Table 2.--Effect of HCTZ on Simultaneously Determined Na and Cl Tracer Fluxes

	Mucosa-to-Serosa, n=8			Serosa-to-Mucosa, n=7		
	G_T (mS/cm^2)	J^{ms}_{Na} ($\mu M/cm^2 h$)	J^{ms}_{Cl}	G_T (mS/cm^2)	J^{sm}_{Na} ($\mu M/cm^2 h$)	J^{sm}_{Cl}
Control	0.669 ±0.147	1.70 ±0.25	2.45 ±0.30	0.346 ±0.035	0.52 ±0.07	1.34 ±0.12
HCTZ (0.1 mM)	0.951 ±0.186	0.40 ±0.05	0.69 ±0.24	0.665 ±0.115	0.23 ±0.02	0.29 ±0.04
p	<0.01	<0.005	<0.005	<0.02	<0.002	<0.001

p value represents significance between control and HCTZ treated group by paired analysis. Net fluxes are the differences between the mean unidirectional fluxes.

These data, when considered together with other information regarding NaCl absorption by the urinary bladder of the winter flounder, indicate that NaCl absorption occurs by an electrically neutral process probably involving an interdependent entry mechanism across the apical membrane. This process is relatively insensitive to loop diuretics but can be inhibited by thiazide-type diuretics. The reduction of the backflux of Na and Cl by mucosal hydrochlorothiazide indicates that a portion of this backflux component occurs transcellularly, perhaps through the same transport process by which absorption occurs. Although the mechanism for the increase in G_T is not clear, it might be secondary to an increase in cellular conductive pathways located on the apical membrane. This thiazide-sensitive NaCl transport system might be a model for NaCl absorption by the distal renal tubule. (Supported in part by NIH AM 25231.)

MORPHOLOGICAL EVIDENCE FOR IONOCYTES IN THE GILL EPITHELIUM OF THE HAGFISH, MYXINE GLUTINOSA L.

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The anatomy of the gill pouches of the Myxinoidea (Hofbauer, Biologia gen. 12:330, 1934; Rauther, Morph. Jb. 75:613, 1935) is fundamentally different from the arrangement of gill lamellae in all other aquatic vertebrates (Dunel and Laurent, in: Epithelial Transport in the Lower Vertebrates, B. Lahlou, ed.). The gill folds, which are irrigated with venous blood in a countercurrent manner to the perfusing water (Pohla et al., Zool. Scripta 6:331, 1977), are generally accepted to represent structures for gas exchange (Morris, J. Exp. Biol. 42:359, 1965). Electron microscopic analysis of gill epithelia in various species of euryhaline fish revealed different types of epithelia in relation to the adaptation to either the freshwater or the salt water habitat (Shirai and Utida, Z. Zellf. 103:247, 1970; Doyle and Epstein, Cytobiol. 6:58, 1972; Sardet et al., J. Cell Biol. 80:96, 1979); and physiological experiments demonstrated the importance of the chloride cells (Keys-Willmer cells) for ion-regulating processes. The present study was intended to decide whether similar cells are present in the epithelium of the gills of the hagfish.

Materials and Methods

Adult hagfish were obtained through the kindness of Dr. Foster, St. Andrews, Canada. After a few days of acclimation in a recirculating cooled seawater tank the animals were prepared for electron microscopy: The hagfish were exposed to MS 222 for ten minutes. A catheter was introduced into the ventral aorta and the vascular system was perfused with ice-cold physiological saline. The ice-cold fixation fluid (2% paraformaldehyde, 1% glutaraldehyde, 0.5% picric acid, 0.2 M cacodylate buffer adjusted to 980-1000 mOsm/l) was added continuously with constant perfusion pressure. After ten minutes the tissue was excised and fixed in the same mixture for an additional hour, rinsed in chilled buffer and postfixed in buffered 1% OsO_4. Tissue samples from all regions of the gill pouch were dehydrated and embedded in EPON 812. Thin sections were stained with uranyl acetate and lead citrate.

Results

The gill folds, projecting from the wall of the gill pouch, exhibit secondary and tertiary folding in the central region. These folds are drained by a capillary network (Fig. 1) and obviously are the site of gas exchange; epithelium and endothelium are very thin in this region.

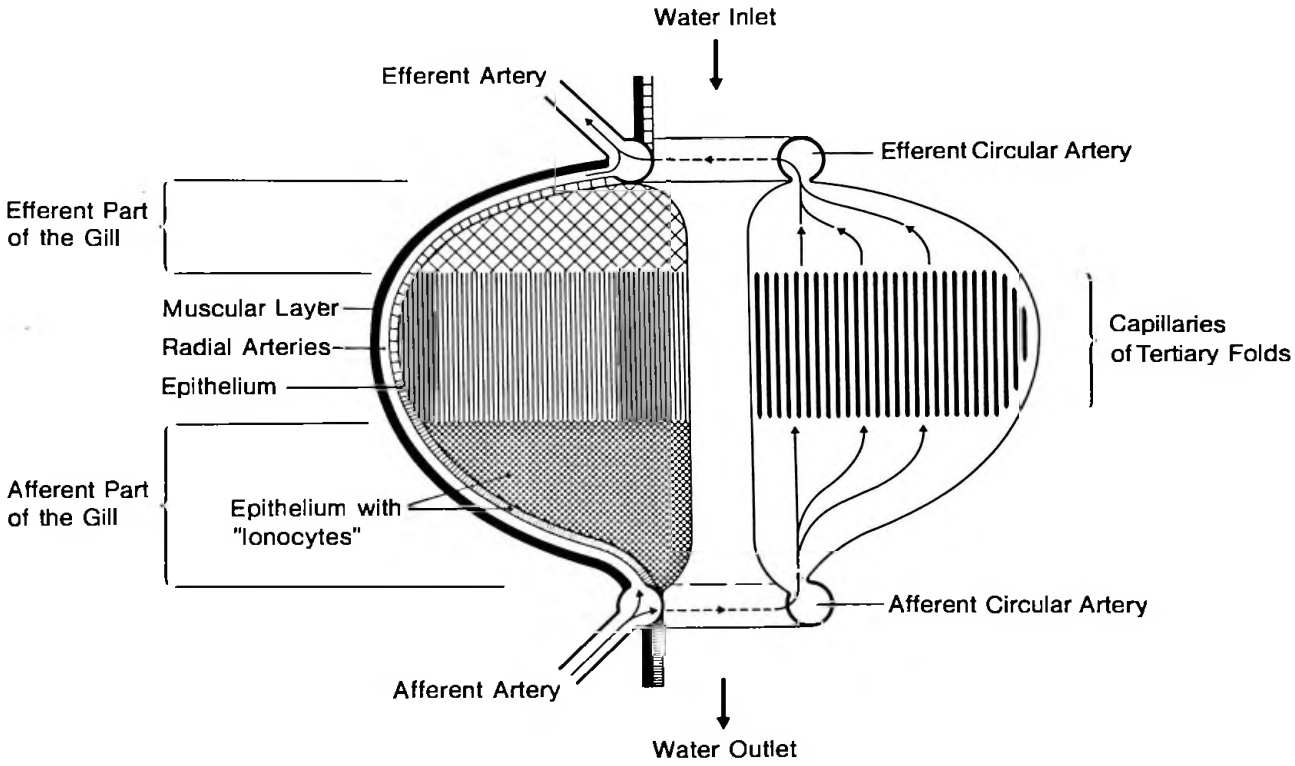


Figure 1.--The gill pouch of Myxine glutinosa, sagittal section. Ionocytes are present in the epithelial layer of the wall of the gill pouch and of the primary folds only at the afferent side. Left side: Cross section of the wall of the pouch; and surface of a gill fold extending almost to the mid-axis of the pouch. Right side: Circulation pattern of a gill fold.

The other parts of the gill pouch which are in contact with the seawater - water inlet and outlet, the unbranched base of the folds (primary folds) - are covered by a well developed multilayered epithelium. The electron microscopic investigation revealed a different type of epithelium on the afferent and efferent side of the gill pouch.

Afferent side of the gill. The wall of the gill pouch and the primary folds show a very high stratified epithelium which consists of several cell types.

The outermost layer is made up of pavement cells which display microvilli with a well stained cell coat. Numerous electron dense secretory vesicles accumulate beneath the apical cell membrane. The cells often possess a conspicuous Golgi apparatus, rough Endoplasmic Reticulum, and a nucleus which is moderately indented. The morphological features would indicate secretory cells which produce glyco-conjugates presumably for the external mucous layer.

Abundant columnar cells which may extend over the whole width of the epithelium are interspaced between the pavement cells: Cell type a). The apex of the cell has an undulated shape with alternating slight protrusions and indentations, and displays many irregular microvilli sparsely coated with filamentous material. The most prominent feature of these cells is a branched system of tubular membranes associated with mitochondria. The

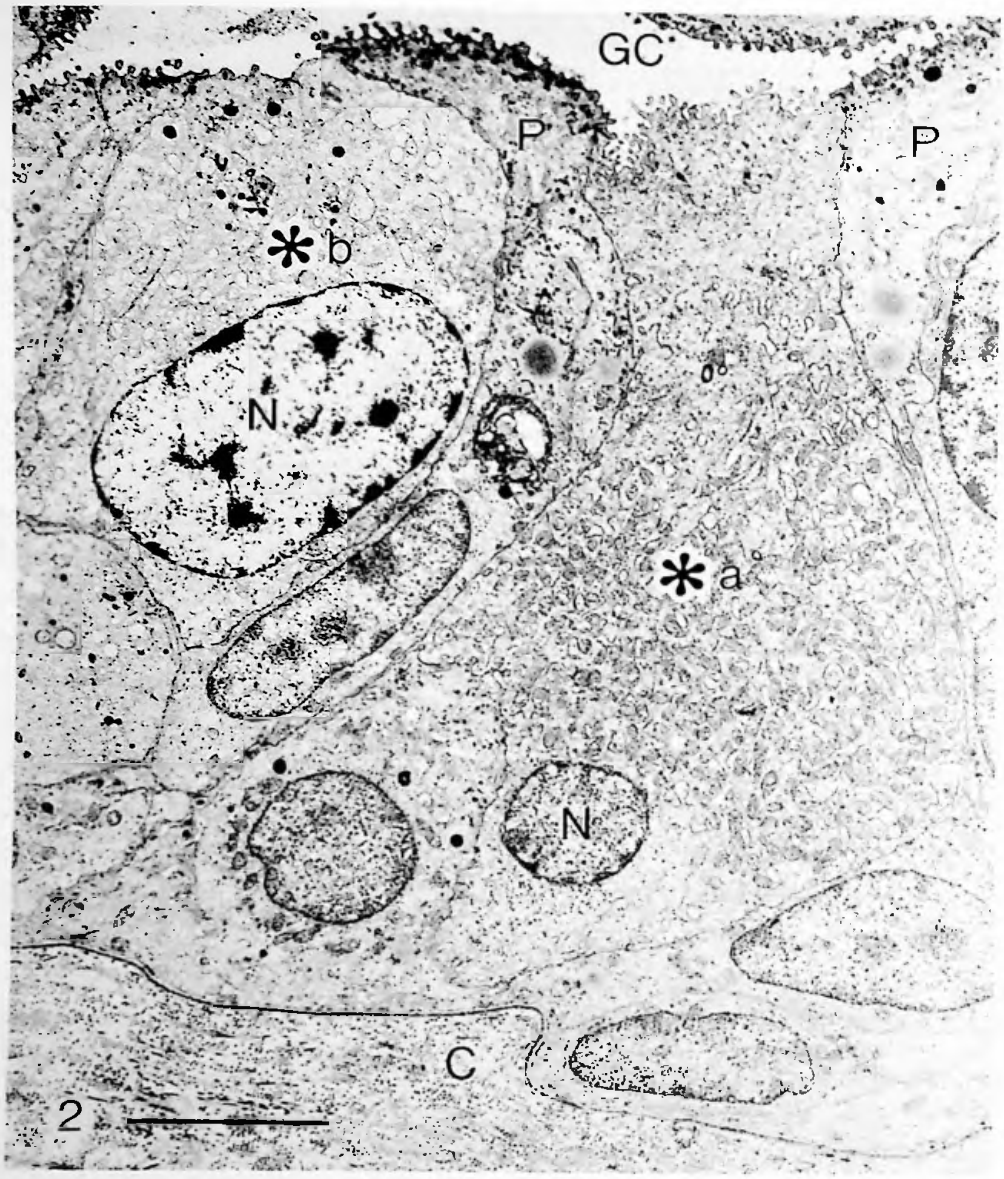
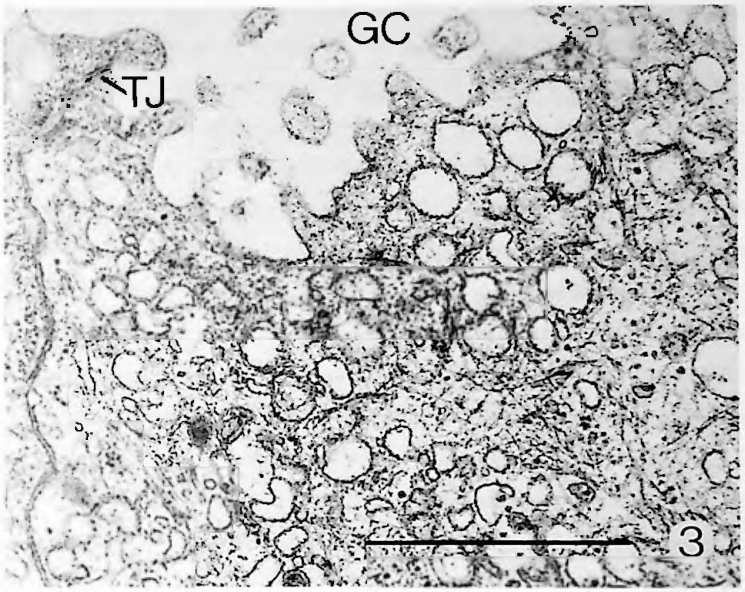


Figure 2.--Low magnification electron micrograph of the epithelium of a primary fold at the afferent side demonstrating the two types of ionocytes. Cell type a) (*a) exhibits an elaborate cell surface with numerous microvilli and spans almost the entire thickness of the epithelium. The nucleus (N) is located in the basal part of the cell. Cell type b) (*b) displays a small free surface with few microvilli. C collagenous layer beneath the basallamina; p pavement cell; GC gill cavity. Calibration bar indicates 5 μm.

tubular system is in open contact with the basolateral cell membrane. The apical cytoplasm is enriched with numerous small clear vesicles. Large areas of the basal perinuclear zone are occupied by masses of glycogen particles. The epithelial cells adhere together by apical junctional complexes (presumably tight).- Cell type b). These cells are similar in position and follow the basic pattern of cell type a), however, they exhibit the following differences: The cell membrane facing the outside is rather small and displays few microvilli which are filled with bundles of filaments. Apical vesicles are less numerous. These cuboidal cells with their inhomogenous nuclear plasma do not penetrate the basal layer of the epithelium. The features described above for the cell types a) and b) are characteristic for the chloride cells (for review see Laurent and Dunel, Am. J. Phys. 238:147, 1980).



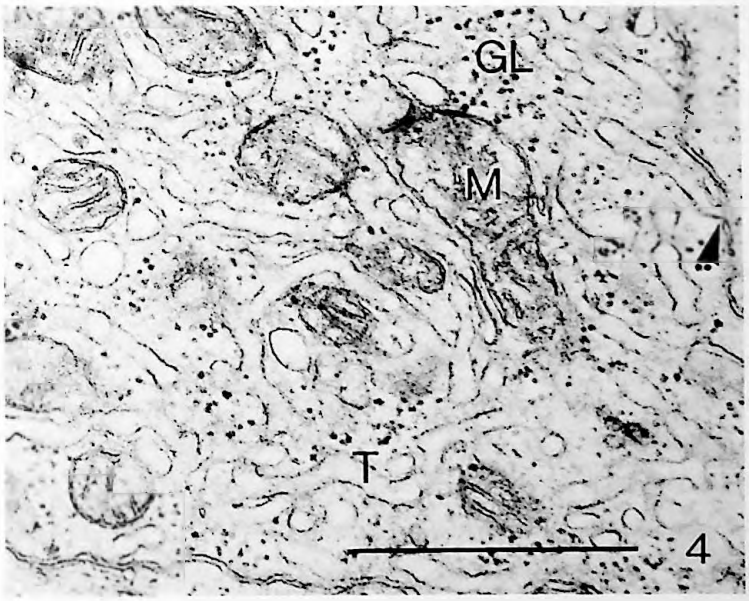


Figure 3.--Part of the apical cytoplasm of the ionocyte cell type a). Abundant vesicles accumulate beneath the apical cell membrane. GC gill cavity; TJ tight junction. Calibration bar indicates 1 μm.

Figure 4.--Part of the basolateral cytoplasm of the ionocyte cell type a). An elaborate system of branched tubules (T) is associated with mitochondria (M) and connected with the basolateral membrane (arrow head). GL glycogen. Calibration bar indicates 1 μm

The inner layer of the epithelium consists of smaller undifferentiated cells, which rest on a thick basallamina.

Efferent side of the gill. The epithelium of the efferent side has a uniform layer of pavement cells with the same morphological features as those from the afferent side. Several cells are columnar and contain numerous vesicles, whereas others exhibit prominent Golgi fields and membranes of the rough ER. The intercellular space between the pavement cells and the basal cells is widened and filled by interdigitating folds of the neighbouring cell membranes. Cells of type a) or b) are not present. Nerves are often to be found beneath the thick basallamina.

Discussion

The electron microscopic investigation demonstrates the presence of ionocytes at the afferent side of the gill pouch: in the epithelium of the primary folds and the epithelium covering the water outlet. In Myxine the chloride cells are not accompanied by accessory cells which are typically found in the pseudobranch (Dunel and Laurent, J. Microsc. Biol. Cell 16:53, 1973), the gill (Hootman and P hilpott, Am. J. Phys. 238:199, 1980), and the operculum (Karnaky et al., Am. J. Phys. 238:185, 1980) of marine teleost and euryhaline teleost in salt water. In these hyposmoregulators chloride cells with their accessory cells are considered to form a functional unit for salt secretion (Sardet et al., ibid.). Furthermore the fine structure of the ionocytes of Myxine resembles that of the chloride cells of hyperosmoregulating fresh water teleost and of the dogfish Squalus acanthias (Doyle, this bulletin Vol. 15:27-28, 1975). Fresh water fish are able to accumulate ions via the chloride cells. We would like to conclude that the slight hyperosmolar body fluid of Myxine (body fluid 1140 mOsmol/1, Raguse-Degener et al., Contr. Nephr. 19:1, 1980) could be the result of an ion-accumulating function of the ionocytes. The authors wish to acknowledge the use of the equipment kindly provided by Dr. B. Schmidt-Nielsen and Harold H. Church, and Suzanne Taylor, Jackson Laboratory, for operating the electron microscope. This work was supported by the Deutsche Forschungsgemeinschaft.

CELLULAR MECHANISM OF NaCl SECRETION BY THE RECTAL GLAND OF SQUALUS ACANTHIAS. STUDIES ON IN VITRO PERFUSED GLANDULAR TUBULES

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Individual glandular tubules (n=200) were dissected from rectal glands (n=60) of specimen of Squalus acanthias of either sex. The glandular tubules were perfused in vitro by the method described recently by Forrest et al. (J. Clin Invest. 1983, 72, 1163-1167). Electrical parameters were measured as described for the thick ascending limb of Henle's loop by Greger and Schlatter (Pflügers Arch: 1983, 396, 315-324). The present study comprises 4 series. A first series was aimed at testing whether the carrier, which mediates secondarily active chloride secretion and which is localized in the basolateral membrane of the rectal gland, has a K^+-binding site. In the second series, we tested the conductivity properties of the lumen and basolateral cell membrane of resting and stimulated glandular tubules. In the third series cellular ion activities for Cl^-, K^+, and Na^+ were measured with double barrelled microelectrodes in resting and in stimulated glandular tubules, and in those treated with 10^{-5} - 10^{-4} mol$\cdot l^{-1}$ furosemide (blood side). In the fourth series we tested the sequence of events occurring during the process of stimulation.

In the first series we measured transepithelial PD (PD_{te}) and the PD across the basolateral membrane (PD_{bl}) as a function of the periglandular K^+ concentration. Pilot experiments revealed that furosemide and ouabain resulted in a reduction of the lumen negative PD_{te}. While furosemide hyperpolarized PD_{bl} rapidly, ouabain lead to a delayed depolarization. The furosemide induced hyperpolarization is caused by a fall in cell Cl^- towards equilibrium. The ouabain induced depolarization is explained by the fall in basolateral K^+-conductance and the increase in cell Cl-. The hypothesis was, that K^+-involvement in the basolateral, furosemide-sensitive carrier, as it has been documented for the thick ascending limb of Henle's loop (Greger and Schlatter, Pflügers Arch. 1981, 392, 92-94), should lead to an initial hyperpolarization followed by a depolarization. This was predicted since K^+ reduction should initially block the carrier, as does furosemide, and only thereafter the ($Na^+ + K^+$)-ATPase. This hypothesis was verified in all 18 experiments.

In the second series K^+-, and Cl^--concentration step experiments (n=108) were performed on the lumen and basolateral cell side. It was shown that a reduction in bath K^+ hyperpolarized PD_{bl} rapidly and completely reversibly