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A NEW SOLID-STATE MICROELECTRODE FOR MEASURING INTRACELLULAR CHLORIDE ACTIVITIES

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Summary

Solid-state microelectrodes for measuring intracellular Cl^- activity (a^iCl) were made by sealing the tips of tapered glass capillaries (tip diameter 0.3 μm), coating them under vacuum with a 0.2—0.3 μm thick layer of spectroscopic grade silver, and sealing them (except for the terminal 2—5 μm of the tip) inside tapered glass shields. 106 microelectrodes had an average slope of 55.0 ± 0.6 mV (S.E.) per decade change in a_{Cl}. Tip resistance was (77.1 ± 3.1) × 10^9 Ω (n=30). Electrode response was rapid (10—20 s), was unaffected by HCO_3^-, $H_2PO_4^-$, HPO_4^{2-} or protein, and remained essentially unchanged over a 24-h period. a^iCl in frog sartorius muscle fibers and epithelial cells of bullfrog small intestine was measured in vitro. In both tissues, a^iCl significantly exceeded the value corresponding to equilibrium distribution of Cl^- across the cell membrane.

Most intracellular Cl^- activity (a^iCl) measurements so far reported have utilized either Ag/AgCl microelectrodes [1,2] or Cl^--selective liquid ion-exchanger (Corning 477315) microelectrodes [3]. Recent studies, together with our own experience in evaluating these microelectrodes, indicate that the values which they yield for Cl^- activity (a_{Cl}) in complex media such as cytoplasm are subject to serious uncertainties. It was reported [4] that liquid ion-exchanger microelectrodes [3] consistently overestimate a_{Cl} in solutions containing Cl^- together with HCO_3^-, propionate$^-$, isethionate$^-$, Br^-, or I^-. Further, when the composition of such mixtures is varied, at constant ionic strength, the calculated selectivity coefficients for these electrodes are not constant [4,5]. We confirmed these results for $KCl/KHCO_3$ mixtures and found similar effects in $KCl/KH_2PO_4/K_2HPO_4$ mixtures.

When used intracellularly, Ag/AgCl microelectrodes appear to be subject to a systematic offset potential which causes a^iCl to be overestimated. In addition, upon withdrawal from a cell after impalement, the potential regis-

tered by such electrodes frequently does not return to its initial extracellular value or does so very slowly [6,7]. This may be due, in part at least, to an irreversible or slowly reversible change in the electrode surface following exposure to an intracellular environment, or may reflect some sensitivity of these electrodes to redox systems in the cell.

These findings prompted us to develop a microelectrode, suitable for measuring a_{Cl} in relatively small cells, and free from the shortcomings associated with liquid ion-exchanger or Ag/AgCl microelectrodes. A Cl^--selective microelectrode [8], formed by the chemical deposition of a small plug of solid silver inside the open tip of a conventional glass microelectrode seemed unaffected by the artifactual potentials found with Ag/AgCl microelectrodes [6], and appeared to be capable of accurate measurements of a^i_{Cl} [8]. However, in our hands, silver deposition by this method proved difficult to control. Frequently, microelectrodes were either incompletely sealed or had excessive deposits of silver on the tip and were, as a result, unsuitable for impaling cell membranes. Further studies were directed towards the fabrication of silver microelectrodes in which the size and shape of the tip could be rigorously controlled.

Fig. 1A is a schematic drawing of a completed microelectrode. Lengths of capillary glass (Kimax-51, outer diameter 1 mm; inner diameter 0.7 mm), previously cleaned in methanol, were drawn in a Kopf 700C vertical electrode puller to very fine tip diameters. The tips were then sealed under high magnification [9]. Sealed tips averaged about 0.3 μm in diameter when inspected in a Phillips 500 scanning electron microscope. Pipettes were then mounted, tip downwards, in a specially designed holder and immersed briefly (in a dust-

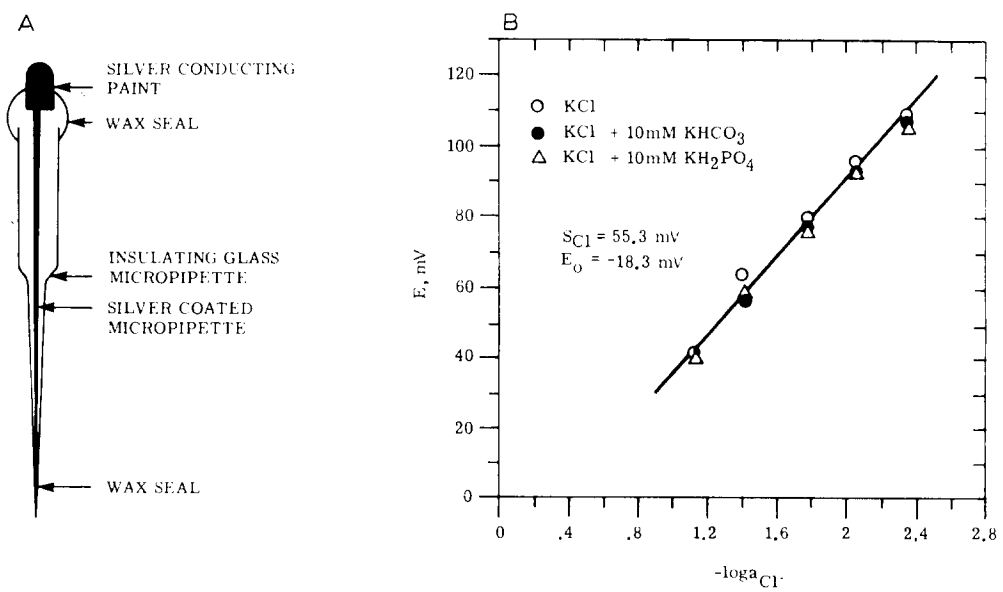

Fig. 1. A. Schematic drawing of a solid-state silver microelectrode. B. Calibration of a silver microelectrode. The line giving the slope of the electrode potential was fitted by least squares analysis. E_0 (the potential that the electrode would register in a solution where a_{Cl}=1), was calculated from the equation of this line.

free atmosphere), first in boiling trichloroethylene and then in a 1/1 (v/v) methanol/isopropanol mixture, maintained just at its boiling point. Then they were transferred to the rotary holder of a Varian NRC Mono 3117 vacuum coater and covered with a uniform layer (0.2—0.3 μm thick) of spectroscopic grade silver.

As described elsewhere for K^+-selective glass membrane microelectrodes [9], all but the tips of the silver coated micropipettes were isolated from the ambient medium by inserting them, under high magnification, in slightly wider glass micropipettes and forming paraffin wax seals at the point where the silver coated tip emerged from the lumen of the glass shield. With practice, exposed tip lengths 2—5 μm are routinely achieved by this method*. Finally, a paraffin seal was made between the open end of the glass shield and the barrel of the silver coated micropipette, and a coating of electrically conducting silver paint was applied to the part of the barrel which protruded from this seal (Fig. 1A). In this form, microelectrodes can be stored indefinitely in a light-tight box.

Fully assembled microelectrodes were calibrated in solutions containing 1, 5, 10, 20, 50, or 100 mM KCl or NaCl. a_{Cl} in solution was calculated from the Debye-Hückel equation [11]. Silver microelectrodes were connected directly to the input of a high-impedance preamplifier (Analog Devices, 311J). Reference electrodes were calomel half-cells connected to the calibrating solution by a salt bridge (3% agar in 1 M potassium citrate) [12]. Electrode potentials were displayed simultaneously on a digital voltmeter (Fairchild 7050 or Keithley 165) and a Brush 240 pen recorder. When relatively rapid phenomena, e.g. electrode response times, were examined, a Tetronix 5103N dual beam storage oscilloscope and C-59 polaroid camera were included in the display system. A Narishige MM 33 micromanipulator was used to advance the microelectrode into the calibrating solution.

The 106 microelectrodes examined had an average slope at 25°C of 55.0 ± 0.6 (S.E.) mV per decade change in a_{Cl}. For each electrode the slopes in KCl and in NaCl solutions were identical. Incorporation of 10 mequiv. HCO_3^-, 2.4 mequiv. $H_2PO_4^-$ plus 7.6 mequiv. HPO_4^{2-}, or 5% (w/v) bovine serum albumin in solutions containing 5—100 mM KCl did not appreciably alter the response of the microelectrodes to Cl^- (Fig. 1B). Erratic responses were obtained when Br^- or I^- (1, 5 or 10 mM) were added to these solutions.

These microelectrodes had relatively rapid response times. Steady-state potentials were attained in 10—20 s after immersion and remained constant, within 1 mV, for immersion periods up to 1 h (the longest time tested). In repeated calibrations the electrode response to Cl^- did not change during at least 3 h and, with 4 electrodes whose response to calibrating solutions was monitored for longer times, slopes remained within 1—2 mV of their initial values over a 24-h period. The average tip resistance recorded for 30 microelectrodes was $(77.1 \pm 3.1) \times 10^9\ \Omega$.

*Very recently, Dr. Waclaw Wojtkowski has developed in our laboratory a promising alternative shielding method. This is based on a method used to insulate tungsten microelectrodes [10], and involves covering the silver coated micropipette with a layer of high molecular weight polymethylmethacrylate (mol. wt. $\approx 1 \cdot 10^6$). The polymethylmethacrylate layer covering the terminal 2—3 μm of silver is then degraded by exposing it to the beam of a scanning electron microscope and dissolved by immersing the tip of the microelectrode in ethanol.

$a^{i}Cl$ was measured in frog (*Rana pipiens*) sartorius muscle fibers and epithelial cells of bullfrog (*Rana catesbeiana*) small intestine. Tissues were equilibrated at 25°C with oxygenated isosmotic Ringer solutions [13,14]. Isolated muscles were pinned in a simple organ bath [9] which had a total capacity of about 50 ml. The small intestine was stripped of its underlying muscle layers [15] and mounted as a flat sheet in a perfusion chamber [12] which permitted continuous recording of the transmural PD. Epithelial cells were impaled through their mucosal membranes. Membrane potentials were recorded in the same experiments with conventional microelectrodes [9,12], connected through agar bridges to a calomel half-cell, a second 311J preamplifier and another digital voltmeter. Narishige MO-10 hydraulic micromanipulators were used to advance both microelectrodes into the cells and a single (grounded) half-cell connected to an agar bridge in the bathing medium served as a reference for both. In experiments with the small intestine the reference electrode was placed in the mucosal medium.

Figs. 2A and 2B are oscilliscope tracings of impalements in a sartorius muscle fiber and an epithelial cell of the small intestine. Table I summarizes the results obtained in these experiments. In this table the mean measured $a^{i}Cl$ values we obtained are compared with the equilibrium values ($a^{eq}Cl$) calculated from the relationship $a^{o}Cl/a^{eq}Cl=\exp(\bar{E}F/RT)$ where $a^{o}Cl$ is the extracellular chloride activity, $\bar{E}$ is the mean membrane potential (mucosal membrane potential in experiments with intestinal epithelial) and R, T and F have their usual meanings. In calculating $a^{eq}Cl$, the equilibrium condition, $\bar{E}=E_{Cl}$, where E_{Cl} is the chloride equilibrium potential across the cell membrane, was assumed.

Table I shows that, under the conditions of our experiments, the observed value of $a^{i}Cl$ was significantly greater ($P<0.001$) than that predicted for electrochemical equilibrium across both the muscle fiber membrane and the mucosal membrane of the intestinal absorptive cell. In the latter, since the serosal membrane potential in a sodium chloride medium without external substrate is usually not more than 1—3 mV greater than the mucosal membrane potential [12,13], it is clear that $a^{i}Cl$ also exceeds the value required for electrochemical Cl^{-} equilibrium across this membrane. Thus, in the frog,

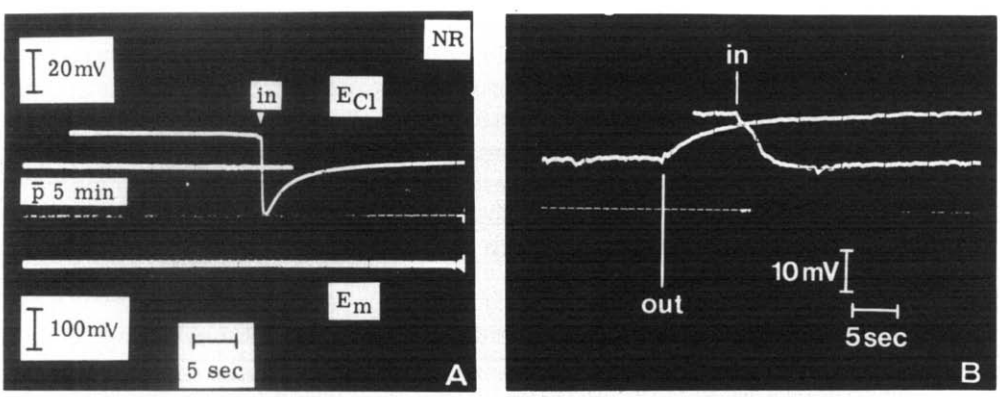

Fig. 2. Impalement with a silver microelectrode of: (A) a sartorius muscle fiber (*R. pipiens*), (B) an intestinal epithelial cell (*R. catesbeiana*). $\bar{P}$ 5 min (A) shows stability of impalement after 5 min. NR, normal Ringer solution (121 mequiv. Cl^{-}/l).

TABLE I

Mean observed Cl^- activities (a^iCl) ± S.E., calculated equilibrium Cl^- activities ($a^{eq}Cl$), and mean membrane potentials ($\bar{E}$) in frog sartorius muscle fibers (I, II) and intestinal epithelial cells (III). Tissues equilibrated in Ringer solutions containing Cl^- at the concentrations and calculated [11] activities (a^oCl) indicated. Figures in parentheses are number of animals followed by total number of impalements. $\bar{E}$ in mV. Other parameters in mequiv./l.

	$[Cl]_o$	a^oCl	$\bar{E}$	a^iCl	$a^{eq}Cl$
I	121	92.3	−82.7 ± 0.9(12:61)	7.0 ± 0.4(12:54)	3.3
II	76.2	58.1	−85.3 ± 1.0(8:15)	6.0 ± 0.1(8:28)	2.1
III	105	81.1	−24.0 ± 1.1(9:44) (mucosal)	71.0 ± 4.7(9.38)	31.5

both skeletal muscle fibers and epithelial cells of the small intestine appear to have the ability to accumulate Cl^- against an electrochemical potential gradient. These results are in general agreement with reported measurements of a^iCl in rabbit ileum [16,17] and confirm earlier measurements of a^iCl in frog (*Rana temporaria*) sartorius muscle [18].

The mechanism underlying Cl^- accumulation in muscle is still unclear. In an earlier study from this laboratory [13], an uphill Cl^- pump from lumen to cell in the brush border membrane, together with a downhill movement of Cl^- across the basolateral cell membrane, was invoked as one possible explanation for active transepithelial Cl^- transport in isolated bullfrog small intestine. The present results provide strong support for the localization at the apical cell membrane of the primary accumulative step in this process. There is now abundant evidence for active Cl^- absorption by the distal small intestine of several mammalian species including man. In isolated rabbit ileum the intracellular Cl^- concentration of the mucosal cells appears to exceed that of the surrounding medium. It was suggested that this is due to uphill Cl^- transport from the mucosal medium to the cell interior [19], and that the energy for Cl^- accumulation could be derived, in part at least, from the electrochemical potential gradient for Na^+ across the mucosal membrane [20]. The extent to which Cl^- accumulation by the epithelial cells of bullfrog small intestine, under the conditions of our experiments, depends on a concomitant downhill Na^+ transport, or is due to a Na^+-independent active transport of Cl^- [13], is a question that requires further study. Nevertheless the results shown in Table I for bullfrog small intestine may reflect a component of intestinal Cl^- transport which is of widespread occurrence in the animal kingdom.

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References

1 Gayton, D.C. and Hinke, J.A.M. (1968) Can. J. Physiol. Pharmacol. 46, 213—219
2 Neild, T.O. and Thomas, R.C. (1973) J. Physiol. Lond. 231, 7P—8P
3 Walker, J.L. (1971) Anal. Chem. 43, 89—92A
4 Saunders, J.H. and Brown, H.M. (1975) Biophys. Soc. Abstr., p. 323a
5 Owen, J.D., Brown, H.M. and Saunders, J.H. (1975) Biophys. Soc. Abstr., p. 45a
6 Neild, T.O. and Thomas, R.C. (1974) J. Physiol. Lond. 242, 453—470
7 Hinke, J.A.M. and Gayton, D.C. (1971) Can. J. Physiol. Pharmacol. 49, 312—322
8 Kerkut, G.A. and Meech, R.W. (1966) Comp. Biochem. Physiol. 19, 819—832
9 Lee, C.O. and Armstrong, W. McD. (1974) J. Membrane Biol. 15, 331—362
10 Skrzypek, J. and Keller, E. (1975) I.E.E.E. Trans. Bio-Med. Eng. 22, 435—437
11 Armstrong, W. McD., Byrd, B.J. and Hamang, P.M. (1973) Biochim. Biophys. Acta 330, 237—241
12 White, J.F. and Armstrong, W. McD. (1971) Am. J. Physiol. 221, 194—201
13 Quay, J.F. and Armstrong, W. McD. (1969) Am. J. Physiol. 217, 694—701
14 Adrian, R.H. (1960) J. Physiol. Lond. 151, 154—185
15 Field, M., Fromm, D. and McColl, I. (1971) Am. J. Physiol. 220, 1388—1396
16 Henriques de Jesus, C., Ellory, J.C. and Smith, M.W. (1974) J. Physiol. Lond. 244, 31P—32P
17 Zeuthen, T. and Monge, C. (1975) Phil. Trans. R. Soc. Lond. B71, 277—281
18 Kernan, R.P., Mac Dermott, M. and Westphal, W. (1974) J. Physiol. Lond. 241, 60P—61P
19 Frizzell, R.A., Nellans, H.N., Rose, R.C., Markscheid-Kaspi, L. and Schultz, S.G. (1973) Am. J. Physiol. 124, 328—337
20 Frizzell, R.A. (1976) in Intestinal Ion Transport (Robinson, J.W.L., ed.), pp. 101—109, MTP Press Ltd., Lancaster, U.K.