sea raven contains few chloride cells, that from the euryhaline killifish adapted to pond water, 100%-, and 200% artificial seawater is predominantly chloride cells. It is important to note that the teleost gill has never been reported to contain more than 10% chloride cells (Karnaky et al., J. Cell Biol., 70:157-177, 1976). An early study on the electrophysiological properties of the sea raven opercular skin suggested that this epithelium warranted serious attention as a third approach to the study of salt transport mechanisms in teleosts (Karnaky, Bull. Mt. Desert Isl. Biol. Lab., 12:60-61, 1972). Recent electrophysiological investigations have demonstrated that the short-circuit current technique can be applied profitably to the opercular epithelium of *Fundulus heteroclitus* (Karnaky et al., Science, in press; Degnan et al., Bull. Mt. Desert Isl. Biol. Lab., this volume). Thus the opercular epithelium of the killifish can serve as a useful model to study the adaptive role of the chloride cell in euryhaline teleosts.

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FINE STRUCTURE OF WINTER FLOUNDER (*Pseudopleuronectes americanus*) INTESTINE

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Physiological studies (Field and Smith, MDIBL Bulletin, Vol. 15) have revealed that flounder intestine, in contrast to mammalian intestine, lacks cAMP-mediated Cl secretion. Also, in contrast to mammalian and amphibian small intestine and mammalian and teleost gallbladder, where the transport of Cl is tightly coupled to that of Na, Cl is absorbed much faster than Na across short-circuited flounder intestine (Field and Smith, MDIBL Bulletin, Vol. 15). These basic differences in electrolyte transport have prompted us to examine the fine structure of the intestine of the winter flounder.

Pieces of intestine were fixed immediately upon removal from the animal or immediately after their study in Ussing chambers. To correlate functional states with corresponding morphological features (e.g., open lateral spaces), tissues were fixed with 1% osmium tetroxide in phosphate buffer. Conventional aldehyde fixatives were employed for ultrastructural studies. To enhance membrane contrast in tight junctions, some tissues were fixed with aldehyde fixatives and postfixed with 1% OsO_4 containing 15 mg potassium ferricyanide/ml. To distinguish strictly intracellular membrane profiles from those connecting with the extracellular space, lanthanum salts were added to conventional fixing solutions to provide an electron dense precipitate in extracellular spaces of the tissue.

The histology of the flounder intestine conforms to the general pattern observed in a number of teleost species (Yamamoto, Z. Zellforsch., 72:66-87, 1966; Iwai, Z. Zellforsch., 91:366-379, 1968). It is composed of four layers: mucosa, submucosa, muscularis externa, and serosa. The absorptive surface of the human small intestine is increased by a combination of plications and secondary, finger-like villi. In flounder intestine large plications are not present but the mucosa is thrown into small folds which are similar in size to villi of mammalian intestine. The mucosal folds are covered with a simple columnar epithelium, and contain a core, the lamina propria, of loose connective tissue, smooth muscle cells, and a rich network of blood capillaries. An additional difference between flounder and mammalian intestine is the complete absence of crypts of Lieberkühn in the flounder. This anatomical difference raises the possibility that active sevretion, which is present in mammalian but absent from flounder intestine, arises from cells in the crypts of Lieberkühn.

The simple columnar epithelium, which rests on a basal lamina, is composed of absorptive cells, scattered mucous cells, basal cells (presumably endocrine in nature), and occasional small lymphocytes (Figure 1). The absorptive cells are long, about 60 μm, and narrow, about 3.5 μm, with a basal nucleus. Absorptive cell cytology is similar whether cells are located at the tip or bottom of mucosal folds, e.g., the brush border seems equally well developed everywhere and there is no evidence that cells found in the bottom of folds are less mature. Mitotic figures were not found.

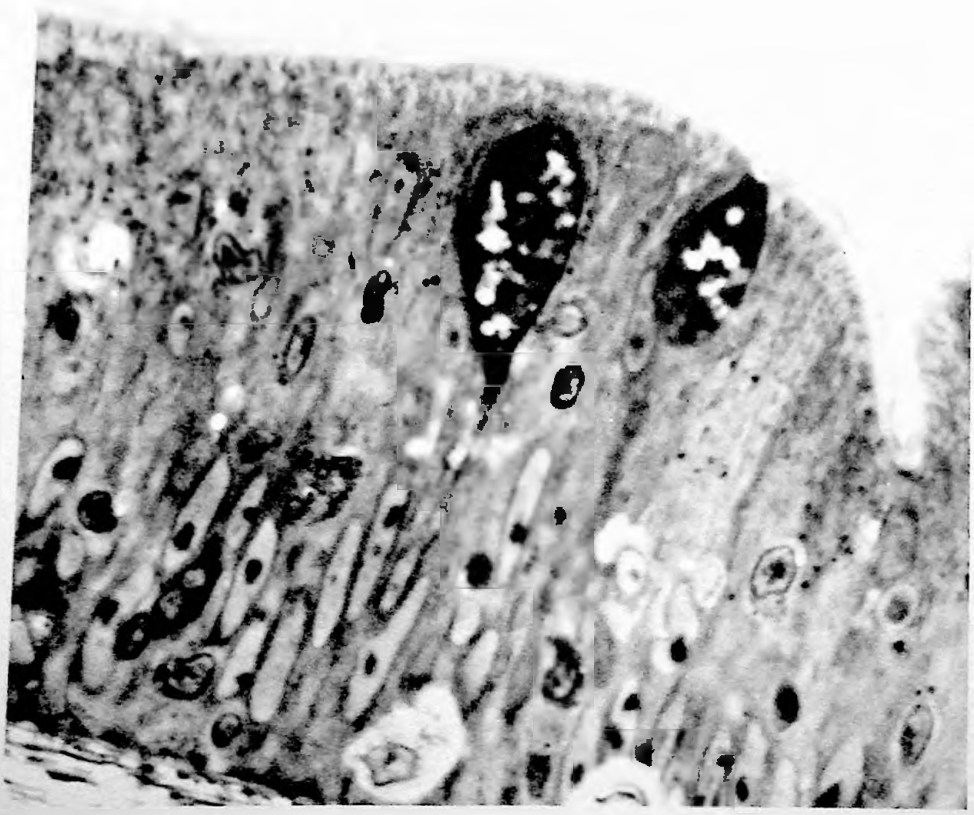

Figure 1. Light micrograph of flounder intestinal epithelium, consisting predominantly of tall, narrow absorptive cells with elongated basal nuclei. A few scattered mucous cells are also present. The absorptive cells exhibit a well-developed brush border. Prominent terminal bars in the apical cytoplasm mark the boundaries of adjacent cells at their luminal poles. The granular appearance of the apical cytoplasm is due mainly to the large population of mitochondria in this region. X 1600

Distended lateral intercellular spaces were consistently present in transporting tissues removed from the chambers and immediately fixed with osmium tetroxide. These spaces were constricted at one or more points, probably due to desmosomes. The area of epithelium exhibiting these distended spaces was estimated in 1 μm plastic sections using an ocular grid. The percent area showed considerable variation from tissue block to tissue block (34 to 92%) with an average of 53 ± 10% SE (observations from a total of 5 blocks from three fish). Distension was most prevalent in epithelial cells located at the tips of folds. Dilitation was only rarely detectable in the apical 12 μm of the lateral space.

Absorptive cells (Figure 2) are attached to each other at their apical poles by junctional complex (zonula occludens, zonula adherens, and desmosome). When conventic (osmium tetroxide or aldehyde) and thin-section staining (lead and uranyl salts) were the zonula occludens could not be demonstrated. However, the zonula occludens was cle strated in tissues prepared with the potassium ferricyanide procedure. Examples of de be found along the full length of the cells, including the basal region. These cell-t attachments, which restrict cell separation, are particularly common in the region of A comparison with electron micrographs of mammalian intestinal absorptive cells (Toner Cytol., 24:233-343, 1968) suggests that desmosomes beneath the junctional complex are frequent in the flounder.

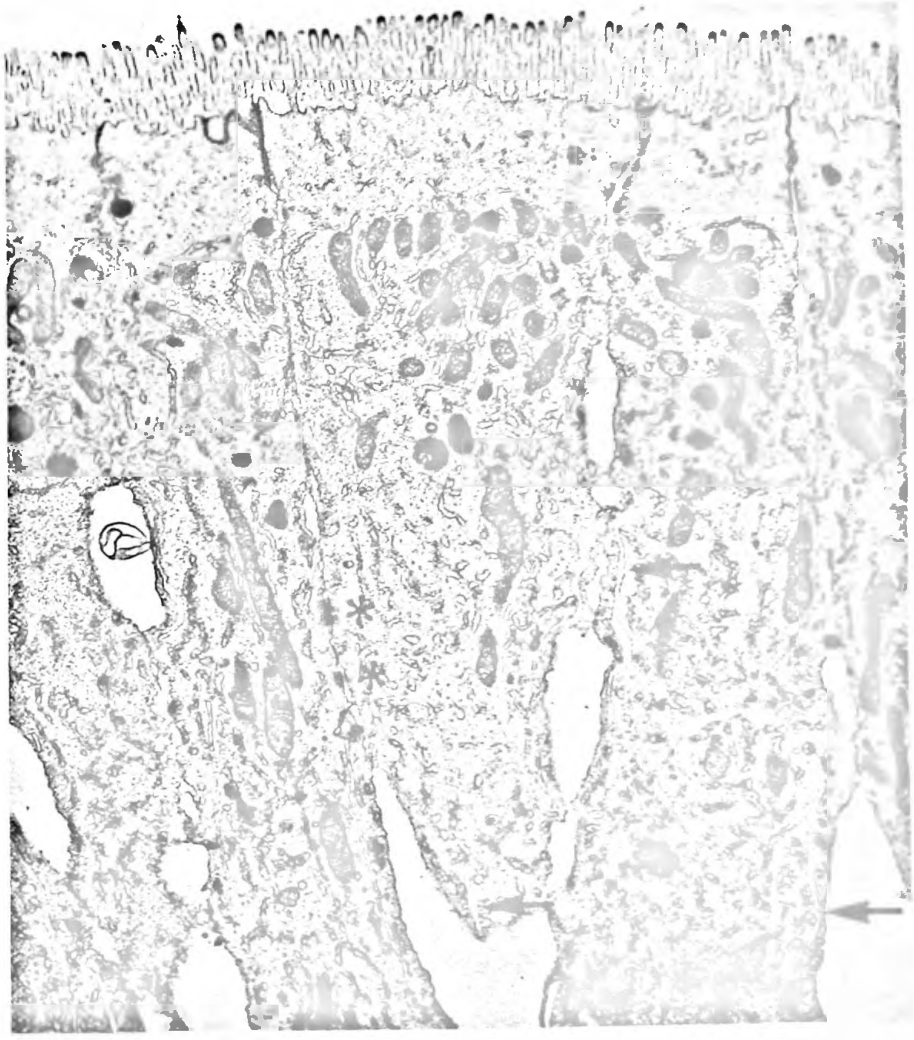

Figure 2. Electron micrograph of the apical region of flounder intestinal epitehlium, showing several absorptive cells. From the apex toward the base, there are, in succession, a brush border, a terminal web region, and a region of mitochondria with scattered elements of rough endoplasmic reticulum. Desmosomes, which restrict the distension of intercellular spaces, are found along the lateral plasma membrane (asterisks). Several examples of lateral infoldings are shown at the arrows. X 16,200.

The lateral surface of the absorptive cell is amplified by surface infolding, rather than by the lateral interdigitations found in mammalian intestine. Connections between the lamellar structures in the basal region and the basal plasma membrane were not observed, but more extensive search of thin sections may have revealed such connections. Further evidence that these lamellar structures represent extensions of lateral (and possibly the basal) cell surface was obtained with the electron-opaque extracellular marker, lanthanum, which penetrated into the folds at all levels of the cytoplasm.

Physiological data suggest that in flounder intestine resistance to intercellular Na diffusion is greater in the lateral space than in the tight junction (see Field, elsewhere in this bulletin and also in Coupled Transport Phenomena in Cells and Tissues. J. F. Hoffman, Ed., Raven Press, New York, in press). The close apposition of cells at their apical ends may provide a morphological basis for this relatively high resistance to Na diffusion.

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AUTORADIOGRAPHIC LOCALIZATION OF ^{3}H-OUABAIN BINDING IN DOGFISH (*Squalus acanthias*) RECTAL GLAND

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One of the most intensely investigated transport enzymes is the ouabain-sensitive, sodium-and-potassium-dependent adenosine triphosphatase (Na,K-ATPase). Associated with most animal cells, the main role of this membrane-bound enzyme is thought to be the active cation transport (Na pump) involved in maintenance of ion gradients at the single cell level, and movement of salt and water across epithelial structures. Na,K-ATPase activity is especially high in a number of osmoregulatory epithelia and appears to be correlated with the Na transport rate. For example, when euryhaline teleosts are adapted to salinites ranging from 50% to 200% seawater (SW), the gill responds with parallel increases in enzyme activity and NaCl secretion. Using ^{3}H-ouabain autoradiography it has recently been demonstrated that the adaptive Na,K-ATPase in teleost gills is located not on the apical membrane of the chloride cells as expected, but on the amplified basal-lateral membrane (Karnaky et al., J. Cell Biol., 70:157-177, 1976). This finding, which indicates that Na is pumped toward the blood side of the transporting cell, poses a major enigma concerning the role of the Na pump in salt secretion by teleost gills. The present investigation with the elasmobranch rectal gland establishes that this enigma extends to an additional osmoregulatory epithelium responsible for secreting NaCl from blood to SW.

In order to establish the subcellular location of the Na,K-ATPase by means of radiolabeled inhibitor bound specifically to the enzyme, both the luminal and basal-lateral cell surfaces must be exposed. Stadie-Riggs slices (200-500 μm thick) of dogfish rectal gland were used to insure that both the lumens of the secretory tubules and the interstitial spaces would be in communication with the in vitro incubation medium containing ^{3}H-ouabain. Furthermore, for autoradiographic localization to be meaningful, ouabain binding by rectal gland slices must be characterized as to rate, specificity, etc. As shown in Figure 1, the binding rate was markedly reduced by high K