fluids are summarized. The effects on eggs of the 10 ppt March 12 sample and the 1 ppt June 26 sample are not clear from the data which show a wide range of toxicity. It would seem plausible that these fluids are on the borderline of toxicity and therefore gametes from different animals demonstrate various susceptibilities.

In an effort to identify the toxic components in the drilling fluids some ions present in large quantities in the most toxic fluids were tested for their effects on sand dollar fertilization. These results are seen in Table 5.

TABLE 5

EFFECTS OF SOME DRILLING FLUID COMPONENTS ON FERTILIZATION OF SAND DOLLAR EGGS

Component[a]	Results
$Na_2Cr_2O_7$	10 mM, Highest concentration tested, no effect
Na_2CrO_4	10 mM, Highest concentration tested, no effect
Ba^{++}	Approx. 10^{-5}M, level of saturated solution of $BaSO_4$, no effect
$ZnCl_2$	0.1 mM or higher concentration, completely inhibits

[a]All solutions were made in seawater and the pH was adjusted to 7.9.

In the case of barium, note that the large quantity of sulfate in seawater limits the quantity of dissolved barium in the medium, whether from the drilling fluid or $BaCl_2$. Zinc is the substance most toxic in these tests and is also present in concentrations which are highest in the most toxic drilling fluids. Values of 606 μg/g and 163 μg/g zinc have been determined for the October 11 and June 26 samples respectively (R.F. Shokes, Science Applications, Inc., reported to E.P.A. April 4, 1980). Zinc levels in the other fluids are less than 100 μg/g. This work was supported by U.S.E.P.A. Grant No. CR-807102010.

JUNCTIONAL COMPLEX STRUCTURE OF SMALL INTESTINAL EPITHELIAL CELLS IN WINTER FLOUNDER (PSEUDOPLEURONECTES AMERICANUS)

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INTRODUCTION

Epithelial cells of the small intestine are interconnected at their apices by junctional complexes. The most apical portion of this junctional zone consists of the tight junction which is characterized by localized sites of fusion of adjacent epithelial cell membranes. It has been suggested that the tight junction may, in part, regulate the flow of small molecules through the paracellular pathway (Schultz et al., Ann. Rev. Physiol. 36:51-91, 1974). Moreover, the complexity of the structure of tight junctions as revealed by freeze fracture may correlate with their ability to regulate paracellular flow (Claude and Goodenough, J. Cell. Biol., 58:390-400, 1973). In mammalian small intestine these structural features vary along the crypt-villus axis and with cell type (Madara et al., Gastroenterology, 78: 963-975, 1980) and may in part be determined by local events within the epithelium such as cell division (Tice et al., Tissue and Cell 11:293-316, 1979). Since cell proliferation in flounder small intestine differs markedly from that in

mammalian small intestine (Trier and Moxey, Cell Tissue Res. 206:379-385, 1980), we examined the structure of junctional complexes between flounder small intestinal epithelial cells.

METHODS

Proximal small intestine from winter flounder was removed and rapidly processed for freeze fracture and electron microscopy.

Specimens for freeze fracture were fixed for 30 minutes in a solution of 2% formaldehyde, 2.5% glutaraldehyde and 0.4% $CaCl_2$ in 0.1 M Na cacodylate buffer at pH 2.4, rinsed in cacodylate buffer, embedded in agar and cut into 150 μm slices on a Smith-Farquhar tissue chopper. After equilibrating in a 20% glycerol - 0.1 M Na cacodylate buffered solution, the tissue slides were mounted between gold discs and frozen in liquid nitrogen. These were then fractured at a stage temperature of -115°C in a Balzer's 360 freeze-etch device and replicated with platinum-carbon. The replicas were then cleaned in commercial bleach and mounted on formvar coated copper grids.

Specimens for electron microscopy were fixed as above but for two hours subsequently rinsed in Na cacodylate buffer, and postfixed in 1% OsO_4. After block staining with uranyl acetate, tissues were dehydrated in graded alcohols and embedded in epon. After examination of 1 μm sections, thin sections were cut, mounted on copper grids stained with uranyl acetate and lead citrate. Sections and replicas were examined in a Philips electron microscope.

RESULTS

In agreement with previous studies (Field et al., J. Membr. Biol., 41:265-293, 1978) we found that the epithelial surface of flounder small intestine is composed of tall 60 μm columnar epithelial absorptive cells and interspersed goblet cells. In contrast to mammalian small intestine the mucosa of flounder intestine, which lacks both crypts and villi, is thrown into mucosal folds.

Electron microscopy of thin sections revealed that the adjacent columnar cells were joined by apical junctional complexes consisting of tight junctions, intermediate junctions, and desmosomes (Figure 1A). The depth of the tight junction zone varied from approximately 200 to 1,000 nm. Additional desmosomes were present below the apical junctional complex. In addition, gap junctions were observed below the apical junctional complex of many of the cells. These were characterized by zones where the adjacent lateral membranes closely paralleled each other, being separated by a gap of only a few nanometers. Focally electron-dense connexons could be seen bridging the gap (Figure 1A). Gap junctions varied in length from approximately 50 to 800 nm.

Freeze fracture replicas also revealed tight junctions with characteristic P face strands and E face grooves (Figure 1B). However, the P face strands were frequently highly segmented. The tight junctions varied both in depth (approximately 50 to 1,000 nm) and in number of horizontal strands or grooves (3 to 14). In addition, many cells showed complex arrays of aberrant strands which extended down from the tight junction zone and meandered over large segments of the lateral membrane. Desmosomes were seen both immediately below the tight junction and scattered over the lateral membrane. In addition, gap junctions with complementary E face pits were frequently observed (Figure 1B). Individual plaques contained as few as 50 particles or as many as several hundred.

DISCUSSION

We have shown that the structure of tight junctions between flounder small intestine epithelial cells is strikingly different from that of tight junctions between epithelial cells in mammalian small intestine. Variability in tight junction structure in mammals is greatest in the crypts and on villus goblet cells (Madara et al.,

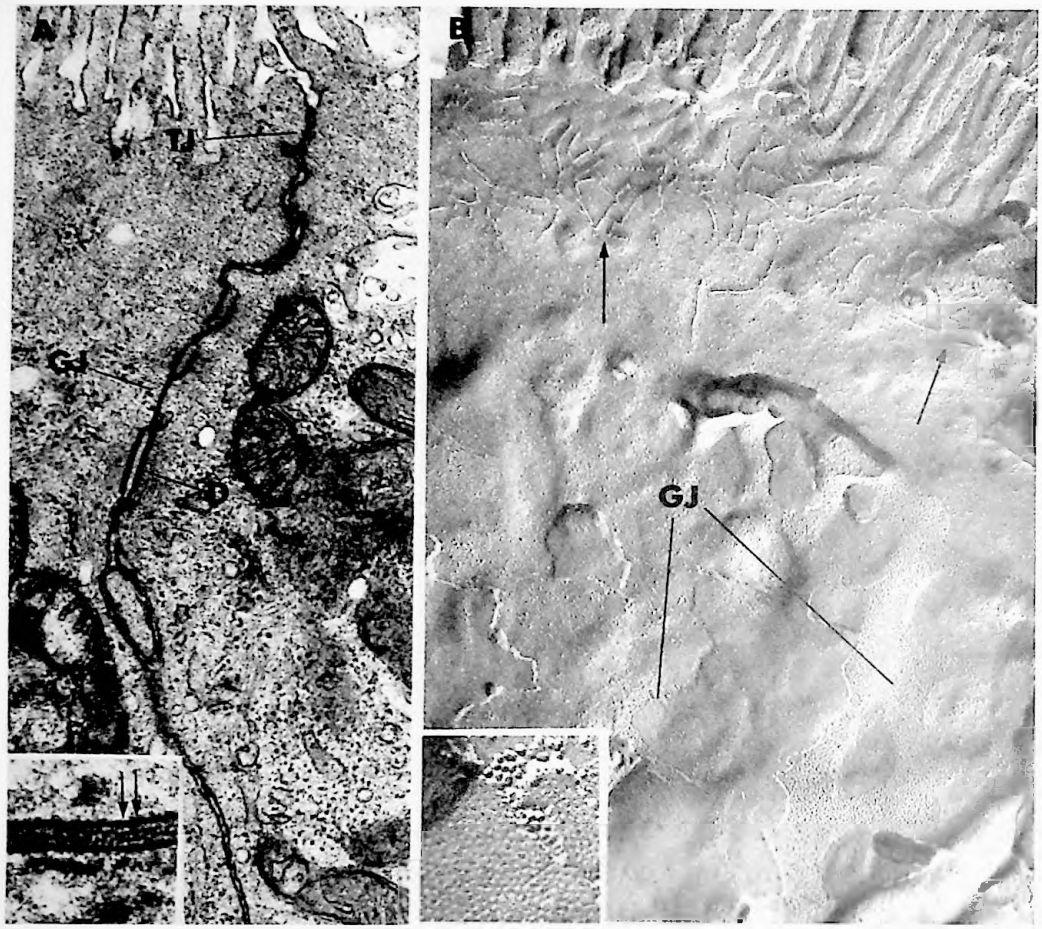

Figure 1A--Electron micrograph of apical junctional complex between two epithelial cells in flounder small intestine. In addition to the tight junction (TJ) and desmosomes (D) a gap junction (GJ) is also seen (x 4,000). Inset: High magnification of a gap junction shows closely opposed lateral membranes separated by a narrow gap. Focally (arrows) this gap appears to be bridged by narrow slightly electron dense structures (arrows) (x 125,000).

B--Freeze fracture replica of flounder small intestine epithelial cell lateral membrane. The tight junction varies in depth (arrows) and the P face strands tend to be highly segmented. In addition to the apical tight junction, large areas of gap junction (GJ) can also be seen (x 40,000). Inset: High magnification of a gap junction showing P face particles and E face pits (x 100,000).

Gastroenterology 78:963-975, 1980 and Tice et al., Tissue and Cell 11: 293-316, 1979) while tight junctions between villus absorptive cells are more uniform. In contrast, great variability in tight junction structure exists throughout the flounder small intestinal epithelium. It is not entirely clear what factors influence tight junction structure but the membrane jostling accompanying cell proliferation is thought to be related to the variation in tight junction structure occurring in the crypts of mammalian small intestine (Tice et al. Tissue and Cell 11: 293-316, 1979). Thus, it is relevant that mitoses occur throughout the flounder small intestinal epithelium (Trier and Moxey, Cell Tissue Res. 206:379-385, 1980), a pattern which correlates with the distribution of marked tight junction structural variation.

Previous studies suggest that tight junction structure may correlate with transepithelial resistance (Claude and Goodenough, J. Cell. Biol., 58:390-400, 1973 and Pricam et al., Lab. Invest. 30:286-291, 1974). Tight junctions consisting of only a few strands of poorly organized and sparsely interconnected strands, or of highly segmented strands are thought to represent sites of low resistance to transepithelial ionic flow (Claude and Goodenough, J. Cell Biol. 58:390-400, 1973 and Humbert et al., J Cell Biol. 69:90-96, 1976). The extreme variability of tight junction structure between flounder epithelial cells thus suggests that this epithelium may have a highly heterogeneous popoulation of ionic permeability sites. Sites of low resistance, if they do exist, would presumably be sites of greater Na backleak in the model proposed by Field et al for Na and Cl absorption in winter flounder intestine (J. Membr. Biol., 41:265-293, 1978).

The presence of large gap junctions between epithelial cells in flounder small intesting is of interest. Gap junctions are seen only rarely in the epithelium of primate intestine (Madara et al., Gastroenterology 78:963-975, 1980) and when present consist of only a few clustered connexons. Although gap junctions are more abundant in rodent small intestine (Staehelin, Proc. Natl. Acad. Sci. (USA) 69:1318-1321, 1972), they are still uncommon and very small. Gap junctions are believed to function as low resistance pathways for intercellular flow of small molecules (Loewenstein et al., Fed. Proc. 37:2645-2650, 1978). Thus, our finding of frequent and extensive areas of gap junction in flounder small intestine suggests that this epithelium might permit substantial intracellular flow of ions and other molecules of low molecular weight and therefore might behave electrically as a syncytium. This study was supported by NIH Research Grant AM 17537, NRSITP AM 02171 (JLM and RLC) and NRSA AM 06192 (JLM).

RENAL HANDLING OF ANIONIC AND CATIONIC ANTIFREEZE PEPTIDES IN THE GLOMERULAR WINTER FLOUNDER

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During the winter months several species of the fishes living in the Gulf of Maine synthesize a group of biological antifreeze compounds which allows them to inhabit coastal ice-laden waters (Petzel and DeVries, Bull. MDIBL 19: 17, 1979). The antifreeze compounds of winter flounder Pseudopleuronectes americanus are a group of 3 acidic peptides with molecular weights ranging from 3000 to 8000 daltons. The winter flounder responds to envrionmental cues by initiating the synthesis of antifreeze peptides during the months of October-November reaching peak concentrations of 30 mg per ml of serum in January-February (Petzel et al., J. Exp. Zool., 211:63, 1980). Additionally the winter flounder is known to possess a glomerular kidney (Nash, Am. J. Anat., 47:425, 1931; Marshall and Smith, Biol. Bull., 59:135, 1930), suggesting that these small molecular weight peptides could be filtered and either reabsorbed at the proximal segment of the nephron or passed directly into the bladder urine. The fact that these peptides are not found in the bladder urine suggests that the acidic peptides are not filtered at the glomeruli. The results of our experiment suggest that the lack of filtration might be due to the charge repulsion between the negatively charged antifreeze peptides and the subendothelial layer of the glomerular basement membrane.

Winter flounder renal clearance experiments were performed as described previously (Petzel and DeVries, Bull. MDIBL., 19:17, 1979). Briefly, an umbilical catheter was inserted through the urinary papilla into the bladder and sutured in place. Urine was collected continuously from free swimming winter flounder into calibrated tubes. Blood was drawn from the caudal vein via a 30 gauge needle at various intervals after the injection of different substances. Trititated polyethylene glycol (mol. wt. 4,000), ^{3}H-alanine (New England Nuclear) and