (in parentheses) ±SEM.

[b] Reference electrodes (barrels) filled with 0.06 M Na_2SO_4 for potassium and 0.05 M K_2SO_4 for chloride measurements. The apparent a_{Cl} probably reflects cytoplasmic phosphate.

[c] Calculated increment 10 min after penetration.

[d] Calculated as the initial rate of increase between 10 and 40 sec after penetration.

[e] Calculated from values of Δa_i/min and the mean cell radius (10.7 μm), assuming spherical geometry. Activity coefficients are assumed to be unity, and hence the leakage rates are minimal estimates.

ignored, since our main concern lies in the change in a_{Cl} with time following impalement. Figure 2 (*compare also* Tables 2 and 3) shows a strong correlation between the recorded values of V_m and R_m and salt leakage. In general, an increase in a_{Cl} of 40 mM or more was accompanied by a noticeable decline both of V_m and of R_m. Almost complete depolarization and loss of membrane resistance were observed as a_K and a_{Cl} rose to 100 mM and above.

Electrode Tip Potentials

It is evident that the customary 1 and 3 M KCl electrolytes must be abandoned in order to study small cells. The results in Fig. 1 suggest that low concentrations of KCl give satisfactory results; but Fig. 2*B* shows that even 0.1 M Cl^- in the microelectrode produced physiological changes, though steady-state membrane potentials and resistances were high enough to be considered "normal" by comparison with previous values from *Neurospora* hyphae. Substitutions of SO_4^{2-} or MES^- provide alternatives to KCl, but the use of salts other than KCl to fill microelectrodes raises other uncertainties, chiefly associated with the diffusional asymmetry and resistance of the electrode tip itself. Microelectrodes filled with solutions of isomobile ions (K^+ and Cl^-) can show substantial tip potentials (Adran, 1956; Slayman, 1965*a*). These potentials are a source of uncertainty in measurements of membrane potential, primarily because tip potentials change as microelectrodes pass from one recording solution to another (i.e., from bathing medium to cytoplasm). The question, then, is whether this problem – inherent to measurements with fine microelectrodes – is compounded by unconventional ions such as MES^- or SO_4^{2-}.

Examining the tip potentials of electrodes filled with different solutions, before and after impale-

Table 4. Membrane potentials (V_m) and resistances (R_m) from spherical cells of *Neurospora* strain P27A measured simultaneously by double impalement (*P*) or by impalement with double-barrelled electrodes (*D*)[a]

Impalement No.	Electrolyte	Electrode(s) paired/double	Tip potential (mV)	V_m (mV)	R_m (kΩ cm²)	Cell radius (μm)
1	0.1 M KCl	*D*	−15	−163	41	11.4
			−3	−165	41	
2	0.05 M K_2SO_4	*D*	−9	−215	103	10.8
			−4	−210	105	
3	0.06 M Na_2SO_4	*D*	−10	−210	81	11.4
			−15	−210	81	
4	0.06 M Na_2SO_4	*P*	−6	−220	62	11.4
			−3	−225	62	
5	0.05 M K_2SO_4	*P*	−6	−168	41	11.4
	0.1 M KCl		−18	−168	41	
6	0.1 M KCl	*P*	0	−179	88	10.8
	0.05 M K_2SO_4		−8	−182	88	
7	0.06 M Na_2SO_4	*D*	0	−195	55	11.4
	0.1 M KCl		−12	−192	55	
8	0.1 M KCl	*P*	−3	−204	47	10.8
	0.06 M Na_2SO_4		0	−202	47	
9	1.4 M K-MES	*D*	−62	−255	62	12.0
	0.1 M KCl		−15	−263	62	
10	0.1 M KCl	*D*	−6	−184	65	12.5
	1.4 M K-MES		−48	−180	68	
11	1.0 M Na-MES	*P*	−30	−184	60	10.3
	0.1 M KCl		−12	−186	60	
12	0.1 M KCl	*D*	−3	−215	61	10.8
	1.0 M Na-MES		−64	−215	61	

[a] In each case, one electrode (barrel) was connected in series with a bridge circuit for passing current (±5 pA). Measurements obtained with this electrode (barrel) are listed first. In each of the first four impalements both electrodes (barrels) were filled with the same solution. For the remaining impalements, one electrode (barrel) – first passive and then current-passing – was filled with 0.1 M KCl as a standard. Values of V_m and R_m given are stable maxima (V_m referenced to the recording zero, *see* Fig. 1, inset). Tip potentials were measured in Standard DMG (25 mM K^+) prior to impalement.

ment (Table 1), leads to the following conclusions: (i) Electrode tip potentials measured before puncture and after withdrawal scattered within 5 mV of each other, so they did not introduce systematic drift artifacts, regardless of the filling solution tested. (ii) Microelectrodes filled with KCl generally had small tip potentials (*ca.* 10 mV), which scattered independently of the KCl concentration. (ii) Substitution of SO_4^{2-} for Cl^- probably increases electrode tip potentials, at least at the higher concentrations (0.5 and 0.6 M), but the effect was not large nor statistically very significant. (iv) Substitution of MES^- for Cl^- did systematically produce large tip potentials, which could compromise measurements of membrane potential even from cells, like *Neurospora,* with potentials of −200 mV or greater.

Of the salts tested, K_2SO_4 and Na_2SO_4 appear to be most satisfactory for filling microelectrodes. Tip potentials of (−)20 mV correspond to a possible error of 10% in the membrane potential of *Neurospora.* However, in order to assess any relative errors introduced by the various salt solutions, we monitored the membrane potentials and resistances of individual cells simultaneously with two electrodes (or with double-barrelled electrodes) filled with different electrolytes. In these experiments 0.1 M KCl was chosen as a standard because of the small difference in the mobilities of the potassium and chloride ions, and because it is tolerated by the spherical cells. Also, the near-isotonicity of the solution was expected to minimize any matrix or "pre-tip" potential artifacts (Davis et al. 1970; Nelson et al. 1978).

Table 4 summarizes the results of several simultaneous measurements with 0.1 M KCl and a test electrolyte as well as similar measurements in which both electrodes (or barrels) were filled with identical solutions. In general, the membrane potentials recorded differed by 3–5 mV between electrodes (barrels), despite the often large difference in tip potentials. The difference in measured membrane potential never amounted to more than 3% of the membrane potential registered with the standard 0.1 M KCl fill. Small variations in recorded membrane potential were also observed from electrode pairs or double-barrelled electrodes filled with identical solutions (impalements 1–4). Hence, while electrode tip potentials may introduce errors into the measured membrane potentials, these errors are largely independent of the electrolyte chosen.

It is also worth noting that the membrane resistances obtained with the bridge circuit and by the two-electrode technique are in close agreement. These observations provide an important check on our earlier use of single electrodes for resistance measurement. No large or systematic variations in R_m between electrodes (or barrels) were observed, which indicates that electrode resistances were stable during measurements and argues against any large changes of electrode resistance with penetration.

Discussion and Conclusions

Two conclusions can be reached on the basis of our observations. First, leakage of KCl from microelectrodes raises cytoplasmic salt concentration appreciably, even when cell turgor visibly pushes cytoplasm into the electrode tip. Second, increases in salt content affect measurements of membrane potential and resistance, and the physiologic status of cells, even with low membrane ionic permeabilities.

Salt Leakage

From direct measurements of intracellular potassium and chloride activities we have observed salt leakage from 1 M KCl-filled electrodes to raise a_K and a_{Cl} in spherical cells of *Neurospora* with initial rates as high as 70 and 72 mM min^{-1}, respectively, to values above 0.5 M in 8 min! This increment in ionic activity is equivalent to a threefold increase in $[K^+]_i$ and at least a 40-fold increase in $[Cl^-]_i$ above normal levels.

Much of this leakage can be accounted for by the diffusion of KCl from the electrode tip. The small current pulses employed in estimating membrane resistance do not contribute significantly to the passage of electrolyte into the cell. Even allowing for a transport number of 1.0, iontophoretic injection of KCl could account for only 0.05 fmol KCl $(pulse\ pair)^{-1}$ for a pulse amplitude of 5 pA. Given a pulse frequency of 0.1 sec^{-1} and averaging over time, this value reduces to 0.005 fmol KCl sec^{-1}. By contrast, we estimate initial leakage rates of K^+ and Cl^- from 1 M KCl-filled electrodes to be 5.0 ± 0.8 and 4.4 ± 0.4 fmol sec^{-1}, respectively. These values are in good agreement with previous estimates of salt diffusion from microelectrodes (Coombs et al. 1955; Isenberg, 1979; Fromm & Schultz, 1981; Page et al. 1981).

Physiological Implications

For cells bathed in Standard DMG (25 mM K^+, 2 mM Cl^-) the equilibrium diffusion potentials for

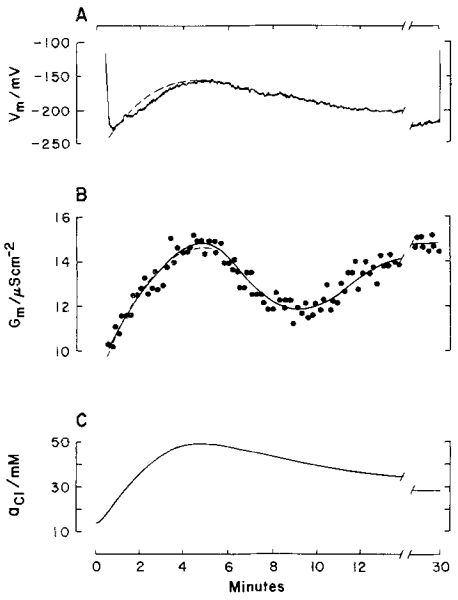

Fig. 3. Effect of 0.1 M KCl leakage upon the membrane potential and resistance of a spherical cell (data from Fig. 2*B*). (*A*): Retraced membrane potential (V_m) recording with the pulses deleted. (*B*): Membrane conductance (G_m) calculated as $1/R_m$ (*see* legend to Fig. 1). (*C*): Point plot of apparent cytoplasmic chloride activity (a_{Cl}). The solid line in *B* was drawn by eye. The dashed lines in *A* and *B* were obtained by fitting Eqs. (1), (2), (3b) and (5) to V_m and G_m, assuming the cytoplasmic chloride concentration to be the change of a_{Cl} after time zero. The small discrepancy between the measured and fitted membrane potentials may reflect drift noise at the electrode tip. No attempt was made to extend the fit beyond 5 min

K^+ and Cl^- are roughly -50 and $+40$ mV, respectively. Salt leakage from the microelectrode into the cytoplasm drives E_K to more negative and E_{Cl} to more positive values. Hence, the decay of membrane potential in cells impaled with KCl-filled electrodes could be accounted for by assuming that the chloride conductance comes to dominate the overall membrane conductance as $[Cl^-]_i$ rises. As such, measured membrane potentials and resistances should be sensitive to chloride, and not to potassium leakage from the electrode.

This prediction is borne out in experiments in which cells were impaled with electrodes filled with alternative electrolytes. If we substituted either SO_4^{2-} or MES^- (pH 7.1) for the chloride anion both V_m and R_m remained high, despite the near molar concentrations of potassium in the electrode. Replacing potassium with sodium had only marginal effects on the membrane parameters. The permeant anions NO_3^- and SCN^- resulted in a loss of membrane potential and an increase in membrane conductance in a manner similar to that observed with electrodes containing 1.0 M KCl.

Thus, with respect to the physiological impact of salt leakage, these observations indicate a finite chloride conductance in the plasma membrane of *Neurospora*. More detailed analyses of the data from Fig. 2*B* and *C* are provided in Figs. 3 and 4. For the first 5 min of impalement with an 0.1 M KCl electrode (Fig. 3), both the increase in membrane conductance and the decrease in membrane potential (upward, positive) can be quantitatively described as results of cytoplasmic chloride loading, without any *change* of membrane permeability.

The plasma membrane of *Neurospora* can be represented by an equivalent circuit containing two parallel limbs: one for the proton pump, with an apparent reversal potential (E_P) and a series conductance (G_P); and the other for the ensemble of passive ionic pathways or leaks, characterized by a diffusional emf (E_L) and conductance (G_L). [Previous electrical analysis of the *Neurospora* membrane indicates that about half of the total membrane conductance can be attributed to the proton pump and about half to the leaks, over the voltage range -230 to -160 mV. In fact, within this range both conductances are approximately linear. By the same analysis, E_P and E_L have been estimated near -400 and 0 mV, respectively (Gradmann et al. 1978; Gradmann, Hansen & Slayman, 1982; Slayman, 1982).]

From the equivalent circuit, the following equations can be written for membrane potential and conductance:

$$V_m = (E_P\, G_P + E_L\, G_L)/G_m \tag{1}$$

$$G_m = G_P + G_L. \tag{2}$$

If the electric field through the membrane is assumed to be approximately constant, and chloride is assumed to permeate passively, then the leak emf can be written as described by Hodgkin and Katz (1949):

$$E_L = \frac{RT}{F} \ln \left[\frac{\Sigma P_x [X^-]_i + \Sigma P_y [Y^+]_o + P_{Cl}[Cl^-]_i}{\Sigma P_x [X^-]_o + \Sigma P_y [Y^+]_i + P_{Cl}[Cl^-]_o} \right] \tag{3a}$$

where the Σ terms represent products of permeability and concentration for all other diffusible anions and cations. Since E_L is normally near zero (*see* above), the Σ terms in numerator and denominator can be equated and held fixed ($=K$) for the present; and since, in the experiments of Figs. 3 and 4, chloride was omitted from the external medium, Eq. (3a) reduces to:

$$E_L = \frac{RT}{F} \ln \frac{K + P_{Cl}[Cl^-]_i}{K}. \tag{3b}$$

Total current flow through the membrane leaks can also be written by the Constant Field Theory as:

$$I_L = \frac{F^2}{RT} V_m \left[\frac{K - (K + P_{Cl}[Cl^-]_i)\, e^{-V_m F/RT}}{1 - e^{-V_m F/RT}} \right] \quad (4)$$

and the leak conductance, $G_L = dI_L/dV_m$.

This latter conductance must be solved for nonzero current, because the proton pump drives a net current through the leak component of the *Neurospora* membrane. The derivative reduces to the following form when the large membrane potential is taken into account:

$$G_L \simeq \frac{F^2}{RT} (K + P_{Cl}[Cl^-]_i). \quad (5)$$

Equations (1), (2), (3b) and (5) can be fit by least-squares (Marquardt, 1963) to the data of Fig. 3*A* and *B*, incorporating the change in apparent chloride activity measured after zero time (Fig. 3*C*) for $[Cl^-]_i$. When this operation was carried out with equal weighting of the V_m and G_m data (G_m scaled to be numerically about the same magnitude as V_m), the dashed curves in Fig. 3 and the following parameter values were obtained: E_P (apparent) $= -465$ mV, $G_P = 5.29$ μS cm^{-2}, and $K = 1.13 \times 10^{-6}$ mM cm sec^{-1}. The calculations also yielded a chloride permeability, P_{Cl}, of 4.1×10^{-8} cm sec^{-1}.

A comparison of the chloride efflux (J_{io}^{Cl}), calculated from P_{Cl} and the parameters above, with the salt leakage from the electrode does suggest that P_{Cl} might be greater than calculated, which could indicate the presence of an electroneutral chloride efflux across the *Neurospora* membrane. Five minutes after impalement, the intracellular chloride activity reaches a plateau, and hence the chloride efflux across the plasma membrane must equal the rate of salt leakage from the electrode. From the Constant Field approximation, the chloride efflux,

$$J_{io}^{Cl} = \frac{FV_m}{RT} \left[\frac{P_{Cl}[Cl^-]_i\, e^{-V_m F/RT}}{1 - e^{-V_m F/RT}} \right] \simeq \frac{FV_m}{RT} P_{Cl}[Cl^-]_i. \quad (6)$$

Thus the parameters $P_{Cl} = 4.1 \times 10^{-8}$ cm sec^{-1}, $[Cl^-]_i = 35$ mM (corrected for a background signal of 14 mM), and $V_m = -157$ mV give a steady-state chloride efflux of 9.2 pmol cm^{-2} sec^{-1}. From the initial increase of cytoplasmic chloride activity (8 mM min^{-1}) and the cell radius (11.4 μm), the leakage rate from the microelectrode is 0.8 fmol sec^{-1}, which would require a balancing chloride efflux across the membrane of 50 pmol cm^{-2} sec^{-1}. However, this figure might be reduced to 30 pmol cm^{-2} sec^{-1} if the chloride leakage rate is assumed to be proportional to the concentration difference between the cytoplasm and the bulk solution in the electrode, and depletion of salt from the electrode tip (Purves, 1979) might account for the additional discrepancy.

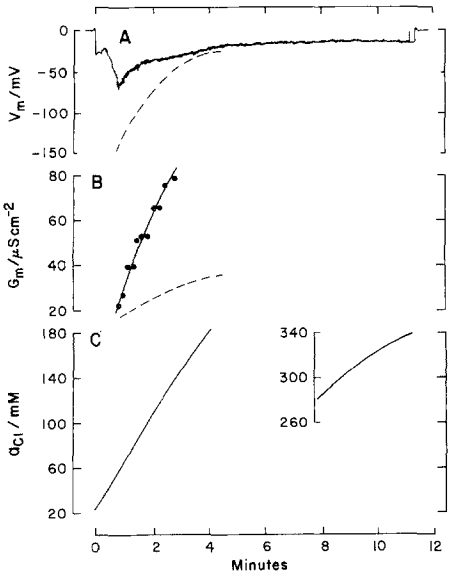

Fig. 4. Effect of 1.0 M KCl leakage upon the membrane potential and resistance of a spherical cell (strain P27A). Data from Fig. 2*C* presented as in Fig. 3. The dashed curves were drawn from Eqs. (1), (2), (3b) and (5), with the voltage, conductance and chloride permeability parameters obtained from analysis of Fig. 3 (*see* text). Lack of agreement between the data and the calculated voltage and conductance values indicates that extreme elevation of $[Cl^-]_i$ results in altered membrane permeability

Clearly, membrane potential and conductance in Fig. 3 are affected by factors other than the elevated cytoplasmic chloride level after 5–6 min. Subsequent recovery of V_m and G_m is disproportionately large compared with the small decrease of $[Cl^-]_i$. The behavior of V_m and G_m in response to severe Cl^- leakage (Fig. 4) also requires more elaborate interpretation. The dashed curves in Fig. 4 are the results of V_m and G_m calculations with the parameter values obtained from Fig. 3. The fact that Eqs. (1)–(5) predict conductance slopes (*vs.* $[Cl^-]_i$ or *vs.* time) that are much too small and voltage slopes that are too large suggests that chloride levels above 40–50 mM cause a large increase of membrane permeability.

It should be emphasized that a chloride permeability of 4×10^{-8} cm sec^{-1} is a modest value, being within an order of magnitude of the resting chloride permeability for *Nitella* (*cf.* Slayman, 1970). The P_{Cl} of *Neurospora* is 10-fold lower than

values accepted for a variety of cultured animal cells (Lamb & MacKinnon, 1971; Nelson, Peacock & Minna, 1972) and is 100-fold lower than the P_{Cl}'s estimated for frog muscle (Hodgkin- & Horowicz, 1959) and rabbit descending colon and urinary bladder basolateral membranes (Wills, Lewis & Eaton 1979; Lewis, Wills & Eaton, 1978).

Yet despite the low P_{Cl} of the *Neurospora* membrane, chloride leakage from impaling electrodes into the spherical cells rapidly depresses the membrane potential and increases membrane conductance. Large cell volumes will act to buffer the effects of electrolyte leakage and, in impalements of plant cells (which generally result in penetration of the central vacuole), the tonoplast could provide a barrier between the cytoplasm and salt from the electrode tip. However, most animal cells are small, with volumes of 20 pl or less, and in several cases are known to be subject to salt leakage artifacts (Nelson et al., 1978, Davis et al., 1970). Comparably sized plant cells, such as stomatal guard cells, also occur, the tonoplast properties of which remain uncertain (Moody & Zeiger, 1978). Perhaps observations of low membrane potentials in yeast (*see* review by Borst-Pauwels, 1981), stomatal guard cells (*cf.* Moody & Zeiger, 1978), plant protoplasts (Racusen, Kinnersley & Galston, 1977), subcellular organelles (Maloff, Scordilis, Reynolds & Tedeschi, 1978), and cultured mammalian cells (Okada, Tsuchiya & Inouye, 1979; Nelson et al., 1972), bear re-examining.

Although salt loading from the microelectrode can overwhelm the normal *Neurospora* membrane potential and permeability, the collapse of membrane resistance is not permanently damaging. We have already noted that, following withdrawal of 1 M KCl-filled electrodes and their replacement by electrodes containing 0.1 M KCl, both V_m and R_m of the spherical cells recover over a period of 10–20 min. Salt loading can also induce rhythmic depolarizations – the "action potentials" previously described in *Neurospora* hyphae (Slayman, Long & Gradmann, 1976). In the hyphae such events occur only sporadically, and all previous attempts to evoke them failed. We now find that action potentials follow upon penetration of the spherical cells with electrodes containing high concentrations of potassium (e.g., K_2SO_4). The relative frequency of occurrence and the association with salt loading may indicate that action potentials in *Neurospora* represent a mechanism for regulating cytoplasmic ion balance or osmotic pressure. Similar spontaneous activity is observed in the marine alga *Acetabularia,* where it has been suggested as a mechanism for regulating cytoplasmic potassium levels (Mummert & Gradmann, 1976). A detailed study of the action potentials in spherical cells of *Neurospora* is now in progress.

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