sides it is clear that the later uterus is able to sustain a serosal positive potential which presumably indicates either net uptake of cations from the uterine fluids or net secretion of anions into the lumen contents. These alternatives cannot be distinguished until tracer flux studies are performed. Further, the TEP across the young uterus is rather refractory to substantial alteration of the ionic content of the mucosal solutions except that removal of external Cl^- did hyperpolarize the membrane by 8 mV, indicating some sort of finite, but low, Cl^- permeability. In contrast, the later uterus displays more substantial changes in the TEP when the dominant cations (Na^+ and K^+) or the dominant anion (Cl^-) are removed from the mucosal solutions. Thus, the relative ionic permeabilities have increased during development of the uterus, and, as in the case with the "pup" (see above), Cl^- appears to be the most permeant.

In summary, our preliminary investigations of some aspects of osmoregulation by developing *S. acanthias* embryos and juxtaposed uteri indicate that the pups are able to maintain blood Na^+ and Cl^- levels below uterine fluids (and seawater) because of low ionic permeability and cloacal (presumably rectal gland) extrusion of these ions (the typical adult pattern). In addition, the uterus maintains a rather low ionic permeability, despite extensive vascularization, with Cl^- being more permeant than Na^+. Most interestingly, the older uteri are able to develop measurable TEPs in elasmobranch Ringer's solutions, which indicates that some sort of electrogenic ionic transport must be taking place across this epithelium. Further experiments on the ontogeny of osmoregulation by elasmobranchs are certainly warranted. Research supported by NSF PCM 77-09915.

EPITHELIAL CELL PROLIFERATION IN FLOUNDER INTESTINE: A PRELIMINARY REPORT

Jerry S. Trier,* Pamela C. Moxey* and Michael Field,** Departments of Medicine, Harvard Medical School (Peter Bent Brigham Hospital)* and The University of Chicago**

It has been suggested that cholera toxin-induced secretion in mammalian small intestine occurs primarily at the level of the immature proliferating undifferentiated cells in the crypts. Unlike mammalian small intestine, the intestine of the winter flounder, *Pseudopleuronectes americanus* does not respond to cyclic AMP with fluid and electrolyte secretion (Field and Smith, MDIBL Bull. Vol. 15). Moreover, unlike mammalian small intestine, morphologically defined crypts are not present in the mucosa of flounder small intestine or in other fish studied to date (Field et al., J. Memb. Biol. 41:265).

To determine whether immature proliferating epithelial cells are present in the small intestine of the winter flounder and to determine their distribution in the mucosa, the DNA precursor, [^{3}H]-thymidine (2 μCi/g body wt) was injected intravenously into fasted (at least 72 hr) and fed flounder. Fasted and fed fish were compared because the rate of active chloride absorption was found to be appreciably greater in the fasted fish (M. Field, D. Clayton and R.A. Frizzell, unpublished observations). Fish were killed 2 to 48 hours after thymidine injection and samples of proximal, middle and distal intestine were fixed in Bouin's solution and embedded in paraffin. Tissues were serially sectioned, dipped in photographic emulsion, incubated, developed and stained with hematoxylin and eosin. Adjacent samples of proximal, middle and distal intestine were fixed with glutaraldehyde, post-fixed in osmium tetroxide and embedded in epoxy resin.

To date we have completed preparation and initial examination of radioautographs of the proximal middle and distal intestine of fasted flounder. The general morphology of flounder intestine was the same at all levels. Whereas flounder intestine is not characterized by crypts and villi as is mammalian small intestine, it is covered by mucosal folds which, when cross-sectioned, give an appearance similar to that of villi. Within 2 hours after exposure to [^{3}H]-thymidine, labeled epithelial cells in fasted

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*

Michael E. Duffey, Raymond A. Frizzell and Stanley G. Schultz, Department of Physiology
University of Pittsburgh, School of Medicine, Pittsburgh, Pennsylvania

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.