In contrast to the situation in very leaky epithelia such as the renal tubule where lanthanum has been observed to penetrate the tight junctions, the present study demonstrates that ionic lanthanum added to the perfusate does not penetrate the tight junction of the rectal gland either under basal conditions or during stimulated secretion. These findings differ from those of van Lennup who showed lanthanum within the rectal gland tight junction following fixation of tissue by immersion, a process that may have altered properties of the junction and allowed colloidal lanthanum hydroxide to penetrate this barrier. The findings of the present study are consistent with a permeability barrier capable of restricting the passage of relatively small molecules, specifically urea, throughout the long lengths of tight junction in this tissue. Supported by NIH grant AM 17433 and a grant from Lederle Laboratories.

CALCIUM SELECTIVE MICROELECTRODES

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Polar lobe formation in the fertilized egg of the marine mudsnail, Ilyanassa obsoleta, is a microfilament-dependent process that results in the formation of a prominent constriction slightly below the cell equator. Intracellular calcium (Ca^{++}) has been implicated in conformational changes in the actin-containing microfilaments during this shape change (Conrad and Davis, Develop. Biol. 74:152-172, 1980). The role of Ca^{++} in this process is difficult to assess since cytoplasmic ionic Ca^{++} activities are unknown and greatly influenced by the presence of efficient intracellular sequestering systems. We have completed initial attempts to make ion-selective microelectrodes for intracellular free Ca^{++} activity measurements $(Ca)_c$.

The difficulty in fabricating such an electrode is in achieving the very low Ca^{++} sensitivity ($10^{-7} - 10^{-9}$M) and high selectivities necessary for intracellular measurements. Recently a Ca^{++} selective compound that may satisfy these requirements was synthesized and formulated into a liquid ion exchanger (Simon et al., in Calcium Transport and Cell Function, Annals NYAS, Scarpa and Carafoli (eds), 307:52-70, 1978). The use of this ion exchanger in a microelectrode has been reported (Youmans et al., Fed. Proc. 38:963, 1979).

Open tip microelectrodes (tip diameter < 1 μM) were fabricated from 1 mm OD borosilicate glass using a Kopf pipette puller. The glass tip was siliconized to eliminate Na^+ ion selective sites on the glass interior surface and make the surface hydrophobic. This was done by drawing into the tip ~300 μM of a 5-10% solution of siliconizing compound dissolved in an organic solvent. Five siliconizing compounds were tested (chlorotrimethyl silane, dichlorodimethyl silane, methyltrichloro silane, Prosil [PCR Research Chemicals], and Dow Corning 500) in three different solvents (n-chloronapthalene, 1-octanol and ethyl acetate). Pipettes were then baked for 1 hour at 125°C to evaporate the solvent. Approximately 200 μM of the Ca^{++} ion exchanger (kindly supplied by Dr. W. Simon) was drawn into the pipette tip by applying a vacuum to the open end of the pipette while the tip was immersed in the exchanger.

Several electrodes were successfully fabricated and calibrated in Ca^{++} standard solutions made by adding known weights of $CaCl_2$ to doubly distilled water. These standards were buffered to pH 7.1 with 20 mM TRIS buffer and contained background ion concentrations of 22 mM M_g^{++}, 20 mM Na^+, 200 mM K^+, 254.5 mM Cl^-, 11.5 mM HCO_3^-. The background ions were present to simulate the intracellular marine egg environment for electrode selectivity determinations. All standards below 10^{-5}M Ca^{++} contained 3 mM EGTA to buffer trace Ca^{++} contamination in the distilled water. Ca^{++} concentrations in the EGTA solutions were calculated from published data (Portzehl et al., Biochim. Biophys. Acta 79:581-591, 1964).

A calibration plot of potential vs log Ca^{++} for one electrode is shown in Figure 1. The calibration circuit has been described previously (Duffey, et al., J. Memb. Biol. 42:229-245, 1978). The linear portion of the plot has a slope of +29 mV/decade and detection limit of 3×10^{-7} M Ca^{++}. The selectivity constants (Simon, et al) for Na^+, Mg^{++}

and K^+ were $k_{CaNa}=5\times10^{-4}$, $k_{CaMg}=1\times10^{-5}$, $K_{CaK}=5\times10^{-6}$. These correspond to selectivities of 2×10^3:1 for Ca^{++}:Na^+, 1×10^5:1 for Ca^{++}:Mg^{++}, and 2×10^3:1 for Ca^{++}:K^+.

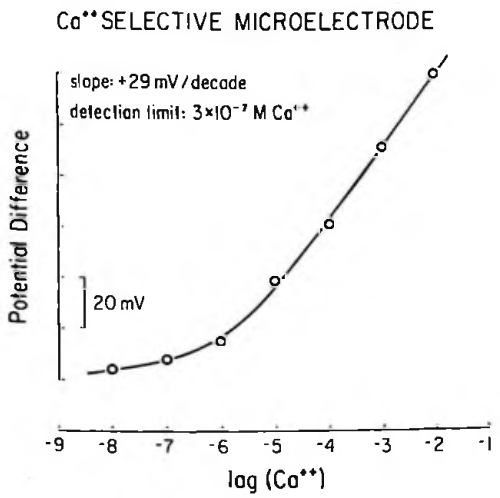

Figure 1. Calibration plot for a calcium-selective microelectrode siliconized with 5% DC 500 plus 5% Prosil in n-chloronapthalene. The calibrating solution contained a fixed level of background ions (see text).

The detection limit and selectivities of these electrodes were not adequate for intracellular measurements. The apparent difficulty is in the siliconization step. The siliconizing agent should react and "cover" glass surface silanol sites which are normally Na^+ selective. However, moisture in room air and the organic solvents may partially precipitate the siliconizing agent and thereby limit this reaction. Further development of the electrodes will require careful dehydration of the solvents and a dry (e.g., nitrogen) working environment. Since macroelectrodes using this ion exchanger have adequate characteristics for intracellular measurement, further microelectrode development is warranted.

No Ilyanassa egg intracellular Ca^{++} measurements were made. Supported by research grants from the NIH (HD 07193-06 and SO7 RRO5764) and the NSF (PCM 77-26790).

CORRELATION OF CHLORIDE CELL NUMBER AND SHORT-CIRCUIT CURRENT IN CHLORIDE-SECRETING EPITHELIA OF FUNDULUS HETEROCLITUS

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It has long been assumed that chloride cells are responsible for the majority of the extrarenal osmoregulatory Na^+ and Cl^- secretion in seawater teleosts. The opercular epithelium lining the gill chamber of the killifish, Fundulus heteroclitus adapted to artificial seawater, contains a high density of chloride cells (Burns and Copeland, Biol. Bull. Woods Hole, Mass., 99:381-385, 1950), which are identical to those described in the gill epithelium (Karnaky and Kinter, J. Exp. Zool. 199:355-364, 1977). When isolated, mounted as a membrane, and short-circuited, this epithelium actively transports chloride ions from the blood side to the seawater side of the preparation (Karnaky et al., Science 195:203-205, 1977; Degnan et al., J. Physiol., 27:155-191, 1977). Active chloride secretion by an epithelium rich in chloride cells suggested, but did not prove, that chloride cells are responsible for this chloride secretion. In their original histological description of the opercular epithelium, Burns and Copeland (1950) noted that chloride cells were most prominent near rich capillary beds. Our qualitative observation that richly-vascularized opercular epithelia generally exhibited the greatest short-circuit currents (a direct measure of active chloride secretion) also suggested that chloride cells are responsible for active chloride secretion.