Studies on Sharks — XX

Epithelial Cells of the Intestine in *Mustelus manazo* and *M. griseus* Embryos

Shigeki OKANO, Tsuguo OTAKE*, Kazuyuki TESHIMA**
and Kazuhiro MIZUE*

Electron microscopic observations were made on the epithelial cells of the intestines in *Mustelus manazo* and *M. griseus* embryos in order to examine their structure and function. The intestinal epithelial cells in the embryos of both species are characterized by microvilli on the free surface, intermicrovillous invaginations of the plasma membrane, and a number of vesicles, granules, and vacuoles. These characteristics suggest that the intestinal epithelial cells in *M. manazo* and *M. griseus* embryos may be engaged in absorption. It has been clear that interdigitations of the lateral plasma membrane at the intercellur junctions, which are not seen in the intestine of teleostean fish, are observed to occur in the *M. manazo* and *M. griseus* embryos.

In shark embryos yolk is considered to be digested and absorbed by the endodermal epithelial cells of the external yolk sac during the early embryonic development, and by intestinal epithelial cells during the late development (TeWinkel, 1943; Jollie and Jollie, 1967). Yolk, which is to be digested and absorbed by the intestinal epithelial cells, is transported into the intestine from the external yolk sac through the ductus vitellointestinalis and the internal yolk sac (TeWinkel, 1943; Iwai, 1957; Teshima, 1981a). In addition, yolk appears to be absorbed by epithelial cells of the ductus vitellointestinalis through which the yolk passes (Teshima, 1981b, unpublished). The structure and function of intestinal epithelial cells in *Mustelus manazo* and *M. griseus* embryos were studied.

Materials and Methods

Live specimens of mature female *Mustelus manazo* and *M. griseus* were obtained from the fish market in Yoshimo near Shimonoseki, Japan during the period from May through June, 1979. In the uteri of the specimens embryos, ranging from 275 to 335 mm in total length, were found. The intestines were removed from these embryos. The intestines were fixed in 10 % formalin, sectioned by the usual paraffin methods, and stained with haematoxylin and eosin, and the PAS method was also

* Ocean Research Institute, University of Tokyo, Minami-dai, Nakano, Tokyo, 164, Japan.
** Shimonoseki University of Fisheries, Yoshimi, Shimonoseki, 759—65, Japan.

used. For electron microscopy, small pieces of the intestines were fixed in 2 % glutaraldehyde and 2 % formaldehyde buffered with Millonig's solution, post-fixed in 1 % osmium tetroxide, dehydrated with ethanol, and embedded in Epon 812. Thin sections were stained with uranyl and lead citrate and observations were made with a JEM-100CX microscope.

Observations

The internal surface and both surfaces of a spiral-shaped internal wall of the intestine in *Mustelus manazo* and *M. griseus* embryos are lined with a simple epithelium. The epithelium is composed of columnar cells among which goblet cells are scattered. The free surfaces of columnar cells are covered with the striated border (Fig. 1).

Through electron microscopic observations epithelial cells in the intestines of *Mustelus manazo* and *M. griseus* embryos each have similar structures (Fig. 2, Fig. 5). Microvilli are formed on the free surfaces of columnar cells which cover the internal surface and the surfaces of the internal wall of the intestine. Microvilli are seen to be regularly arranged on the free surfaces of the cells. Invaginations of the apical plasma membrane are observed to occur between the microvilli of the cells (Fig. 3). Terminal webs are visible in the apex of the cell beneath the microvilli. Cell organelles are rarely found within the terminal web, but a small number of vesicles are observed there. Throughout the cytoplasm beneath the terminal web various types of cell organelles exist. Granular endoplasmic reticula are seen in the cytoplasm around a nucleus and in the basal region of the cell while granular endoplasmic reticula are scattered throughout the cytoplasm. Mitochondria are visible throughout the cytoplasm and are especially abundant in the basal region of the cell (Fig. 4). Golgi complexes are located around the nucleus. A number of vesicles are found at a slightly deeper level than the terminal wed. Multivesicular bodies and various sizes of granules and vacuoles are seen in the upper region of and around the nucleus. The granules show varying structures, i. e., those covered with a single membrane, those with two membranes, and those containing dense substances. Vesicles, granules, and vacuoles are observed much more frequently in epithelial cells of the posterior portion than in those of the anterior portion of the intestine in embryos of the both species (Fig. 2, Fig. 5). Three types of attachment apparatuses are found at junctions of adjacent cells; these are the zonula occludems, the zonula adherens, and the macula abherens. At the lateral junctions of abjacent cells the plasma membranes are seen to be complicatedly interdigitated with each other.

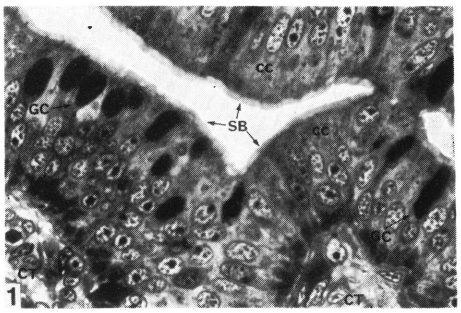

Fig. 1. A light micrograph of a part of the epithelium of the mid intestine in *M. griseus* embryo. x 480
The epithelium is composed of columnar cells among which goblet cells are scattered.
cc:columnar epithelial cell, CT: conective tissue, GC: goblet cell, SB: striated border.

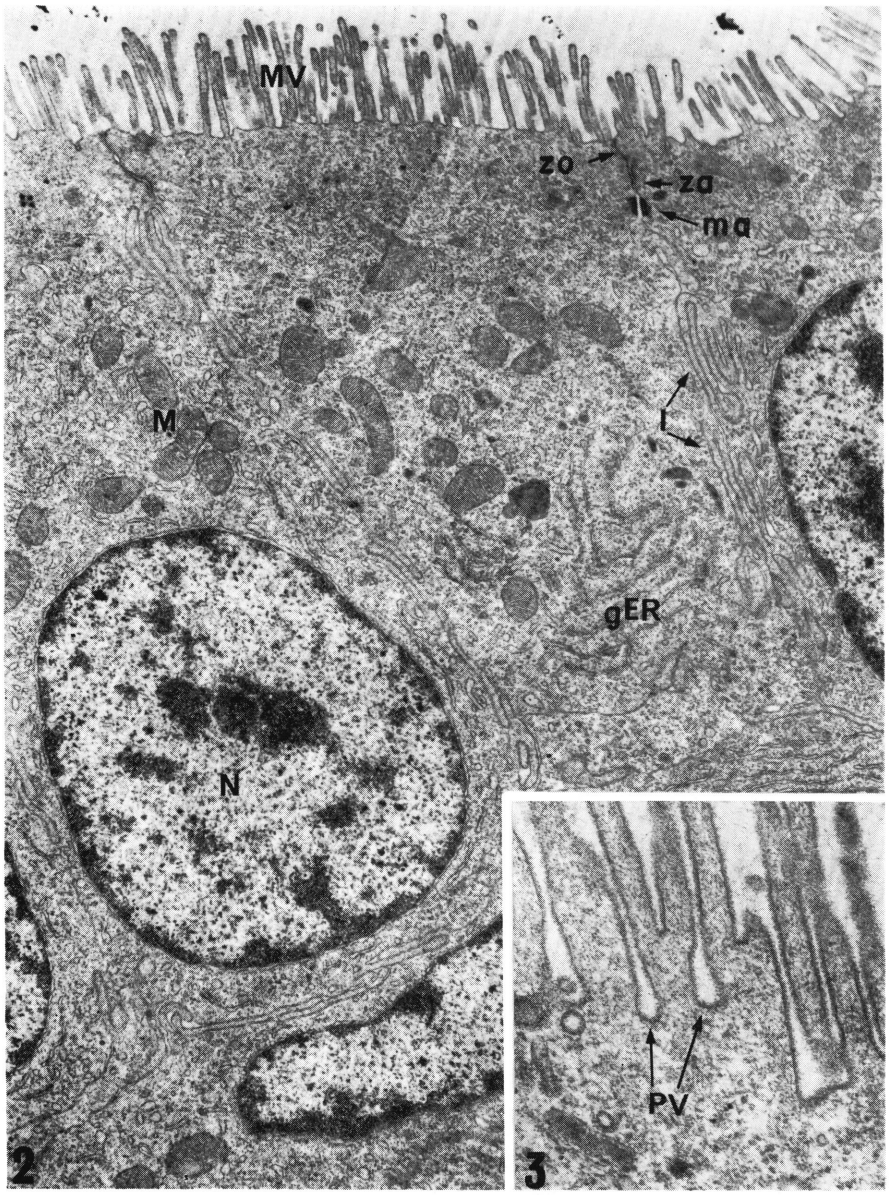

Fig. 2. An electron micrograph of the upper half of columnar epithelial cells of the anterior intestine in *M. griseus* embryo. x 7,600
The free surfaces are covered with microvilli. The interdigitations of the lateral plasma membrane are formed. gER: granular endoplasmic reticulum, I: interdigitattion of the lateral plasma membrane, M: mitochondria, ma: macula adherens, MV: microvilli, N: nucleus, za: zonula adherens, zo: zonula occludems.

Fig. 3. An electron micrograph of the apical edge of a columnar epithelial cell of the anterior intestine in *M. griseus* embryo. x 30,000
Pinocytotic vesicles are seen. PV: pinocytotic vesicles.

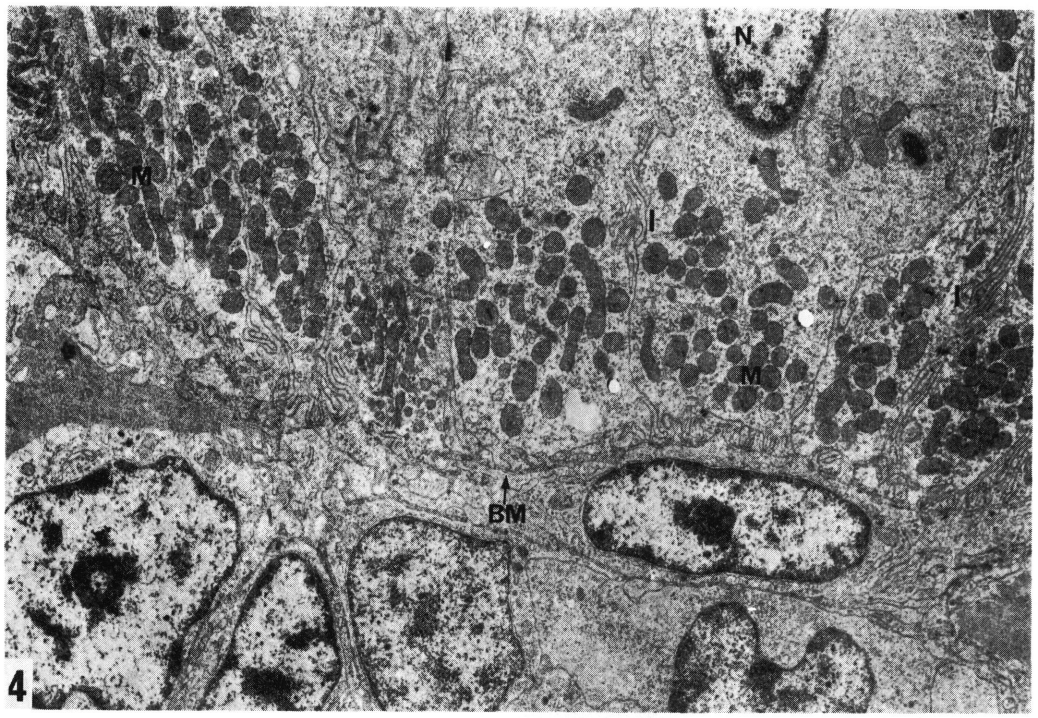

Fig. 4. An electron micrograph showing the basal region of columnar epithelial cells of the anterior intestine in *M. griseus* embryo. x 5,000
A number of mitochondria are observed in the basal region of columnar cells. BM: basal plasma membrane.

Goblet cells are scattered in the intestinal epithelium. The goblet cell contains a mass of globules which appear in the supranuclear region, and no microvilli are found on the free surface of the cell.

Discussion

Intestinal epithelial cells in *Mustelus manazo* and *M. griseus* embryos are characterized by the microvilli formed on free surfaces, intermicrovillous invaginations of the plasma membrane, terminal webs, a number of vesicles, granules and vacuoles, and attachment apparatuses. These characteristics closely resemble those of absorptive cells in the intestine of the teleostean fish and rat (Yamamoto, 1966; Iwai, 1967a, b, 1968a, b; Iwai and Tanaka, 1968; Mendoza, 1972; Sandborn, 1974; Rodewall, 1980). The intermicrovillous invaginations of the intestinal epithelial cells in *M. manazo* and *M. griseus* embryos thus appear to be pinocytotic vesicles. A number of vesicles in the apex of the cell seem to be closely associated with the pinocytotic vesicles, i. e., the vesicles in the apex of the cell are those formed by the pinocytosis. It is therefore considered that the columnar cells with microvilli in the intestinal epithelium of *M. manazo* and *M. griseus* embryos are involved in absorption.

Iwai (1967a, b), working with the intestines in *Plecoglossus altivelis* and *Hypomesus olidus*, described that ciliated cells are intermixed with cells possessing microvilli. However in the intestinal epithelium of *Mustelus manazo* and *M. griseus* embryos

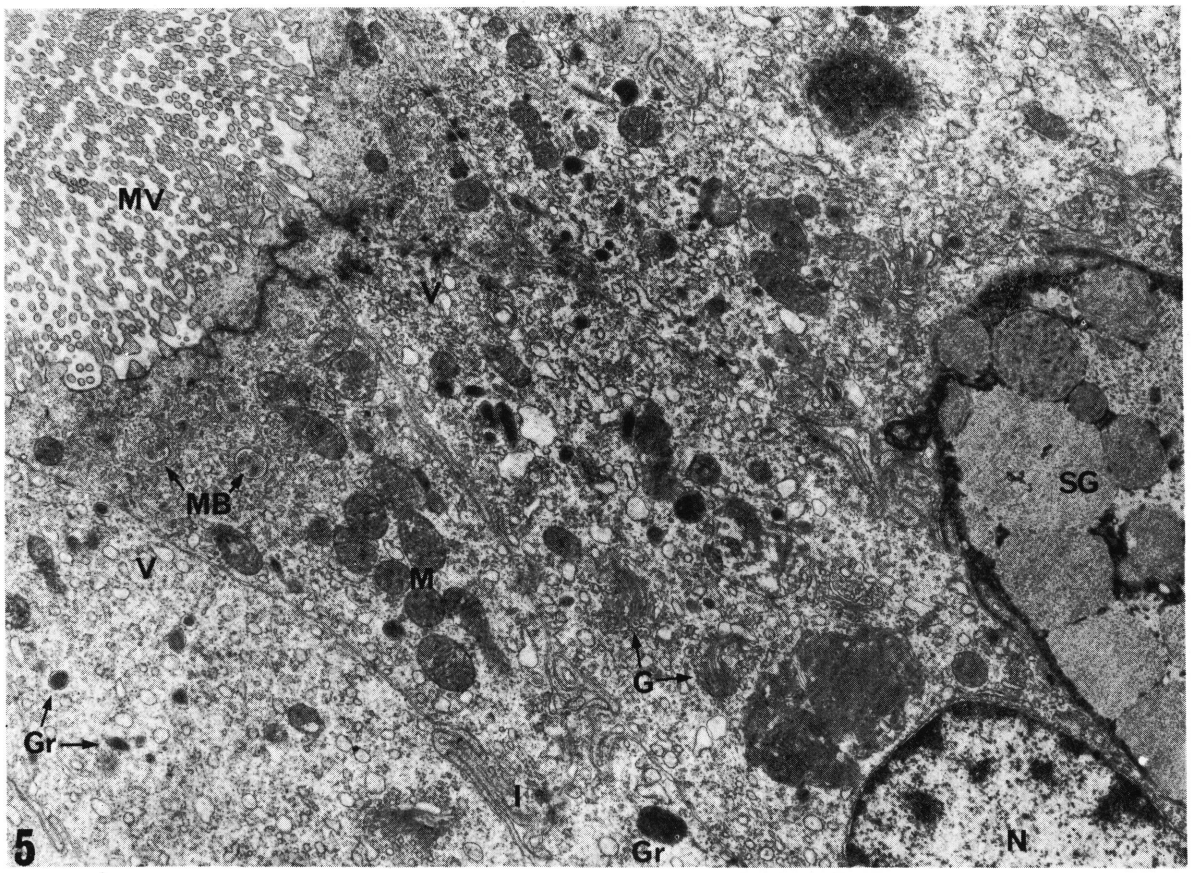

Fig. 5. An electron micrograph of the upper half of columnar epithelial cells of the posterior intestine in *M. griseus* embryo. x 7,200
The intestinal epithelial cells are characterized by microvilli, intermicrovillous invaginations, a number of vesicles, granules, and vacuoles.
G: Golgi complex, Gr: granule, MB: multivesculaied body, SG: secretory granules, V: vacuole.

such ciliated cells, as found in teleostean larvae, are not observed; throghout the whole area the internal surface of the intestine is covered with cells having microvilli.

In both *Mustelus manazo* and *M. griseus* embryos vesicles, granules and vacuoles are observed more frequently in epithelial cells of the posterior portion than in those of the anterior portion of the intestine. This may indicate that substances associated with the vesicles, granules and vacuoles are more actively absorbed in the posterior than in the anterior portion of the intestine in embryos of both species.

The membraneous lamellar structures are known to be developed in the intestinal epithelial cells of fish, especially of marine fish (Ozaki, 1965; Yamamoto, 1966, 1974; Iwai, 1967a, 1968a, b). The lamellar structures appear to be involved in the transportation of water and ions (Yamamoto, 1966; Iwai, 1968b). On the other hand, such the lamellar structures found in fish are not formed in the intestinal epithelial cells of the mammalian, but interdigitations of the lateral plasma membranes are ob-

served to occur at junctions of adjacent cells. The interdigitations are also considered to be engaged in the transportation of water and ions (Yamamoto, 1974). In the epithelial cells of the intestine of both *Mustelus manazo* and *M. griseus* embryos, the lamellar structures are not formed, but the lateral plasma membranes are seen to be interdigitated with each other. It is worthy to note that the interdigitations of the plasma membranes which are usually found in mammalians are formed in the intestinal epithelium of embryos of the two species of sharks even though they are fish. Unlike teleostean fish both *M. manazo* and *M. griseus* are of viviparity, and the embryos are retained in uteri for about ten months before they are born (Teshima, 1981). The formation of interdigitations of the lateral plasma membranes in the intestinal epithelium of *M. manazo* and *M. griseus* embryos may be ralated to their viviparous reproductive pattern.

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サメ類の研究 − XX

ホシザメとシロザメ胎児の腸管上皮細胞について

岡野茂喜・大竹二雄・手島和之・水江一弘

ホシザメとシロザメ胎児を用いて，それらの腸管上皮細胞の構造と機能を電子顕微鏡的手法により究明した．両種の胎児の腸管上皮細胞は遊離表面上の微絨毛，微絨毛間の細胞膜の陥入，多数の小胞，空胞，顆粒等により特徴づけられる．これらの特徴により，ホシザメとシロザメ胎児の腸管上皮細胞は吸収細胞として機能すると考えられる．また，硬骨魚類の腸管上皮には見られない隣接細胞の側面および基底面の細胞膜のかみ合いの形成がホシザメとシロザメ胎児において観察された．