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Author(s)	TAKANO, Kazunori
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FINE STRUCTURE OF THE WALL OF THE OVARIAN LUMEN IN THE TELEOST, *ORYZIAS LATIPES**

Kazunori TAKANO**

The ovary of teleosts shows various degrees of the structural differentiation which may be related to the breeding habits of the species. Except for some fishes such as salmon and lamprey (Kendall, 1921; T.S. Yamamoto, 1955a), the ovary of almost all oviparous teleosts usually assumes a sac-shaped organ involving a hollow lumen. The lumen has been regarded as a stockroom in which ovulated eggs are stored temporarily until they are spawned. Although many investigations on the ovarian structure have been performed, little attention has been given to the wall of the ovarian lumen. Especially the role and function of the wall during reproduction remain almost untouched. Recently, K. Yamamoto (1963) found in *Oryzias latipes* that marked changes of the wall of the ovarian lumen occur in parallel with the ovarian maturity, and he suggested that the wall may, perhaps, take a role in the secretion of fluid. The present author studied the fine structure of the wall of the ovarian lumen of *Oryzias latipes* to clarify the function and mechanism of its structural changes.

Before going further, the author wishes to express his hearty thanks to Professor Kiichiro Yamamoto for his kind guidance and revision of the manuscript. Thanks are also offered to Messrs H. Onozato and I. Oota for their friendly help in the course of the present study.

Materials and Methods

The fish used in the present study, the wild type fish of *Oryzias latipes*, were collected from a pond at Yunokawa Hot Spring, Hakodate, Hokkaido. They were reared in an outdoor pond in the campus of Hokkaido University until needed. At the time of pre-ovulation, post-ovulation and post-oviposition respectively, the fish were decapitated and their ovaries were quickly removed. Subsequently the wall of the ovarian lumen was dissected and trimmed into small pieces. These tissues were fixed for two hours in Millonig's solutions adjusted to about pH 7.4 by a phosphate buffer. After dehydration in graded ethanols, the tissues were embedded in Epon epoxy resin mixture. Sections were cut with a Porter-Blum microtome, and stained with uranyl acetate or stained double with uranyl acetate

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** Laboratory of Fresh-Water Fish-Culture, Faculty of Fisheries, Hokkaido University
(北海道大学水産学部淡水増殖学講座)

and lead monoxide of Karnovsky (1961). They were examined with a Hitachi HS-7 electron microscope.

Observations

The ovary of adult *Oryzias* is a single oviform sac and lies on the intestinal duct in the post-dorsal portion of the peritoneal cavity. The ovarian lumen is a cavity found between the ovarian lamellae and the caped covering wall of the ovary and spreads over the dorso-lateral portion of the ovary. Light microscopic observations revealed that the wall of the ovarian lumen consists of three main components, viz., an inmost epithelial layer, a middle layer of tunica albuginea and an outmost peritoneal epithelium (K. Yamamoto, 1963). In the present study, the author mainly observed the former two layers which will be described in the following sections as epithelial layer and subepithelial layer.

1. Epithelial layer

The epithelium usually is composed of a single layer of the cuboidal or columnar cells, but in some portions the shape and arrangement of the cell are not regular (Figs. 1 and 6). The free surface of the epithelial cell is provided with microvilli projecting into the ovarian lumen. They are cytoplasmic projections of various shapes and sizes, showing no special internal structure (Fig. 2a). The basal surface of the cell is limited from the subepithelial layer by a thin basement membrane and a relatively thick collagenous layer (Figs. 1, 6 and 11). Frequently, the basement membrane is not distinguishable due to the production of complicated protuberances from the subepithelial layer (Figs. 6, 7 and 8).

A characteristic feature of the epithelial cell is the well developed tonofilaments. The tonofilaments distribute almost the whole cytoplasm, but they are found more densely in the basal portion (Figs. 1, 2b and 11). They are meandering among the cytoplasmic organelles as bundles of various thickness. Oil droplets showing irregular contour and variable density are observed in the cytoplasm (Figs. 1 and 11). The mitochondria are usually oval or rod-shaped, and show a fairly regular and parallel arrangement of cristae. The matrix of the mitochondria is moderately electron-dense and often involves a few intra-mitochondrial granules. Although the mitochondria are distributed throughout the cell, they are often found more abundantly in the basal and peri-nuclear cytoplasm than in other parts (Fig. 1).

The granular endoplasmic reticulum develops moderately and is scattered throughout the cytoplasm. Their profiles are seen as tubules or somewhat distended vacuoles (Fig. 2b).

The Golgi apparatus is commonly demonstrable in all epithelial cells. It consists of lamellar elements with many vesicles and vacuoles of various sizes (Figs. 1 and 2b). The Golgi lamellae assume flattened sacs arranged in parallel and

frequently distended at their end. Large vacuoles limited with agranular membranes are often seen near the Golgi area. Their contents are mostly clear or moderate in density. These findings suggest that these vacuoles are derived directly from the dilated cisterns of the Golgi lamellae. Vesicles such as those found in the Golgi area are also seen numerously throughout the cytoplasm. These vesicles show striking differences in size and electron lucidity.

In the specimens preserved in the pre-spawning season, the distended cytoplasmic process is observed at the apical portion of the epithelial cell (Fig. 3), but no microvilli are seen there. These processes consist of the lucent cytoplasm without any notable cellular inclusions except for free ribosomes and a little amount of vesicles or vacuoles (Fig. 4). The cytoplasmic membrane covering the process is continued to that covering the cell body proper but they show very complicated forms (Fig. 5). From their features and position, it is logical to interpret these processes as apocrine process. In the basal portion of apocrine process and the apical portion of main cell body, however, there are a large amount of vacuoles and vesicles with agranular limiting membranes (Figs. 4 and 5). These vacuoles and vesicles might fuse together to form gradually large cisterns and then make the apocrine process by releasing the contents into the apical cytoplasm. The facts, as mentioned above, suggest that the epithelial cell of the wall of the ovarian lumen has a secretory function.

In the specimens obtained at the post-ovulation phase, the epithelial cell often shows an extreme swelling in the apical portion (Fig. 6), and form a cap-like process which is more lucent than the apocrine process. The processes have many vacuoles of various sizes and sometimes the endoplasmic reticulum and mitochondria.

On the other hand, in the specimens, except those obtained just after oviposition, a notable feature of the epithelium is the occurrence of wide intercellular spaces (Figs. 2b, 3 and 6). They are various in size and filled with liquid substances of a low or moderate electron density. The liquid substance is surmised to be originated from the subepithelial layer and flowing into the intercellular space between the epithelial cells. In close proximity to the ovarian lumen, the well developed junctional complexes are located between adjacent cells (Figs. 1 and 6), and seal up the intercellular space to prevent the outflow of the liquid substance. Sometimes, the intercellular substance is accumulated considerably and makes the epithelial cells deform in their arrangement.

After oviposition, the typical swelling processes or the apocrine ones of the epithelial cells have almost disappeared, and the cytoplasmic matrix also becomes considerably lucent in electron density. In the intercellular spaces, only a little amount of the liquid substance remains here and there.

2. Subepithelial layer

Just beneath the epithelium, a thick layer mainly composed of smooth muscle cells is located. The smooth muscle cells are spindle-shaped and have the nucleus showing complicated contour and deep invaginations. Very thin filaments running parallel to the long axis of the cell occupy the major part of the cytoplasm. Together with the mitochondria, the endoplasmic reticulum which is represented by agranular vesicles and tubular elements are found clustered near the nucleus (Fig. 7).

The most conspicuous structure in the muscle cell is the presence of numerous vesicles in the peripheral region. These vesicles are enclosed with smooth-surface membranes and show flask-like invaginations of the cell membrane (Fig. 9). These are interpreted as evidence of submicroscopic order of pinocytosis (Bloom and Fawcett, 1962). The pinocytotic vesicles often show long, tubular or vacuolar forms (Fig. 9). These vacuoles exhibit the tendency to aggregate in the peripheral cytoplasm and to accumulate the vacuolar contents there. Then, the fluid substance seems to be protruded into the intercellular space (Fig. 10). Since the mitochondria are located in the peripheral cytoplasm, they are often seen in the protruded processes. Such papillary processes gradually may fuse together and form the large prosecretory vacuoles attaining about 8μ in maximum diameter. The prosecretory vacuoles covered with a limiting membrane, contain finely granular and relatively electron-lucent materials. Often these vacuoles cluster near the border of the muscle cell layer just beneath the epithelium (Figs. 1 and 11). It is observed at times that the prosecretory vacuoles are extruded into the epithelial layer from the subepithelial muscle layer (Fig. 8). The extruding vacuoles are still bounded by a limiting membrane and enter the intercellular spaces between the epithelial cells. Subsequently the prosecretory material begins to fill each space as mentioned above. Although such profiles showing the extrusion of vacuoles can be observed in the specimens obtained in the pre-spawning season, the most active protrusion seems to occur at the time of ovulation.

In the subepithelial layer, the blood capillaries develop well. Around the capillary lumen, the endothelial cells form a continuous layer. They attenuate except for the nuclear portion and have the routine structure of the endothelium. The striking features in the cells are the presence of numerous vesicles like those in the muscle cell. Some of the vesicles show the flask-like invaginations of the membrane along both the luminal and the basal surfaces of the endothelial cells (Figs. 11 and 12). The cells are lined with the collagenous fibers which continue to those running among the muscle cells. The nerve cells also can be seen in the inter-muscular connective tissues.

The outmost layer of the wall consists of a row of squamous peritoneal cells.

Discussion

Based on the observation with a light microscope, T.S. Yamamoto (1955b) stated that in *Oryzias* the wall of the ovarian lumen consists of the three components, viz., a vascular fibrous tissue, a muscular middle layer and a ciliated cell layer inward from the outer surface. On the contrary, K. Yamamoto (1963) insisted that the compositions of this wall are a peritoneal epithelium, a tunica albuginea and an epithelial lining, and no ciliated cells are found in the inner epithelial lining. In the present study, the three components, as revealed by the latter author, are confirmed with the electron microscopic observation. The epithelial lining is composed of a single layer of identical type cells with many microvilli. No ciliated cell could be seen among them.

The observations reported here suggest that the wall has a secretory activity as already indicated with the result of light microscopic investigation (K. Yamamoto, 1963). Kurosumi (1965) described the five types of secretory mechanism from gland cells. The ovarian epithelial cell of *Oryzias* obtained in pre-spawning season shows a typical profile of the macroapocrine secretion (extrusion type II by Kurosumi). A close connection could be seen between the vacuoles and vesicles in the Golgi area and the vacuoles in the apical cytoplasm. It is suggestive, therefore, that the secretory vacuoles originate in the vacuoles and vesicles in the Golgi area. This apocrine projection and its formation mechanism observed in the present study appear to be analogous to that of the superficial cell in human eccrine sweatglands (Ito and Shibasaki, 1966). As to the origin of secretory vacuoles, Munger (1961) suggested that they are formed in the Golgi zone and migrate to the apical cytoplasm in human eccrine sweat glands.

In the specimen obtained at the time of post-ovulation during the spawning season, on the other hand, the apical process of the epithelial cell is somewhat different from that seen in pre-spawning season. In the apical portion of cell, the cap-like process made of markedly lucent cytoplasm could be observed instead of the typical apocrine projection. In the present study, the author could not confirm the fate of the liquid substance accumulated in the intercellular space of the epithelium. It seems, however, that this liquid substance might be taken into the cell and extruded into the ovarian lumen by a way such as that of the apocrine secretion, because there is found the liquid substance deeply invaded into the cell body and almost disappeared from it soon after oviposition.

Some females of *Oryzias* reared at about 25°C show a daily spawning cycle if not throughout breeding season. At the time of spawning, the wall of the ovarian lumen secretes a large bulk of liquid substance to facilitate the extrusion of eggs. In such daily secretion, the liquid substance accumulated in the intercellular spaces of the epithelium may take a more important role than the secretion produced in the epithelial cell itself.

K. Yamamoto (1963) reported that the epithelial lining and the tunica albuginea are changed markedly along with the development of ovarian maturity. Also he suggested that the vacuoles formed in the epithelium have some relation to the contents of the oedema-like tissue in the tunica albuginea. As mentioned above, the liquid substance presented in the intercellular space of the epithelium could be traced reversely to the prosecretory vacuoles in the subepithelial layer, which are corresponding to the oedema-like tissue described by K. Yamamoto (1963). In the formation of the prosecretory vacuoles in the smooth muscle layer, the pinocytotic mechanism may take an important role. Although it is suggestive that there is an intimate connection between blood capillaries and muscle cells through the collagenous layer, the detailed processes of transference and formation of prosecretion remain for a further study.

In the present study, it could be observed that the prosecretory vacuoles formed in the subepithelial muscle layer extrude into the epithelial lining. Although these extrusion profiles were seen in the ovary of pre-spawning season, the evidence suggests that it occurs most actively at the time of ovulation. It is an interesting fact that the movement of ovary gradually increases up to the time of ovulation and after that tends to decrease in *Oryzias latipes* (Hosokawa and Nambu, 1966).

Summary

The wall of the ovarian lumen in *Oryzias latipes* was examined with an electron microscope and the evidence suggesting its secretory function was confirmed. The epithelium is composed of a single layer of identical cells. The basal surface of the epithelium is limited by a thin basement membrane and a collagenous layer. Just beneath the epithelial layer, a thick smooth muscle layer develops well. The epithelial cell is characterized by many microvilli seen on the luminal surface, well developed tonofilaments, abundant agranular endoplasmic reticulum and the Golgi apparatus. The secretory vacuoles formed in the epithelial cell extrude into the ovarian lumen by the mode of macroapocrine secretion. At the same time, a large bulk of prosecretory vacuoles arises in the subepithelial muscle layer and extrudes into the intercellular spaces of the epithelium. It may take an important part in secretion at the time of daily spawning during breeding period.

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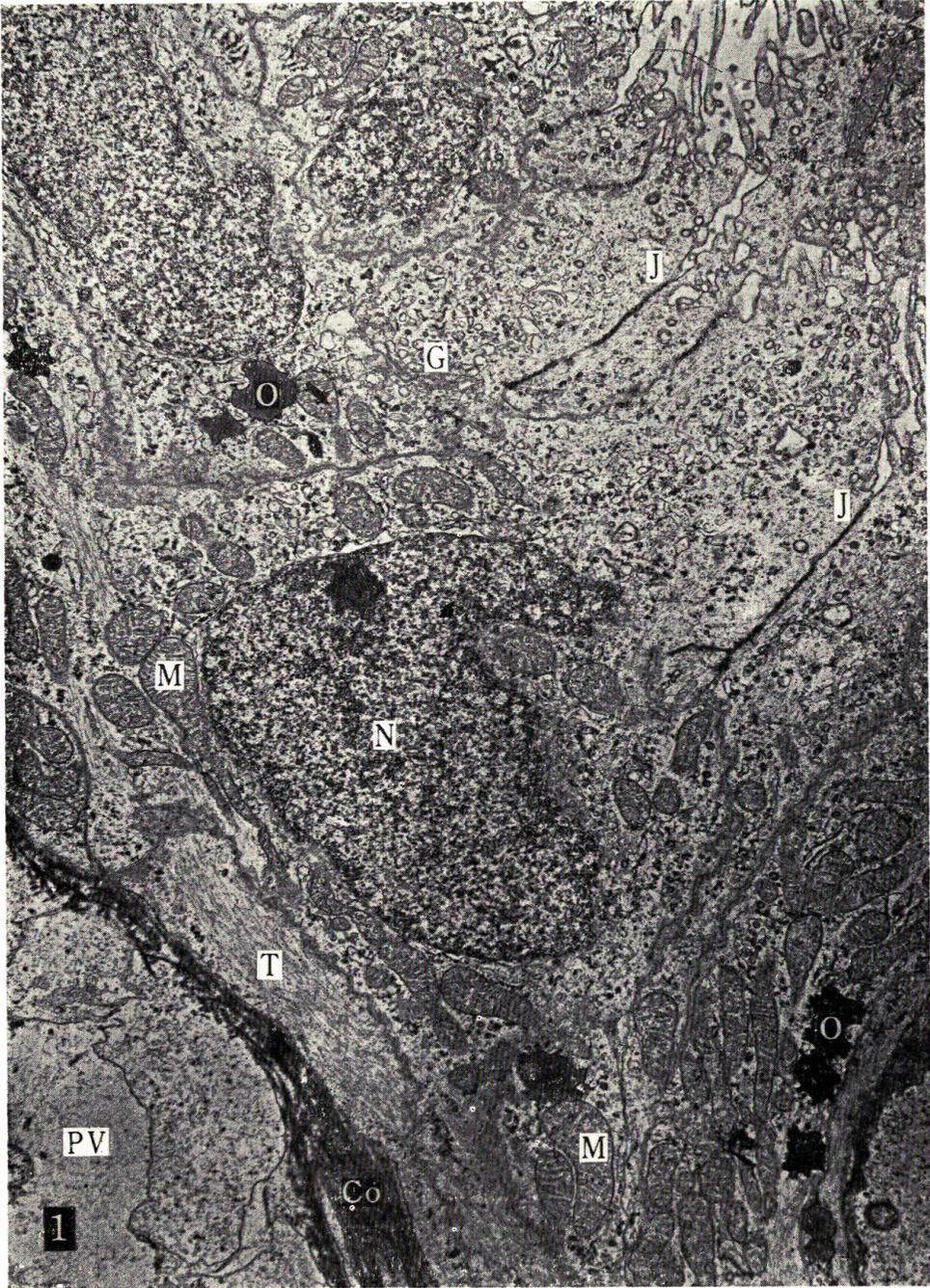
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Explanation of Plates

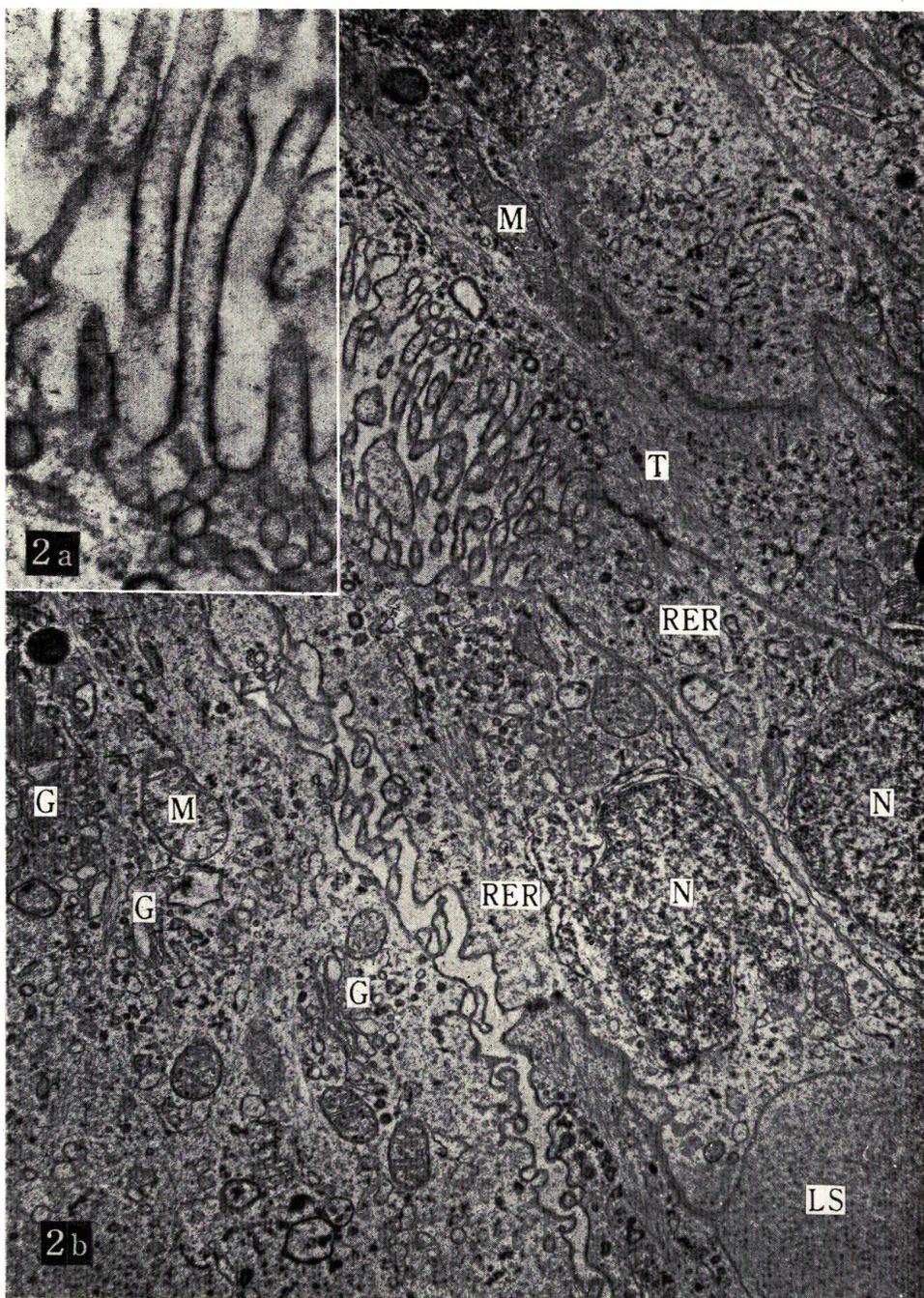
PLATE I

Fig. 1. Epithelial cells of the wall of the ovarian lumen : Facing the ovarian lumen (the upper right-hand portion), many microvilli are seen. Junctional complex (*J*) located between adjacent cells. In the basal portion of cell, tonofilaments (*T*) are well developed. Through a collagenous layer (*Co*), prosecretory vacuoles (*PV*) are represented in the subepithelial layer. *G* Golgi apparatus, *M* mitochondria, *N* nucleus, *O* oil droplets.

× 9,000



K. TAKANO : Wall of ovarian lumen in *Oryzias*



K. TAKANO : Wall of ovarian lumen in *Oryzias*

PLATE II

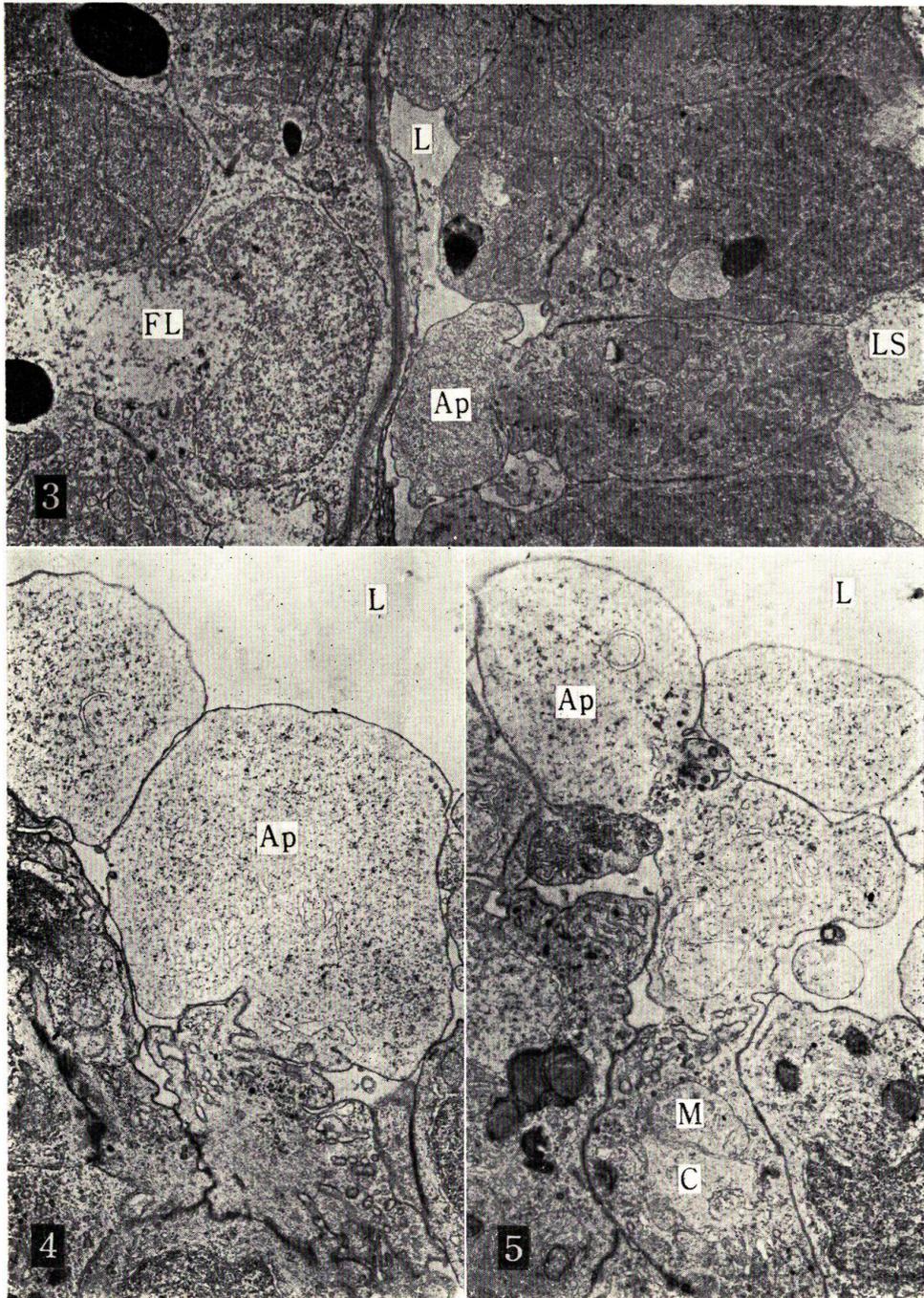
Fig. 2a. Higher magnification micrograph of the microvilli $\times 40,000$

Fig. 2b. Apical portion of the epithelial cells: The Golgi apparatus (*G*) shows dilated cisterns in the end of flattened sacs. Numerous vesicles and vacuoles are scattered throughout the cytoplasm. Granular endoplasmic reticulum (*RER*) are also seen as tubular or somewhat distended vacuoles. Liquid substance (*LS*) for secretion are accumulated in the intercellular space. *M* mitochondria, *N* nucleus, *T* tonofilaments. $\times 12,000$

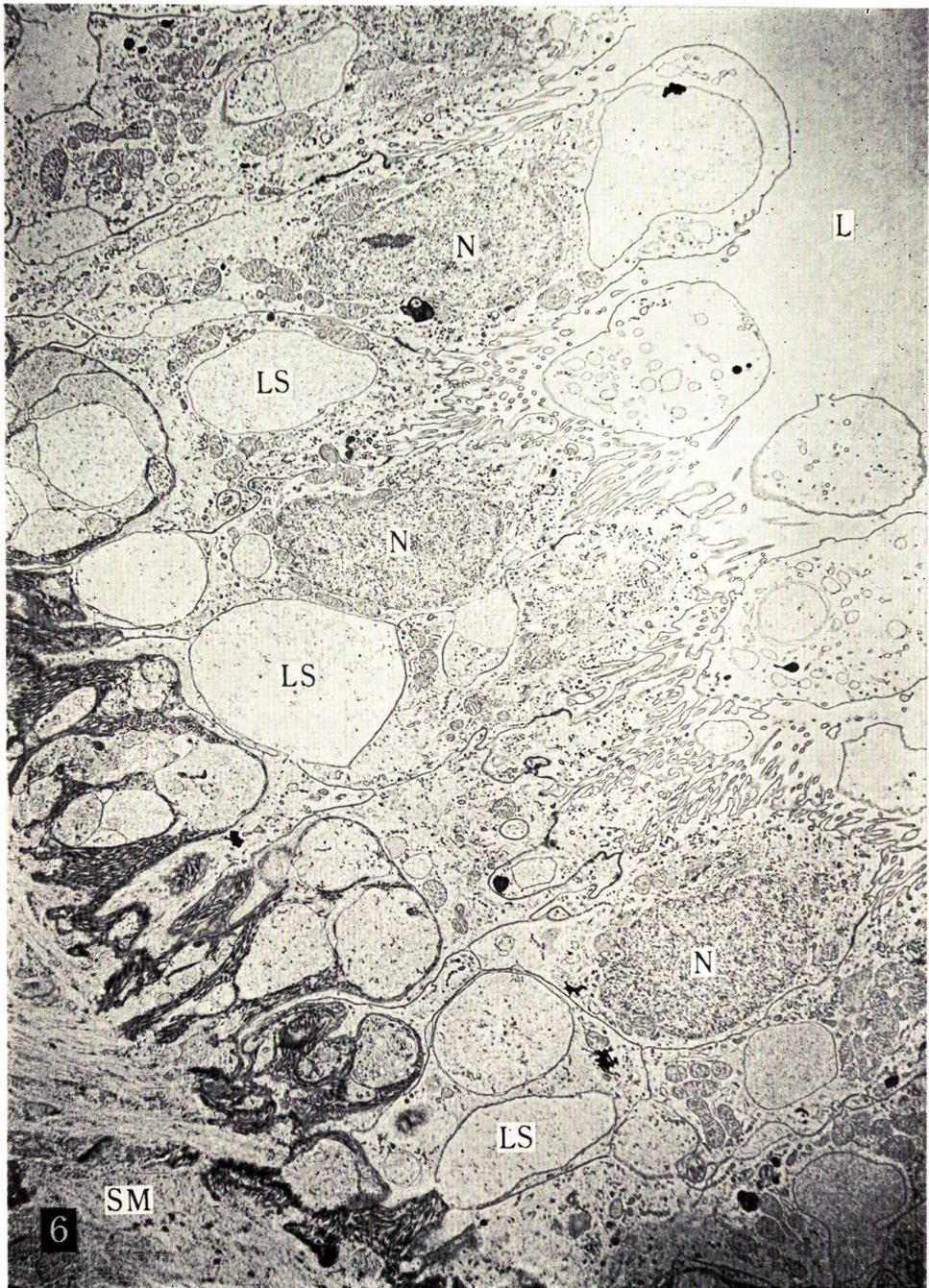
PLATE III

Fig. 3. A survey micrograph of the epithelium in the specimen obtained in pre-spawning season: Epithelial cell has a typical macroapocrine projection (*Ap*) at the apical portion. Through a narrow space of ovarian lumen (*L*) the apical portion of cell faces to ovigerous lamella. *FL* follicular layer of a young oocyte, *LS* liquid substance. $\times 5,200$

Figs. 4 and 5. Higher magnification of apocrine projections (*Ap*) protruded into the lumen (*L*): In the apical cytoplasm of the epithelial cells, there are large cisterns (*C*) containing secretory liquid, numerous vesicles and mitochondria (*M*). $\times 9,000$



K. TAKANO : Wall of ovarian lumen in *Oryzias*



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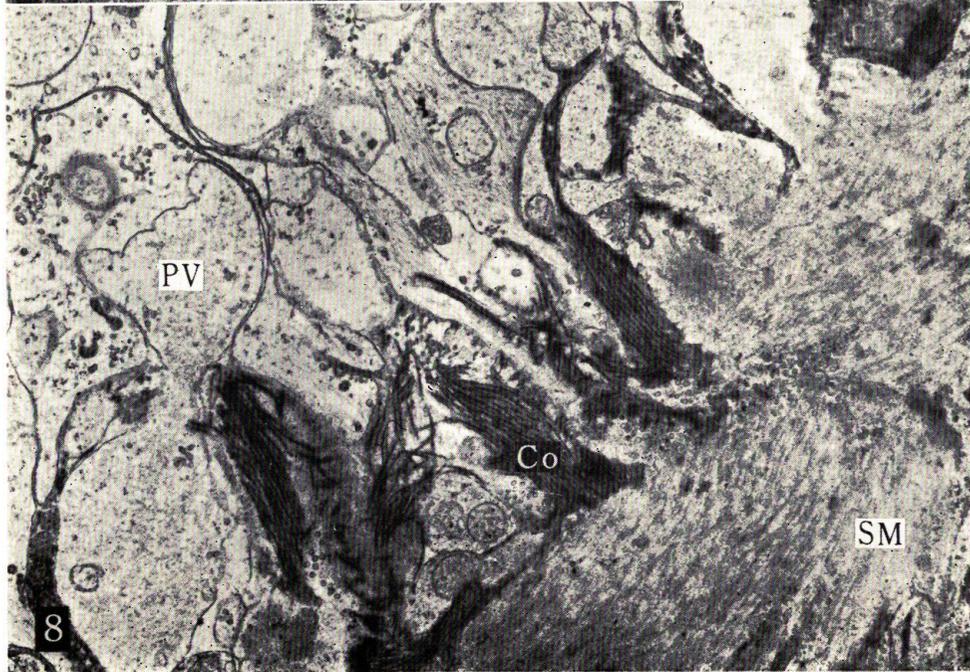
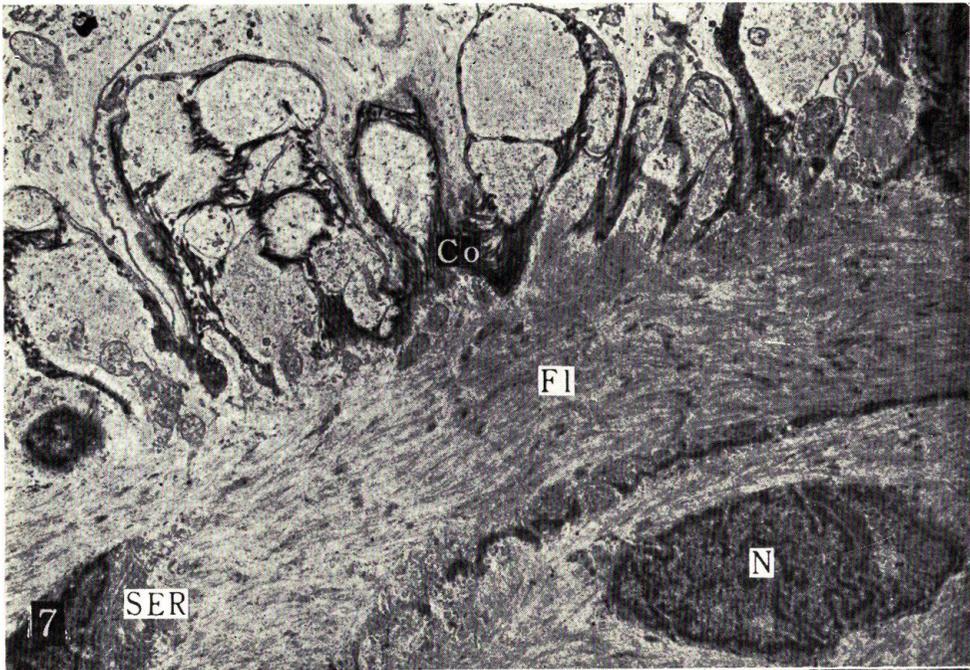
PLATE IV

Fig. 6. Low-power micrograph of the epithelium after ovulation : The epithelial cells show extreme swellings in the apical portion. In intercellular spaces the liquid substance (*LS*) exists here and there. *L* ovarian lumen, *N* nucleus, *SM* smooth muscle of subepithelial layer. × 3,700

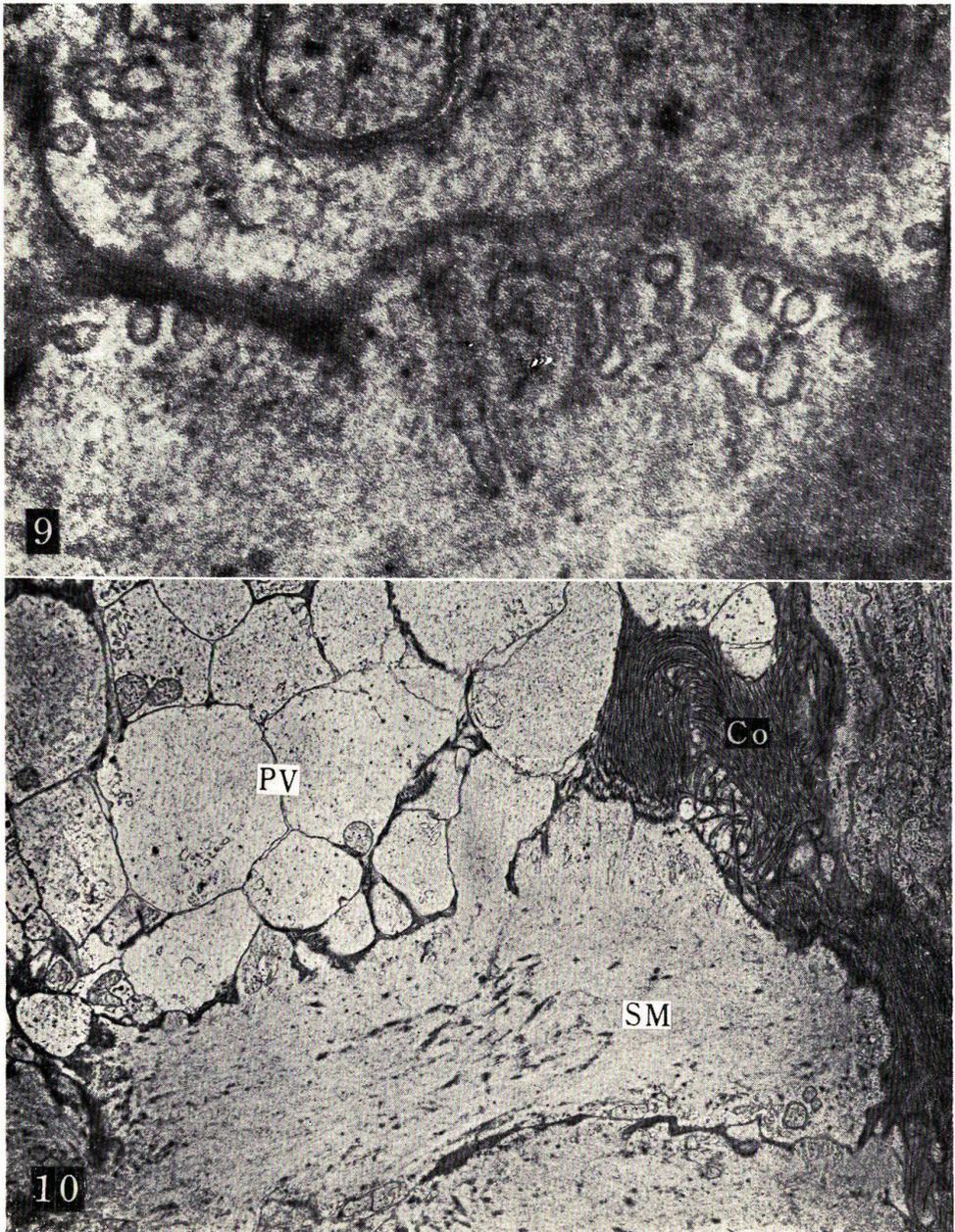
PLATE V

Fig. 7. Subepithelial layers mainly composed of smooth muscle cells: They are characterized by a nucleus (*N*) with complicated contours, thin filaments (*Fl*) running parallel to the long axis of the cell, agranular endoplasmic reticulum (*SER*) clustered near the nucleus and numerous vesicles located along the peripheral margin. *Co* collagenous bundles. $\times 6,000$

Fig. 8. Prosecretory vacuole (*PV*) accumulated in the epithelial layer: The vacuoles are surmised to arise in the subepithelial muscle layer (*SM*), and come to the epithelial layer through the collagenous layer (*Co*). $\times 9,200$



K. TAKANO : Wall of ovarian lumen in *Oryzias*



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PLATE VI

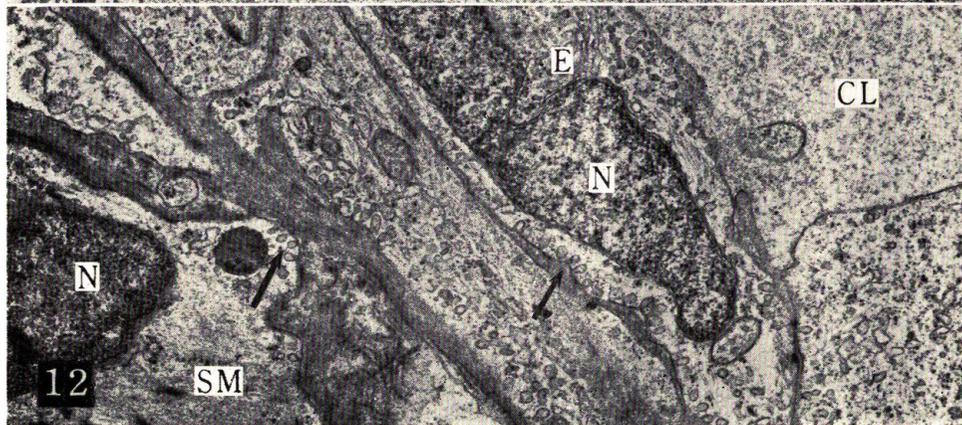
Fig. 9. High magnification micrograph of pinocytotic vesicles in the peripheral region of the smooth muscle cell: These vesicles showing flask-like invaginations of the cell membrane, often give long tubular or vacuolar profiles. $\times 60,000$

Fig. 10. Prosecretory vacuoles (*PV*) accumulated in the peripheral region of the muscle cell (*SM*). *Co* collagenous bundles. $\times 6,000$

PLATE VII

Fig. 11. Capillary lumen (*CL*) and the endothelial cell (*E*) having many small flask-like invaginations of the plasma membrane (arrows): Under the collagenous bundle layer (*Co*), large prosecretory vacuoles (*PV*) are accumulated. In the left-hand portion, the basal regions of epithelial cells are seen with well-developed tonofilaments (*T*), oil droplets (*O*) and mitochondria (*M*). $\times 9,000$

Fig. 12. Vesiculations of the cell surface (arrows) observed at the basal surface of endothelial cell (*E*) and a periphery of smooth muscle cell (*SM*); *CL* capillary lumen, *N* nucleus. $\times 12,000$



K. TAKANO : Wall of ovarian lumen in *Oryzias*