TABLE 2

Effect of Various Tissue Homogenates of Spiny Dogfish (*Squalus acanthias*) on the Growth of L5178Y/AR Cells in Vitro

Organ Homogenate	% Inhibition of Growth After 48 Hours
Plasma	100
Liver	100
Bile	100*
Muscle	50
Others (see text)	0

Legend: Tissue supernatant was sterilized by filtration and added (0.5 mg protein/ml of medium) to the Dulbecco-Vogt medium supplemented with L-glutamine (3.5 g/L) and 5% fetal calf serum. One million cells/ml L5178Y/AR cells were added and the culture flasks were incubated at 37° for 48 hrs in an atmosphere of 95% air and 5% CO_2. After 48 hrs, the cells were counted in the Coulter Counter, Model Fn. The cell concentration had increased to 3-4 millions cells/ml in the control cultures. Trypan blue exclusion counting indicated greater than 90% cells Trypan blue negative in cultures showing no inhibition.

plasma (by 40%). However, the megalocytic principal in plasma was again observed to be retained by cellulose dialysis tubing. Attempts are being made to characterize the megalocytic principla in the spiny dogfish plasma by isoelectrofocusing and ion-exchange chromatography.

THE OPERCULAR EPITHELIUM OF THE KILLIFISH (*Fundulus heteroclitus*) and the Sea Raven (*Hemitripterus americanus*)

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Studies on intact fish and isolated, perfused gills have provided virtually all of our knowledge of osmoregulatory NaCl transport mechanisms in the teleost gill. The severe limitations of these two preparations, e.g., they are not flat sheets and cannot be studied under the ideal thermodynamic conditions achieved with the short-circuit current technique, have prompted us to examine the epithelium lining the gill chamber as a third preparation to study osmoregulatory electrolyte transport mechanisms in teleosts.

Over twenty-five years ago, Burns and Copeland (Biol. Bull. Mar. Biol. Lab., Woods Hole, 99: 381-385, 1950) showed that the opercular epithelium of the killifish, *Fundulus heteroclitus*, contained chloride cells. Recently, we have examined the histology and ultrastructure of this epithelium in both the killifish and the sea raven, *Hemitripterus americanus* (Karnaky and Kinter, J. Expt. Zool., in press). We observed that the opercular epithelium is indeed a flat sheet containing chloride cells. Significantly, the identity of the chloride cell in this epithelium was definitely established with the electron microscope. Furthermore, we observed that whereas the opercular epithelium of the marine

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.