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Light- and Electron-Microscopic Studies on Changes of the Coelomic Epithelium of the Japanese Brook Lamprey, *Lampetra reissneri*, during the Spawning Period

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Abstract

The coelomic epithelium of the Japanese brook lamprey, *Lampetra reissneri*, collected in the Ohno River, southern Hokkaido, was observed histologically and ultrastructurally.

In maturing lampreys of the metamorphic period, the coelomic epithelium in both sexes consisted of a layer of squamous cells which lacked any obvious apical structures such as cilia and well-developed microvilli. In mature females just before ovulation, epithelial cells lining the ventral and lateral coelomic wall were cuboidal in shape, and were light-microscopically furnished with numerous fine cytoplasmic processes on their apical surface. In females after spawning, epithelial cells of the ventral and lateral coelomic wall were remarkably thickened, from which numerous microvilli and many cilia of the 9+2 type projected into the coelomic cavity. In the cytoplasm of these cells, mitochondria with parallel cristae were abundant, but the rough endoplasmic reticulum, the Golgi complex and lysosomes were hardly detectable. It was observed that a large number of small vesicles of about 0.1 μm in diameter and many large vacuoles of about 1 μm in diameter accumulated mainly in the apical region of the cytoplasm. These small vesicles were observed to open often into the coelomic cavity, suggesting the occurrence of eccrine-like secretion. Similar ultrastructural characteristics were detected to occur also in the cells of the coelomic epithelium of male lampreys, during and after the spermiation period.

Discussions were made on the possible roles of liquid material that secreted from the cells of the coelomic wall in the maintenance of gametes which were shed directly from the gonads into the coelomic cavity in female and male lampreys.

Introduction

In some teleost fishes, the wall of the ovarian cavity or coelomic cavity shows conspicuous thickening accompanied by ciliation and secretion in association with ovarian maturation (Ashby, 1957; Yamamoto, 1963; Takano, 1968, 1973; Takahashi and Takano, 1971). In addition, the ovarian cavity or the coelomic cavity accumulates liquid material at the time of ovulation (cf. Ginzburg, 1968), which has been shown to play some essential roles in the maintenance of fertility of ovulated eggs in salmonid fishes (Takano et al., 1973; Billard, 1976).

Female lampreys, like some teleost fishes such as eel and salmon, are lacking in any specialized ovarian cavity, resulting in direct release of mature eggs into the coelomic cavity. Furthermore, lampreys are also very unique in that

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spermatozoa are released into the coelomic cavity directly following rupture of testicular cysts. No report has been concerned hitherto with changes of the coelomic epithelium associated with gonadal maturation in cyclostomes, so far as the present writers know. In the present study, the coelomic epithelium of the Japanese brook lamprey, *Lampetra reissneri*, was observed light- and electron-microscopically in both sexes captured mainly during the spawning period in order to examine morphological and functional characteristics of epithelial cells of the coelomic cavity.

Material and Methods

Japanese brook lamprey, *Lampetra reissneri*, were collected in the Ohno River, southern Hokkaido, in the months of May and June, 1980 and 1981, during the course of their upstream transition for spawning.

To determine the degree of sexual maturity, middle portions of their body were cut off, fixed *in toto* in Bouin's fluid, sectioned longitudinally at 4 to 8 μm in thickness, and stained with Delafield's hematoxylin and eosin for histological examinations of gonads.

Concomitantly, pieces of the coelomic epithelium from identical portions of the body were prefixed in Karnovsky's gultaraldehyde-paraformaldehyde mixture in 0.2 M cacodylate buffer (pH 7.4) for 2–3 hours at room temperature, followed by postfixation in 1% osmium tetroxide in the same buffer for about 2 hours at 4°C, and embedded in Epon. Ultrathin sections stained double with uranyl acetate and lead citrate were observed with a Hitachi H–300 electron microscope. Parallel Epon-embedded sections of about 1 μm were stained with methylene blue for light-microscopic observations.

Observations

In the Japanese brook lamprey, *Lampetra reissneri*, rapid sexual maturation initiates during the metamorphic period from June to September in both sexes (Fukayama, 1982): rapid growth of oocytes occurs following the initiation of vitellogenesis in females, and active proliferation of spermatogonia starts in males. In the next spring, mature adult lampreys start upstream transition for spawning.

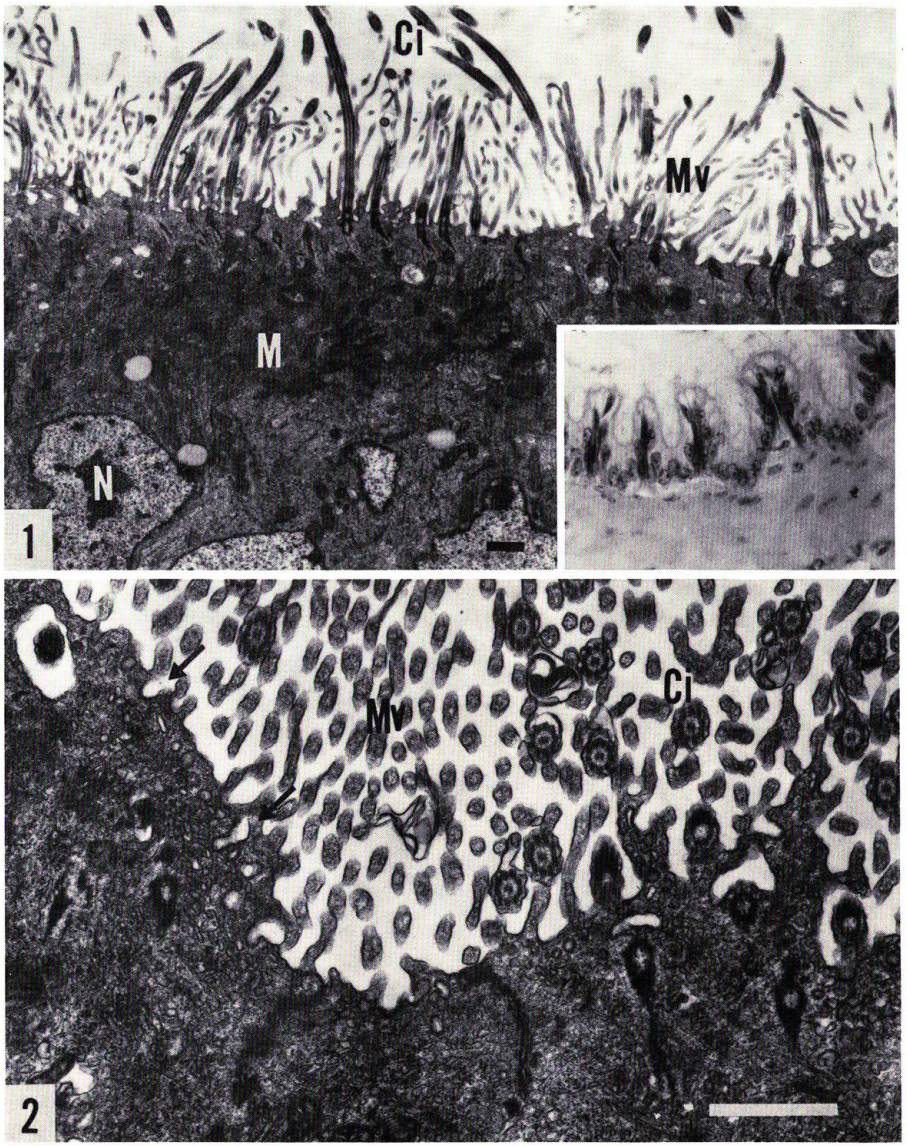
In maturing female and male lampreys captured during the metamorphic period, the coelomic epithelium consisted of a layer of squamous cells lacking in any specialized structures such as cilia and well-developed microvilli. In sexually mature females just before ovulation, ovaries packed with mature eggs occupied the coelomic cavity. At that period, epithelial cells lining the ventral and lateral coelomic wall were cuboidal in shape, about 8 μm in height, with a roundish nucleus in the center, and were furnished with numerous fine cytoplasmic processes projecting into the coelomic cavity from their apical surface. Going from the lateral to the dorsal regions of the coelomic wall, epithelial cells gradually decreased in height and their cytoplasmic processes became obscured, being quite squamous and devoid of ciliation along the dorsal region of the coelomic cavity.

In female lampreys with ovaries emptied after spawning, the coelomic epithelium displayed regular undulation parallel to the body axis, possibly due to shrinkage of

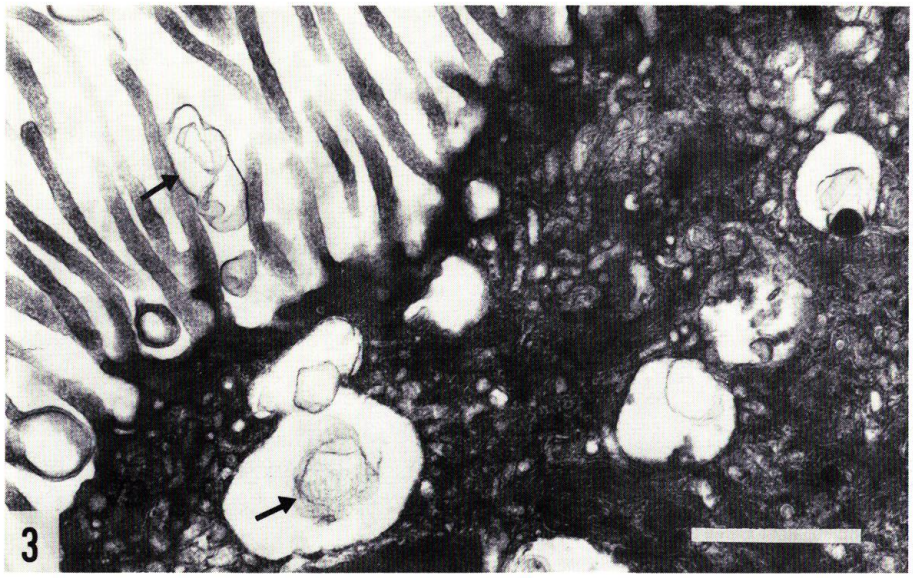
the coelomic wall following the discharge of eggs. Moreover, epithelial cells of the ventral and lateral coelomic wall were thickened conspicuously, attaining to about 25 μm in height, with basally located nuclei in most of them, though those of the dorsal coelomic wall remained still squamous in shape. Electron-microscopic observations showed that, in the thickened cells, many cilia of the 9+2 type together with numerous microvilli were seen to protrude into the coelomic cavity (Figs. 1 and 2). In the cytoplasm of the ciliated cells, many mitochondria with parallel cristae and dense matrix existed assuming varying shapes and sizes (Fig. 1). Development of the rough endoplasmic reticulum, the Golgi complex and lysosomes, was obscure in these cells except an occasional existence of oil droplets. Besides these cytoplasmic organelles, small vesicular structures of round or rod shape were abundantly present in the apical cytoplasm, and were rather frequently seen to open into the coelomic cavity (Fig. 3). Large roundish vacuoles, measuring from 0.7 to 1.2 μm in diameter, occurred also in that region, and were sometimes seen to fuse with the small vesicles (Fig. 3). The large vacuoles often contained membraneous material of undetermined origin, and were occasionally observed to be attached to the plasma membrane to open into the coelomic cavity. In fact, some membraneous material, which was similar in aspect to that contained in the vacuoles, existed in the coelomic cavity near the apical surface of the ciliated cells (Fig. 3). On the other hand, peculiar cytoplasmic processes with membraneous structures on their top were observed in rare cases (Fig. 4).

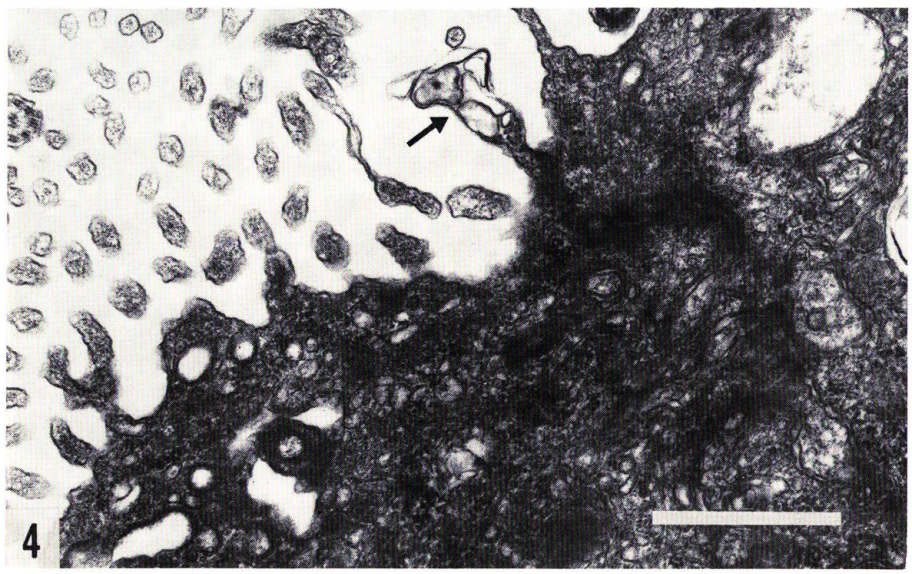
Similar to the case in spawning females, the coelomic epithelium in males with fully matured testes was remarkably thickened except in the dorsal region, attaining as much as 30 μm in thickness in the ventral region. Cells of the thickened epithelium projected numerous microvilli and many cilia from their apical surface into the coelomic cavity (Fig. 5). In the cytoplasm of these cells, mitochondria with parallel cristae were abundantly present, but the rough endoplasmic reticulum did not show obvious development and the Golgi complex were found only rarely. It was remarked that a large number of small vesicles of about 0.1 μm were seen distributed throughout the apical region of the cells. Some cells of the ventral coelomic epithelium possessed strikingly swollen apical cytoplasm where numerous small vesicles and larger vesicles or vacuoles of irregular shapes existed characteristically (Fig. 6). The small vesicles were observed often to open into the coelomic cavity at some places of apical surface of the cells. Moreover, some membraneous material was found in the coelomic cavity near the epithelium as well as in the vacuoles of the apical cytoplasm (Fig. 7), as in the case of mature females mentioned before.

In males with nearly evacuated testes after sperm discharge, epithelial cells of the lateral and ventral coelomic wall became diminished in height to about 7 μm. They were still provided with microvilli and cilia in the apical surface, and no notable changes were observed in their cytoplasmic organelles when compared with the cells of mature males described above. However, apical surfaces of the cells were quite rough with atypical protrusions of the cytoplasm including free ribosomes and vesicles, fragments of various sizes of the cytoplasm occurring frequently in the coelomic cavity near the epithelium (Fig. 8).

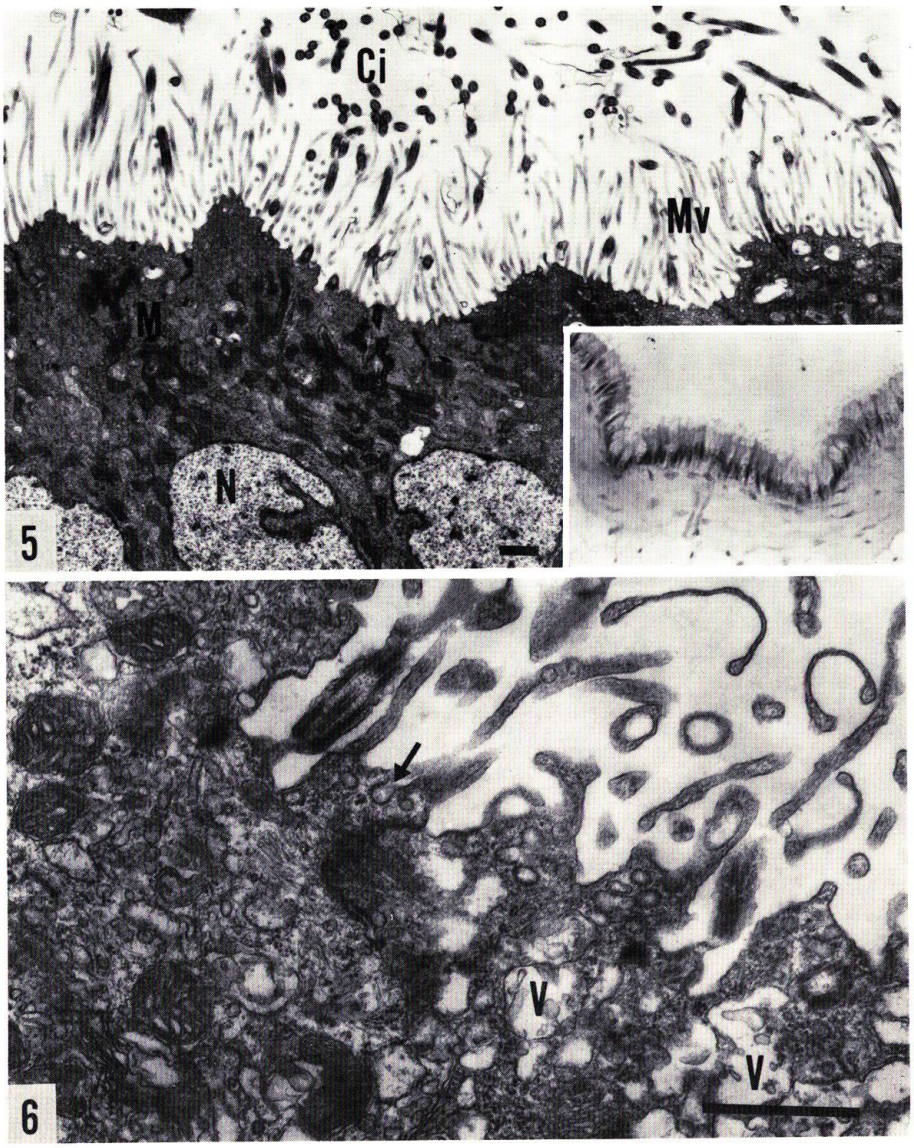


Figs. 1 and 2. Epithelial cells of the ventral coelomic wall of female lampreys after spawning. Numerous microvilli (*Mv*) and many cilia (*Ci*) develop on the apical surface of the cells. Arrows in Fig. 2 indicate openings of small cytoplasmic vesicles into the coelomic cavity. *N*, nucleus; *M*, mitochondrion. Bars indicate 1 μm. Inset in Fig. 1: a light-microscopic figure of the ventral coelomic wall, showing regular undulation running parallel to the body axis. × 270.

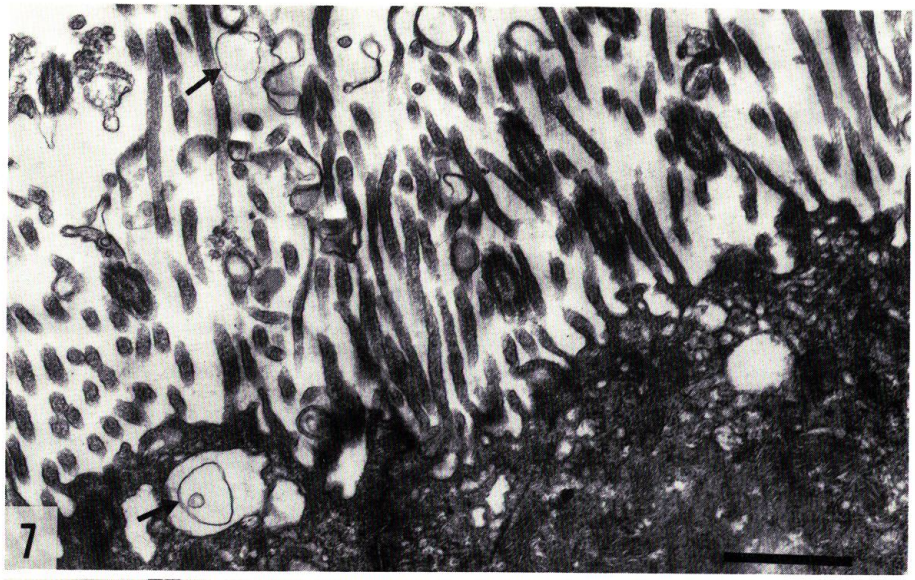


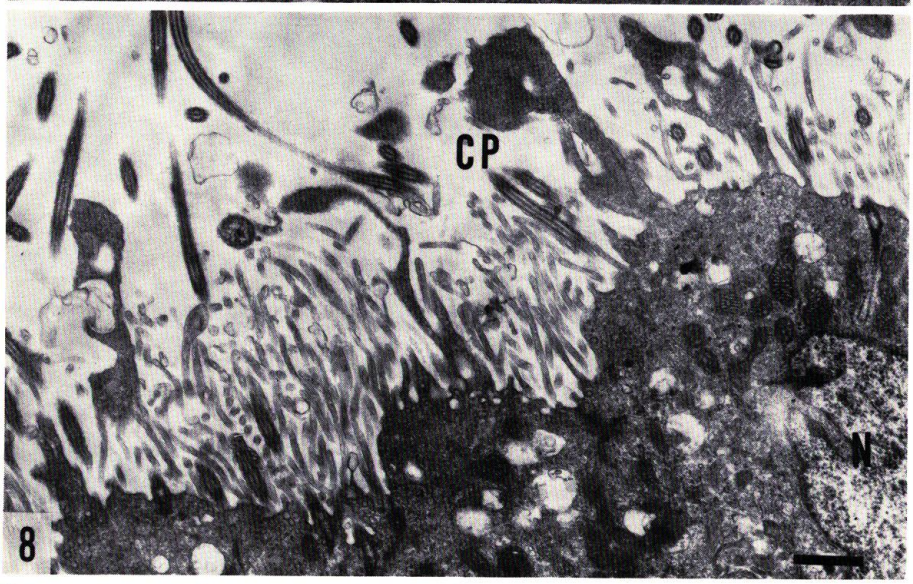


Figs. 3 and 4. Apical region of epithelial cells of the ventral coelomic wall of female lampreys after spawning, with numerous small vesicles and large vacuoles packing the cytoplasm. Arrows indicate peculiar membraneous material in the vacuoles and in the coelomic cavity. Bars indicate 1 μm.



Figs. 5 and 6. Epithelial cells of the ventral coelomic wall of male lampreys at spermiation, with numerous microvilli (*Mv*) and many cilia (*Ci*) projecting into the coelomic cavity. Fig. 6 shows swollen apical region of the cytoplasm filled with small vesicles and large vacuoles (*V*). Some of the small vesicles are seen to open into the coelomic cavity (arrow). *N*, nucleus; *M*, mitochondrion. Bars indicate 1 μm. Inset in Fig. 5: a light-microscopic figure of the ventral coelomic wall thickened remarkably. × 400.





Figs. 7 and 8. Epithelial cells of the ventral coelomic wall of male lampreys at spermiation (Fig. 7) and after sperm-release (Fig. 8). The apical surface of the cells is quite rough in aspect after the release of sperm out of the coelomic cavity, with many atypical cytoplasmic protrusions (*CP*). Membraneous material occurring in cytoplasmic vacuoles and in the coelomic wall are indicated by arrows in Fig. 7. Bars indicate 1 μm.

Discussion

The present study demonstrates for the first time that, in females of the Japanese brook lamprey, *Lampetra reissneri*, the epithelial lining of the coelomic cavity shows thickening and ciliation in association with ovarian maturation. In such teleost fishes as trout and salmon which ovulate their eggs directly into the coelomic cavity just like in lampreys, epithelial cells of the coelomic cavity become thickened and ciliated as they mature to spawn (Ashby, 1957; Takano, 1973). Also, in mature female goldfish, *Carassius auratus*, the coelomic epithelium confined in the ovarian cavity, into which mature eggs are ovulated, is columnar and ciliated, and further shows a sign of apocrine-like secretion into the ovarian cavity (Takahashi and Takano, 1971). In the medaka, *Oryzias latipes*, the epithelial cells lining the ovarian cavity possess numerous microvilli instead of cilia, and show also characteristic features of apocrine-like secretion (Yamamoto, 1963; Takano, 1968). Epithelial cells of the coelomic cavity of the kokanee salmon, *Oncorhynchus nerka*, have many microvilli together with cilia projecting into the coelomic cavity, and show a secretory activity of the microapocrine type at the time of ovulation (Takano, 1973). In postspawning lampreys observed in the present study, development of microvilli and cilia in the epithelial cells seemed more prominent than that observed in the salmon, but signs of apocrine secretion of the cells were not evident. However, numerous small vesicles were characteristically present in the apical cytoplasm of these epithelial cells, and they were occasionally found to open into the coelomic cavity, suggesting the occurrence of eccrine-like secretion. In addition, the small vesicles were seen to fuse with larger vacuoles existing in the same region of the cytoplasm, and the vacuoles were also seen to open into the coelomic cavity. Irregular membraneous material often occurred within the vacuoles and in the coelomic cavity neighboring the epithelium, but the nature of the membraneous material was not determined in the present study. Well-developed Golgi complex and rough endoplasmic reticulum, as observed in the secretory cells of the coelomic wall of the kokanee salmon (Takano, 1973), were hardly detected in the cells of postspawning lampreys observed in the present study.

It is well known in teleost fishes that a liquid is accumulated in the coelomic cavity or in the ovarian cavity at the time of ovulation (Ginzburg, 1968). The liquid, generally termed as the coelomic fluid in salmonid fishes, has been shown to play some essential roles in the maintenance of normal fertility of ovulated eggs (Ginzburg, 1968; Takano et al., 1973; Billard, 1976). It has been suggested that the apocrine-like secretion mentioned above may be a component of the coelomic fluid (Takano, 1973). From repeated field and laboratory observations made by the present writers, it seems that the Japanese brook lamprey may ovulate their eggs simultaneously and discharges them several times during a spawning period. Then the ovulated eggs for successive spawnings may be kept for certain lengths of days in the coelomic cavity. In fact, a large amount of fluid was always accumulated in the cavity to immerse the eggs. It is reasonable to assume that the eccrine-like secretory activity of cells of the coelomic epithelium may be involved in accumulating a liquid in the coelomic cavity of lampreys at the time of ovulation. Whether or not the "coelomic fluid" of the lamprey may be important

in maintaining normal fertility of ovulated eggs, as in the case for teleost fishes, is to be examined further.

On the other hand, epithelial cells of the coelomic wall became thickened and ciliated also in male Japanese brook lampreys as their testes became mature for spermiation. There has thus far been no information about such changes occurring in the cells of the coelomic epithelium in sexually mature males of other cyclostomes or teleost fishes, so far as the present writers know. Furthermore, secretory activities of a possible eccrine type were observed in the epithelial cells of male lampreys during and after spermiation, similar to those found in female lampreys.

Lampreys are unique, when compared with teleost fishes, in that males are completely devoid of the sperm duct and release mature spermatozoa from the testes directly into the coelomic cavity. It was ascertained by field observations made by the present writers that a single male could participate in spawning activities repeatedly during a spawning period. The spermatozoa spermiated from testicular cysts should be maintained in the coelomic cavity to be ejaculated repeatedly. It has been shown in some teleost fishes that gonadal hydration is brought about in mature males resulting in the thinning of milt for its smooth discharge outside the body (Clemens and Grant, 1964, 1965). It seems likely that similar phenomenon may occur in mature male lampreys during the spawning period, and that the possible secretion from the coelomic epithelium may be involved in the preparation of milt for successful dishcharge.

In the present study, the thickening of coelomic epithelium was observed to be prominent in both sexes during and shortly after spawning but not during the process of gonadal maturation. Similarly, in the medaka (Yamamoto, 1963) and the goldfish (Takahashi and Takano, 1971), the wall of the ovarian cavity becomes thickened along with the progress of ovarian maturation. It has been demonstrated experimentally that the development of epithelial cells lining the ovarian cavity or the coelomic cavity may depend upon sex steroids (Ashby, 1957; Takahashi and Takano, 1971). In lampreys, the existence of estrogens in the blood serum of mature females has been revealed by several authors (cf. Hardisty, 1979). Piavis et al. (1975) reported that peripheral blood levels of estradiol tended to increase toward full ovarian maturation in the sea lamprey, *Petromyzon marinus*. In the sea lamprey, the testis was capable of synthesizing estrogen (Callard et al., 1980), and a high concentration of estrogen was detectable in the blood in males as well as females (Katz et al., 1982). These findings may imply that the development of the coelomic epithelium in mature lampreys of both sexes is influenced by endogenous sex hormones. Some series of experiments are now in progress to elucidate this possibility.

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