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^+.

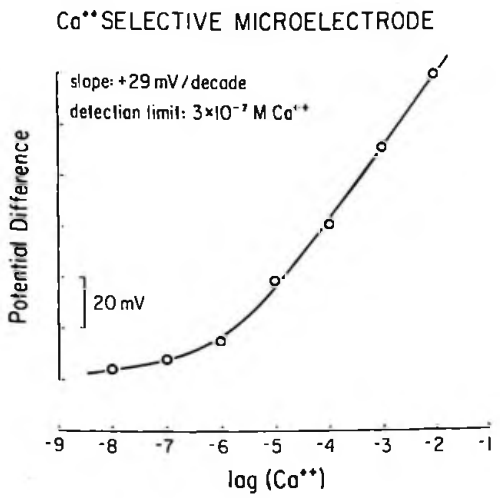

Figure 1. Calibration plot for a calcium-selective microelectrode siliconized with 5% DC 500 plus 5% Prosil in n-chloronapthalene. The calibrating solution contained a fixed level of background ions (see text).

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.

During our initial electrophysiological studies on the chloride cell-rich opercular epithelium we also searched for a second, chloride cell-poor epithelium in the head region of Fundulus heteroclitus. Would such an epithelium, with fewer chloride cells, exhibit a proportionately smaller short-circuit current? Our preliminary investigations revealed that the anterior roof epithelium of the mouth of Fundulus heteroclitus possessed fewer chloride cells and exhibited smaller short-circuit currents. In the present study we report a direct correlation between the number of chloride cells and short-circuit current in pooled data from roof and opercular epithelia.

Epithelia from seawater-adapted killifish, Fundulus heteroclitus, were dissected, mounted in lucite chambers, and short-circuited by methods previously described (Degnan et al., J. Physiol. 271:155-191, 1977). For examination of roof epithelia with the light and electron microscopes, tissue was fixed, embedded, and examined by techniques described in Karnaky and Kinter (J. Exp. Zool. 199:355-364, 1977). To count chloride cells in roof and opercular epithelia each epithelium was first mounted in an Ussing chamber, short-circuited, and the short-circuit current allowed to come to a steady-state value. The plastic sandwich (composed of two flat pieces of x-ray film, one on each side of the membrane) holding the epithelium was removed from the Ussing chamber and placed on a glass surface. A punch which fits snugly to the inside of the 3mm diameter aperture of the plastic sandwich was used to cut out the membrane. The membrane was then incubated for 30 min. in 50 μM DASPMI in oxygenated Ringer solution. DASPMI (dimethylaminostyrylmethylpyridinimiodine) is a fluorescent probe which serves as a vital stain for mitochondria (Bereiter-Hahn, Biochim. Biophys. Acta 423:1-14, 1976). Epithelia were then examined in a fluorescent microscope. An ocular grid was used to count individual chloride cells. All chloride cells were counted in roof epithelia since the number of cells was small (500-1500). Since opercular epithelia contained 15,000-35,000 chloride cells, only a representative area was counted. The number of cells in this representative area was then multiplied by the total surface area to obtain the total number of chloride cells.

Light microscope examination revealed that the anterior roof of the mouth is lined by an epithelium that contains the same four cell types composing the opercular epithelium and the salt-secretory epithelium of the gill (pavement, mucous, nondifferentiated, and chloride). Electron microscope studies revealed that chloride cells in this roof epithelium are identical to those in the gill and opercular epithelium.

Electrophysiological studies on the roof epithelium demonstrated that the mean electrical properties of this chloride cell-poor epithelium are markedly different from those of the chloride cell-rich opercular epithelium. Values (mean $\pm$ S.E.) from fifteen roof epithelia were as follows: potential difference, 8.5 $\pm$ 1.9mV; short-circuit current, 30.1 $\pm$ 3.4 $\mu A/cm^2$; and resistance, 271.8 $\pm$ 42.2 Ω-cm^2. Corresponding values for six opercular epithelia used for chloride cell counting were: 11.4 $\pm$ 2.1mV; 150.5 $\pm$ 35.2 $\mu A/cm^2$; and 97.3 + 26.8 Ω-cm^2. Under short-circuit current conditions, ^{36}Cl flux studies in four roof epithelia showed that the difference between the average net chloride current (40.4 + 8.9 $\mu A/cm^2$) and the average short-circuit current (39.4 $\pm$ 10.0) was not statistically significant. Thus, as is the case for the opercular epithelium, most of the short-circuit current can be attributed to the active transport of chloride ions from the blood to the seawater side of the roof epithelium.

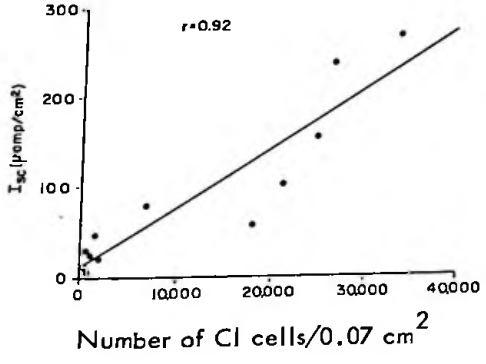

Figure 1. Plot of steady-state short-circuit current versus number of chloride cells in six roof epithelia (six data points with smallest number of chloride cells) and six opercular epithelia (remaining data points). The number of chloride cells was determined by counting DASPMI--labelled chloride cells with a fluorescent microscope. Slope = 6.542 x 10^{-3}; intercept = 12.82; correlation coefficient, r = 0 0.92.

Linear regression analysis of chloride cell counts versus short-circuit currents in pooled data from six roof and six opercular epithelia (Fig. 1) showed that the correlation coefficient was 0.92 (significant at $P < 0.001$) and that the regression line essentially passed through the origin. This close correlation between short-circuit current (i.e., active chloride secretion) and the number of chloride cells provides strong evidence that the chloride cell is responsible for chloride secretion in both roof and opercular epithelia. This work was supported by NIH grants GM 24766 (to K.K.) and GM 25002 and EY 01340 (to J.Z.).

FURTHER CHARACTERIZATION OF HEPATIC ARYL HYDROCARBON HYDROXYLASE INDUCTION IN A NATIVE POPULATION OF WINTER FLOUNDER, Pseudopleuronectes americanus, FROM COSTAL MAINE

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Many toxic agents (PCBs, PBBs, halogenated dioxins, and polycyclic aromatic hydrocarbons [PAH]) cause induction of the cytochrome p-450-dependent monooxygenase system involved in oxidative xenobiotic metabolism, and it has been suggested that induction of this enzyme system in fish could predict exposure to such environmental pollutants. However, before using enzyme induction in fish as a sentinel system for certain classes of chemical contaminants, it is necessary to elucidate clearly the causative factors of this enzyme response (including the contribution of different physiological states). The purpose of this study was to further investigate the PAH-type induction of the hepatic monooxygenase system of large numbers of male and female winter flounder captured near Mt. Desert Island, Maine, during the summer months, paying particular attention to factors that might be responsible for, or related to, the enzyme response.

In two previous successive summer seasons, we (Bend et al., Bull. MDIBL 17:47-49, 1977; Bend et al., Bull MDIBL 18:60-62, 1978) had shown marked heterogeneity of hepatic aryl hydrocarbon hydroxylase (AHH) and 7-ethoxyresorufin deethylase (7-ERF) activities in flounder as well as a differential effect of in vitro 7,8-benzoflavone AHH activity (stimulated AHH activity in fish with low AHH and 7-ERF activities and inhibited AHH activity in fish with higher AHH and 7-ERF activities).

Winter flounder sampled during June-August, 1979, using previously described assay procedures (Ibid, 18:60-62, 1978), again showed a marked heterogeneity in hepatic AHH and 7-ERF activities. The percentage of flounder with PAH-type induced hepatic monooxygenase systems, by year, is shown in Table 1. The data demonstrates that this is a

Table 1. Percentage of native winter flounder examined with polycyclic hydrocarbon type-induced[a] hepatic monooxygenase systems

Date	No. of Fish Assayed	% Induced[a]
June - August, 1977	15	85
June - August, 1978	172	49
June - August, 1979	81	72

[a]Fish with relatively high AHH activity in which AHH activity was inhibited by in vitro addition of 7,8-benzoflavone (0.5 mM).

recurring phenomenon in the Maine flounder population we have investigated. The plot of AHH activity vs. (AHH activity in the presence of 7,8-benzoflavone-AHH activity in the absence of this flavone) for both male and female fish sampled during 1979 (Figure 1) showed a high correlation between these two parameters (correlation coefficient 0.95, N = 81).