flounder were most abundant in the valleys between adjacent folds. However, labeled epithelial cells were also present at all levels including the extreme apex of the folds. This prompt and immediate labeling of epithelial cells at all levels of the mucosal folds was a distinctive and unexpected finding since studies of intestinal epithelial renewal in other fish including the juvenile grasscarp (Stroband and Debets, Cell Tiss. Res. 187:181), goldfish (Hyods, Radiation Res. 36:383), and carp (Gas and Noaillic-Depeyre, C.R. Acad. Sc. Paris, Ser. D. 279:1085) had revealed that labeled cells were confined initially to the epithelium between and at the base of mucosal folds. In studies in the grasscarp it was shown that labeled cells had not migrated to the apex of the folds until 10 to 15 days had elapsed.

Thus, cell proliferation in fasted flounder appears to occur at all levels of the mucosal folds without a consistent pattern of cell replication between folds followed by cell migration and maturation toward the apex.

Tissue from fed flounder collected last summer is now being processed for radioautography. When these radioautographs are completed, cell proliferation in both the fasted and fed flounder will be quantitated and compared. To complement the studies on paraffin embedded tissues, radioautographs of 1 μm thick epoxy resin sections are being prepared. In addition, electron microscopy of the intestinal mucosa of the fasted and fed flounder is currently being carried out in our laboratory. This work was supported by NIH grants AM-17537 and AM-21345.

INTRACELLULAR CHLORIDE ACTIVITIES AND THE MECHANISM OF ACTIVE CHLORIDE ABSORPTION BY SMALL INTESTINE OF THE FLOUNDER, *Pseudopleuronectes americanus*

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Previous studies on an *in vitro* preparation of small intestine from the winter flounder, *Pseudopleuronectes americanus* have demonstrated that both Na and Cl are actively absorbed. In addition, active Na absorption is abolished in the absence of Cl and, likewise, active Cl absorption is abolished in the absence of Na (Field et al., J. Memb. Biol. 41:265, 1978). Further, the unidirectional influx of Na from the mucosal solution across the apical membrane into the cell is reduced in the absence of Cl and the unidirectional influx of Cl is equally reduced in the absence of Na (Frizzell et al., J. Memb. Biol., in press). These findings suggest the presence of a carrier mechanism at the mucosal membrane that is capable of mediating the one-for-one, neutral entry of Na and Cl into the cell. These observations also raise the attractive possibility that the energy required for active transcellular Cl transport may be derived from coupling to the movement of Na into the cell across the mucosal membrane down an electrochemical potential difference as appears to be the case for rabbit gallbladder (Frizzell et al., J. Gen. Physiol. 65:769, 1975; Duffey et al., J. Memb. Biol. 42:229, 1978).

The purpose of the present investigation was to test this notion directly by determining intracellular Cl activities in flounder small intestine using conventional and Cl-selective microelectrodes.

Methods

Segments of flounder small intestine, stripped of the underlying musculature, were mounted mucosal surface up between two halves of a plexiglass chamber which permitted continuous perfusion of both surfaces with electrolyte solutions. The composition of the standard (control) electrolyte solution was (mM): 165 Na; 150 Cl; 20 HCO_3; 5 K; 2 HPO_4-H_2PO_4; 1 mg; 1 Ca; and 10 glucose (serosal solution) or 10 mannitol (mucosal solution). The pH was 8.0 when gassed with a mixture of 99% O_2 - 1% CO_2 at 15°C. Na-free solutions were prepared by isosmotic replacement of Na with choline.

The methods for fabrication of conventional (KCl-filled) and Cl-selective microelectrodes, calibration of the Cl-microelectrodes, and the recording of data have been described in detail previously (Duffey et al., J. Memb. Biol. 42:229, 1978). The conventional microelectrodes were employed to determine the electrical potential of the cell interior with reference to the mucosal solution, ψ_{mc}. The difference between the electrical potential recorded by the Cl-selective microelectrode when the tip was in the mucosal solution and during impalement of a cell is referred to as ΔE_t. The criteria for successful impalements have been described previously (Duffey et al., J. Memb. Biol. 42:229, 1978).

The intracellular Cl activity, $(Cl)_c$, was calculated from the equation

$$\Delta E_t = \psi_{mc} + S \ln [(Cl)_c/(Cl)_m] \qquad (1)$$

where $(Cl)_m$ is the Cl activity of the mucosal solution (113 mM) and S is a measure of the selectivity of the electrodes determined from the calibrations; in these studies, S averaged -24.7 mV, a value that compares very favorably with that predicted for an ideal Cl-electrode, -25.4 mV.

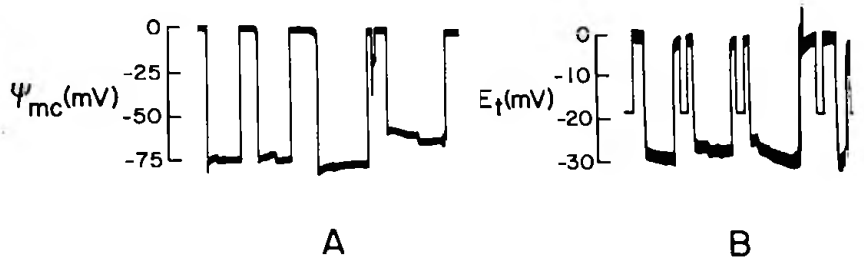

Figure 1. Typical examples of impalements with conventional microelectrodes (A) and Cl-selective microelectrodes (B), made in the same tissue with the position of the electrode changed slightly after each impalement. The square wave tracings between Cl electrode impalements are chart recorder zero.

Results

Typical recordings of ψ_{mc} and ΔE_t are illustrated in Figure 1; these values were found to be remarkably consistent within a single tissue and among different tissues, thus justifying the use of single-barrel microelectrodes for separate measurements of these parameters.

TABLE 1

Intracellular chloride activities in flounder small intestine

	ψ_{ms} (mV)	ψ_{mc} (mV)	ΔE_t (mV)	$(Cl)_c$ (mM)	$(Cl)^e_c$ (mM)	$[(Cl)_c/(Cl^e_c]$
Control	-3.3	-69±2	-29±2	24±3	7±1	3.4
Na-free	-0.8	-52±3	- 6±2	18±3	15±2	1.3
Recovery	-1.3	-51±5	-20±4	30±4	16±3	2.2

All errors are expressed as S.E.M.

Tissues from 15 animals were studied with the results summarized in Table 1. In 8 tissues, data were obtained only in the presence of the normal Na-buffer ("Control"). In 7 tissues, data were first obtained in the presence of the normal buffer ("Control"), then after the perfusate was switched to the Na-free buffer, and then once again when the normal Na-buffer was restored ("Recovery"). The control data did not differ significantly and have been combined.

Under control conditions, ψ_{mc} averaged -69 mV and ΔE_t averaged -29 mV. The value of $(Cl)_c$ calculated using equation (1) is 24 mM. This value is 3.4 times that predicted for an equilibrium distribution of Cl across the mucosal membrane, $(Cl)^e_c$, calculated from the Nernst equation. The transepithelial electrical potential difference, ψ_{ms}, under these conditions averaged -3.3 mV, in good agreement with the values reported by Field et al. (J. Memb. Biol. 41:265, 1978).

In the absence of Na, ψ_{ms}, ψ_{mc} and ΔE_t declined significantly and the average value of $(Cl)_c$ was only 1.3 times that predicted for an equilibrium distribution; in a number of instances the ratio $[(Cl)_c/(Cl)^e_c]$ was statistically indistinguishable from unity.

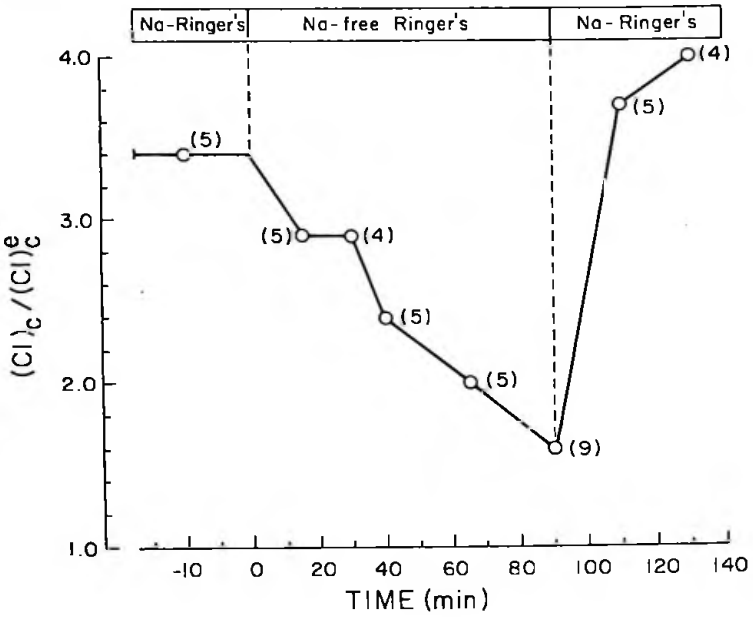

Figure 2. Time course of a typical Na replacement experiment. Values in parentheses are the number of successful Cl electrode impalements.

Finally, when, at the end of an experiment, the perfusate was switched back to the normal Na-buffer, there was a partial but highly significant restoration of ψ_{ms} and a significant increase in $(Cl)_c$; the ratio $[(Cl)_c/(Cl)^e_c]$ increased significantly to a value of 2.2.

A typical time course of the ratio $[(Cl)_c/(Cl)^e_c]$ when the perfusate was switched from the Na-buffer to the Na-free buffer and then back to the Na-buffer is illustrated in Figure 2. The striking feature is that the decline of this ratio toward the value of unity is slow so that it is quite likely that the failure to achieve the precise equilibrium value in many of our studies was simply due to the fact that we did not wait long enough. In contrast, after restoration of the Na-buffer there is a very rapid recovery which is complete within several minutes. The meaning of this repeated finding remains to be elucidated.

Conclusions

The results of these studies (Table 1) indicate conclusively that in the presence of 168 mM Na, the movement of Cl from the mucosal solution into flounder small intestinal cells is directed against an adverse electrochemical potential difference and, thus, must be coupled to a nonconjugate source of energy. Table 1 also shows that replacement of Na in the bathing media with choline leads to a reversible decrease in $[(Cl)_c/(Cl)^e_c]$ toward the equilibrium value of 1. Thus, the accumulation of Cl by the cell against an electrochemical potential difference is dependent upon the presence of Na in the

bathing media. Na-dependent Cl accumulation has also been demonstrated for rabbit gallbladder (Duffey et al., J. Memb. Biol. 42:229, 1978) and renal proximal tubule (Spring and Kimura, J. Memb. Biol. 38:233, 1978).

The intracellular Na activity in flounder small intestine is not known, but is almost certainly much lower than that in the mucosal solution. However, even if $(Na)_m = (Na)_c$, the potential difference derived entirely from ψ_{mc} of 69 mV is more than enough to propel Cl entry against its calculated electrochemical potential difference, i.e.,

$$\Delta\tilde{\mu}_{Cl} = (RT/F) \ln [(Cl)_c/(Cl)_m] - \psi_{mc} = 32 \text{ mV}$$

Thus, the present findings and the findings by Frizzell et al. (J. Memb. Biol., in press) which suggest that the unidirectional influx of Cl is coupled to the influx of Na constitute compelling evidence that the downhill Na-gradient across the mucosal membrane at the very least contributes to the energy necessary for the uphill accumulation of Cl by the cell and is more than sufficient to entirely energize this movement!

The mechanism responsible for Cl exit from the cell across the baso-lateral membranes is unknown but, at present, simple diffusion cannot be ruled out. In any event it seems highly likely that Cl absorption by flounder intestine is entirely energized by coupling to the electrochemical potential difference of Na across the mucosal membrane which, in turn, is established and maintained by a mechanism that actively extrudes Na from the cell across the baso-lateral membrane. While the active extrusion of Na is directly coupled to a source of metabolic energy, no direct coupling between Cl transport and metabolic energy need be invoked. Supported by research grants from the NIH-NIAMDD (AM-16275 and AM-18199) and the Wechsler Research Foundation.

INTRACELLULAR ELECTRICAL POTENTIALS AND CHLORIDE ACTIVITIES IN THE PERFUSED RECTAL GLAND OF *Squalus acanthias*: A REPORT OF PRELIMINARY DATA

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The in vitro perfused rectal gland of *Squalus acanthias* is capable of actively secreting Cl from the perfusate (serosal solution or plasma) into the ductal fluid; many of the central characteristics of this secretory process have been described by Silva et al. (Am. J. Physiol. 233:F298-F306, 1977).

The purpose of the preliminary studies described in this report was to determine the intracellular electrical potential and the thermodynamic activity of cell Cl under control conditions.

Methods

Rectal glands were isolated and perfused through the rectal gland artery with dogfish-Ringer's using the methods described by Solomon et al. (MDIBL Bull. 17:59-63, 1977). The glands were simply submerged in the perfusion fluid at 15°C contained in water-jacketed glass reservoirs. A 3 mm^2 section of the capsule was removed from the outer surface of the gland to expose the contraluminal surface of the tissue for impalements with conventional (KCl-filled) and Cl-selective microelectrodes.

The methods of fabricating and calibrating microelectrodes and recording data have been described in detail (Duffey et al., J. Memb. Biol. 42:229-245, 1978).

Results

Two glands were studied. The electrical potential difference across the contraluminal membrane determined in 7 successful impalements averaged -81 ± 4 mV and was remarkably uniform (average value