# ELECTRON MICROSCOPE STUDY ON THE GROWING OOCYTE OF THE GOLDFISH DURING THE FIRST GROWTH PHASE[1)2)]

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CONTENTS



The growing process of the fish oocyte is commonly divided into two phases, the first phase and the second one. The first growth phase corresponds to the prophase of meiosis. During this phase the germ cell shows the so-called premeiotic phenomena and grows to about 150 micra. The second phase is characterized by the accumulation of yolk and the oocyte increases greatly in size during this phase (cf. Yamazaki, 1965).

Many workers have called attention to the nuclear change in the first growth

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phase of the oocyte and good information about the premeiotic phenomena has been accumulated (cf. Raven, 1961). Concerning the change in the cytoplasmic organelles during this growth phase, a considerable number of studies by means of the routine cytological methods have also been published, and they have reported marked changes in the form and distribution of the cytoplasmic organelles (cf. K. Yamamoto, 1958; Raven, 1961). However, the results reported seem not only to be too divergent, but also to leave unsolved many doubtful points due to the limited resolving power of a light microscope. Naturally the increased resolution obtainable with the electron microscope should yield a solution to this problem.

Recently, the oocytes of various kinds of animals have been investigated with an electron microscope and changes in the fine structure of oocytes during oogenesis have been gradually clarified.

As for teleostean oocytes, several articles reporting valuable findings have also been published hitherto. However, most of them are concerned with special topics of oogenesis; Kemp and Allen (1956), Kemp and Hibbard (1957), Zahnd and Porte (1962), Chambolle *et al.* (1962), Sterba and Müller (1962), K. Yamamoto (1962), Müller and Sterba (1963), M. Yamamoto (1963), Jollie and Jollie (1964) and Anderson (1964) dealt mainly with the formation of the egg membrane, and Porte and Zahnd (1962) and Zahnd and Porte (1963) with some cytoplasmic organelles. The paper of M. Yamamoto (1964) is the only one concerned with the complete oogenesis of teleosts, but it deals rather briefly with each stage. Thus, the knowledge accumulated up to date is too meagre for complete understanding of all aspects of teleostean oogenesis. The presents study has been undertaken to clarify the changes in the cytoplasm and the relationship between the oocyte and the follicle cells during the early phase of oogenesis, using the goldfish as material.

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## MATERIAL AND METHOD

The material used in the present study was the young goldfish, *Carassius auratus* which were hatched and reared in the culture pond of the Faculty of Fisheries, Hokkaido University. The fish, 3 to 5 cm in body length, were dissected and their gonads were removed. After being cut into small pieces, the tissues were fixed in Millonig's solution (1961) for two hours, dehydrated by the routine ethanol method and two changes of propylene oxide, and embedded in Epon Epoxy resin mixture (Luft, 1961). Sections were cut with glass knives at a thickness of

about 500 to 800 Å on a Porter-Blum microtome, stained by Karnovsky's lead method (1961), and were examined with a Hitachi HS-7 electron microscope.

## OBSERVATIONS

**I. Early phase of the chromatin-nucleolus stage*** (Below 20 $\mu$ in diameter) (Pls. I and II)

The oocyte of this stage is almost spherical in form and contains a large nucleus of spherical form. The ground matrix of the nucleus is granular and of moderate density. Nucleoli, several in number, are found embedded in the matrix. They vary in size and show a network pattern due to the dense or loose aggregates of small electron-opaque granules. High magnification of the nucleoli shows that they are composed of densely or loosely packed aggregates of a certain unit structure. The unit structure measures about 80 Å in thickness, which makes small bundles or arrangements in one to several rows. Each unit shows a fibrous structure with a central hole about 60 Å in diameter, the largest fibrous unit measuring approximately 600 Å in long axis. An amorphous substance of low density seems to occupy the space between the fibrous units. The nucleus is enclosed with a double membraned envelope showing a somewhat wavy contour. In some places the outer membrane of the nuclear envelope forms small outpockets protruding into the cytoplasm.

The cytoplasm is occupied with mitochondria, Golgi complexes, endoplasmic reticulum, ribosomes and large electron-opaque substances. The mitochondria are round to oval or rod-shaped. They are comparatively large in size, the large rod-shaped ones measuring more than 3 $\mu$ in length and 0.5 $\mu$ in width and the round ones about 1 $\mu$ in diameter. The cristae of the mitochondria are usually well developed and run vertically across the long axis of the mitochondria. Intramitochondrial granules are recognized only in a few of the small, round mitochondria (Fig. 2). Frequently, several mitochondria take a rosette form by attaching themselves closely to an electron-opaque substance. The core is usually polygonal in shape and below 1 $\mu$ in dimension, and has no limiting membrane. Electron-opaque masses similar in nature to the core substance and variable in size and shape, are commonly encountered in the juxtanuclear region (Figs. 1 and 2). The endoplasmic reticulum develops moderately and their profiles appear as round or oval vacuoles of about 0.1 $\mu$ in diameter or slender tubules with inflated cisternae (Fig. 5). The smooth form of the endoplasmic reticulum is common, but the rough form is also discernible. The organelle is distributed widely throughout the cytoplasm

* General descriptions of the stage may be found in the paper of Yamamoto and Yamazaki (1961)

and occasionally it is contiguous with the nuclear, cellular and mitochondrial membranes. Golgi complexes are rarely encountered in the cytoplasm. They consist of flattened sacs with small terminal vacuoles and many vesicles. The flattened sacs, three or four in number, about 1 $\mu$ in length and 40 m$\mu$ in width, are arranged parallelly. The vesicles show a dimension of about 75m$\mu$ in diameter and flock together at the ends of the flattened sacs. Golgi vacuoles are not found in this region (Fig. 3). Numerous ribosomes are uniformly distributed in the ground matrix of the cytoplasm, but sometimes they are studded closely to the endoplasmic reticulum.

In this stage, the follicular epithelial cells are small in size and rather cuboidal in shape, and they are situated nearly all around the oocyte except that part directly in contact with another oocyte, but not arranged regularly in a single layer yet. The surfaces of both the oocyte and follicular epithelial cells are generally smooth and run parallel to a narrow intercellular space. No microvilli are still recognized on the surface of the oocyte. At intervals the apposing membranes of oocytes and follicle cells show desmosome-like thickening. The cytoplasm of the follicular epithelial cells is full of agranular endoplasmic reticulum. The profiles of the endoplasmic reticulum appear either in the form of long, slender tubules or small, round vacuoles. Occasionally the infolding of the cell membrane is seen in the follicular epithelial cells. Mitochondria, round or oval in shape, small in size and very few in number, are also encountered in the cytoplasm. Their cristae are few in number and show a somewhat irregular arrangement. Surrounding a group of oocytes and follicular epithelial cells there is found a basement membrane which is of moderate electron density and about 200 m$\mu$ in thickness. In this stage it is difficult to find well-defined theca cells.

**II. Late phase of the chromatin-nucleolus stage** (20–30 $\mu$ in diameter) (Pls. III to V)

The nucleus, round or oval in shape, still holds a relatively large volume to the cytoplasm. The nuclear envelope composed of double membranes becomes smooth and the two membranes run almost parallel to a narrow interspace. The small outpocketing of the outer membrane is rarely found. The nucleoplasm is granular and of moderate density. The nucleoli, large in size and few in number, retain the network form. Just inside and outside of the nuclear envelope, there are found small clusters of fine, dense granules. The intranuclear clusters are indefinite in form and loosely aggregated, while the extranuclear ones are usually thread-like in form and densely aggregated. The fine, dense granules are also found deposited within the nuclear envelope, and appear as fine threads across the nuclear envelope (Fig. 13).

As the cytoplasm increases in volume, mitochondria, electron-opaque masses, endoplasmic reticulum and Golgi complexes take positions in the inner part of the cytoplasm, and the outer part is mainly filled with ribosomes distributed densely

and homogenously (Fig. 13).

During this stage mitochondria increase slightly in number and become numerous simultaneously with a marked change in shape and size. The mitochondria are generally round to oval or rod-like in shape, but almost all are more or less deformed. They decrease clearly in dimension, even large rod-shaped ones measuring about 1.3 $\mu$ in length and 0.4 $\mu$ in width. Moreover, in the majority the mitochondrial cristae are sometimes curved concentrically. There are found some mitochondria which are elongated and have a constricted part in the middle region (Figs. 7, 8 and 9), dumb-bell shaped ones whose outer membranes are constricted at the part where the circular inner cristae appear to transverse the organelle (Figs. 7, 10 and 11), and those which have the obscure limiting membranes at one end as if they have just finished division (Figs. 11 and 12). These figures suggest that mitochodria increase in number by their division. The changes in size and shape of the mitochondria may, perhaps, be attributed to the multiplication of mitochondria.

Mitochondrial rosettes become more numerous and are situated mostly in the juxtanuclear region. The features of the mitochondrial rosettes are about the same as in younger oocytes. The core shows a granular appearance. In highly magnified figures (Figs. 14a and 14b), however, it can be seen that the core as well as the nucleoli is also composed of both minute fibrous units and an amorphous substance. The fibrous unit measures about 75 Å in thickness and has a light central part about 45 Å in width. The arrangement of the fibrous unit is so complicated that the profiles of the units appear as a single fibre, fibrous bundles or vesicle clusters in a variety of sizes. The amorphous substance is of low density and occupies the space between the fibrous units. The electron-opaque masses without attached mitochondria increase also in number and are located in the juxtanuclear region. They vary widely in shape; thread-lik, semi-circle, polygonal, and also in size, ranging from 0.1 to 1 $\mu$ in dimension. The development of the endoplasmic reticulum is about the same as in the last stage. The profiles of the endoplasmic reticulum, slender tubules or round to oval vacuoles, are found sparsely in the whole cytoplasm. In most cases the endoplasmic reticulum has many attached ribosomes and takes the rough form (Fig. 6). In the advanced oocyte of this stage, the profiles of the endoplasmic reticulum appear in the inner part of the cytoplasm as long and narrow tubules concentric to the nuclear envelope. Between the narrow tubules, many mitochondria, mostly round and small, are found embedded making a regular row (Fig. 13). At about the central part of the cytoplasm there is found a large, round body of high density. It measures about 3 $\mu$ in diameter, shows a densely packed network structure, and has no limiting membrane (Fig. 13). High magnification of the body shows that the body is also composed of a certain fibrous unit structure with a light centre, the entire unit measuring about 90 Å in thickness

and the light centre some 50 Å in width. And the spaces between the fibrous units are filled with an amorphous substance of low electron density. The arrangement of the fibrous units appears to be complicated and this makes it difficult to identify the unit structure clearly (Fig. 15). The body rather resembles the core substance in appearance and structure, but the former differs clearly from the latter in the larger size of the fibrous unit. From the shape, size and position, this body is interpreted as yolk nucleus.

The follicular epithelial cells become somewhat elongated and have almost completely enclosed the oocyte by the late phase of this stage, but they are not arranged regularly in a row. They have the flattened nucleus and the cytoplasm is filled with well developed endoplasmic reticulum and several, small mitochondria. The relation between the follicular epithelial cells and the oocyte is almost the same as in the younger oocytes. The surfaces of both the oocyte and follicular epithelial cells are smooth and run parallel to a narrow intercellular space. Neither the oocyte nor follicular epithelial cells have microprojections on their surfaces. A basement membrane, 200–300 mμ in thickness, surrounds a group of both the oocyte and follicular epithelial cells closely. External to the basement membrane, several non-germ cells may be found, but they cannot yet be defined as theca cells.

**III. Early phase of the peri-nucleolus stage** (30–50 μ in diameter) (Pls. VI to IX)

With the growth of the oocytes, the volume of the nucleus in relation to the cytoplasm becomes small and the development of cytoplasmic organelles becomes more conspicuous.

In the oocyte of this stage the nucleus is about the same as in the last stage. It is round in shape, smooth in contour and contains a few large nucleoli showing a network pattern. The small clusters of fine granules are numerous just inside and outside of the nuclear envelope.

In order to clarify the relationship between the clusters seen in both sides of the nuclear envelope some detailed observations have been done on the nuclear envelope. The nuclear envelope is composed of two membranes and an interspace. In the transverse sections the inner membrane of the envelope runs rather smoothly, while the outer membrane is somewhat wavy. The two membranes join each other at intervals to form both pores and perinuclear spaces respectively. The cylindrical structure of the pores is suggestive but not clear. Sometimes figures are found suggestive of the nucleo-cytoplasmic exchange, in which a short and fine electron-opaque substance of thread form is seen passing through the pore from the nucleoplasm to the cytoplasm (Fig. 20). A close inspection reveals a vesicular structure of the thread-like substance, the vesicle being approximate 200 Å in diameter. Not only such vesicles are seen near the inside and outside of the nuclear envelope, but also the vesicular threads are further in continuity with the

electron-opaque mass in the cytoplasm. Therefore, it is most probable that the minute vesicles within the nucleoplasm flow out into the cytoplasm through the pore and coalesce to form large electron-opaque masses. In tangential sections many annuli are found regularly arranged. The individual annulus is approximate 1500 Å in outer diameter and 1000 Å in inner diameter, and the ring wall is composed of small particles. In about the central area of the annulus there is frequently found a small granule which seems to be composed of fine particles and which show somewhat an uneven contour. Occasionally, the central granule appears as a small ring, composed of very minute particles and suggesting the profile cut a vesicle, the ring being measured about 200 Å in diameter (Fig. 21).

Mitochondria increase in number steadily, thus the inner half of the cytoplasm becomes densely filled with mitochondria. The size and shape of the mitochondria are about the same as in the last stage. In addition to the mitochondria seemingly in the process of division and just after division, there are found many mitochondria which show a regular, round or rod-lik shape and have straight and parallel cristae. The formation of mitochondrial rosettes is a common feature of this stage oocyte. The rosettes are mostly situated in the perinuclear region and they vary in the size of cores and the number of attached mitochondria. Occasionally primitive appearing mitochondria whose cristae develop poorly, whose limiting membranes seem incomplete, and whose matrix appears to have direct communication with the core substance are found in the rosettes (Figs. 17 and 18). Besides these primitive appearing mitochondria, there are found many large ones whose limiting membranes against the core are obscure and whose matrix shows in part the same electron density as that of the core substance. These findings suggest that the formation of rosettes may be related to taking of the core substance of nuclear origin into the mitochondria, that is nucleo-mitochondrial exchange.

The endoplasmic reticulum forms parallel loops embracing mitochondria between them. Many Golgi complexes are found located mostly in the peri-nuclear region. Now, the Golgi complex consists of three components, i.e., flattened sacs, vesicles and vacuoles. In some Golgi zones flattened sacs develop well, and three or four sacs, about 1 $\mu$ in length, are arranged in parallel. Many vacuoles, usually round to oval, sometimes polygonal and variable in size from 100 to 500 m$\mu$ in diameter, are situated in the vicinity of the sacs. They are mostly clear and empty, occasionally they are moderate in density. The direct communication between the end of the sac and the nearest vacuole suggests that the vacuoles may be derived from the terminal vacuoles of the flattend sacs by their swelling and separation. A fair number of Golgi vesicles, 70 m$\mu$ in diameter and moderate in density, are also seen in the zones (Fig. 19). In addition to such Golgi zones, there are found many Golgi complexes with poorly developed sacs. The greater part of the zone is occupied

with numerous vacuoles of various sizes and shapes and a fair number of vesicles. The sacs located are only one or two in number and small in length (Fig. 19). Besides the sacs there are found some elongated vesicles arranged in a row, as if they are in the way of a new formation of the sac. These Golgi complexes are interpreted as the primitive and developing ones.

Besides these organelles there are found 'multivesicular bodies'. They are few in number and round in shape, scattered at random in the cytoplasm. The bodies vary in size, from 2 to 1 $\mu$ in diameter, and consist of many vesicles below 80 m$\mu$ in diameter and are surrounded with a limiting membrane. Near the central part of this body, a structure composed of a few rods of high density, about 300 to 100 m$\mu$ in length and 90 to 40 m$\mu$ in width, is located (Figs. 25 and 26).

Follicular epithelial cells are arranged regularly in a row and enclose the oocyte completely. In general, the surfaces of both the oocyte and follicle cells are smooth and run almost in parallel with a very narrow interspace. However, in some places ovular microvilli can be seen. They are short and slender, few in number and stick in the follicular epithelial cells. The follicular epithelial cells are flat and contain a flattened nucleus. The cytoplasm of the cells is occupied with mitochondria, Golgi complexes, well developed endoplasmic reticulum and ribosomes. Mitochondria are round or oval in shape, comparatively large in size and few in number. Golgi complexes are found here and there in the cytoplasm. They consist of flattened sacs and vesicles, but Golgi vacuoles are obscure. The endoplasmic reticulum develops well and occupies the greater part of the cytoplasm. The profiles of the endoplasmic reticulum are seen as long, slender tubules or small, round vacuoles. Usually the ribosomes attach closely to the outside of their membranes, showing a rough form. Besides these organelles, multivesicular bodies of small size are also recognized in the cytoplasm.

A thick basement membrane lies closely on the follicular epithelial cells. The membrane is about 300 m$\mu$ in thickness and shows a pattern of light and dark stripes. Theca cells are also seen outside the basement membrane. They are very flat and usually stand in a row. The cytoplasm of theca cells is embedded with a poorely developed endoplasmic reticulum (Fig. 27).

**IV. Middle phase of the peri-nucleolus stage** (60–70 $\mu$ in diameter) (Pls. X to XII)

In the oocyte of this stage the cytoplasmic organelles develop markedly and they are distributed widely throughout the whole cytoplasm. The nucleus shows about the same features as in the last stage. It takes a round shape and generally shows a smooth contour. The masses of electron-opaque substance are numerous in the juxtanuclear cytoplasm (Figs. 28 and 29).

Mitochondria increase greatly in number, but no marked changed in form and size is recognized in this organelle as compared with that in the last stage. The

formation of mitochondrial rosettes and the juxtaposition of mitochondria to the developed endoplasmic reticulum are also characteristic features of the oocyte of this stage (Figs. 28 to 30).

The endoplasmic reticulum develops further, but it shows about the same profiles as in the last stage oocyte. Here and there the endoplasmic reticulum forms parallel loops and embraces small mitochondria among them (Figs. 29 and 30).

Golgi complexes increase in number. Some of them are similar in form as those seen in the last stage, which consist of a few flattened sacs, a lot of vesicles and vacuoles. Sometimes the vacuoles are polygonal or elongated and are suggestive of sections of short broad channels. Others almost take the form of typical Golgi complex (Figs. 28 and 33).

A certain lamellar structure is encountered in the cytoplasm of some oocytes. This structure is just fit for the 'annulate lamellae' named by Swift (1956). Usually many lamellae, 80–100 m$\mu$ apart, run in parallel and make a peculiar structure, but a single, isolated lamella is encountered occasionally in the juxtanuclear region. An individual annulate lamella consists of two parallel membranes which contain a series of pores or annuli. In this respect this structure is identical with that of the nuclear envelope. In transverse sections, it can be observed that the paired membranes of each lamella join with each other at intervals to form the pores (Fig. 34). In a surface view, each of the pores in the annulate lamellae is seen to be surrounded by an annulus. The outer diameter of the annulus measures approximately 1500 Å and is similar to that of the nuclear envelope (Fig. 35). Vacuoles are situated near the end of several lamellae as seen in *Nectrus* (Kessel, 1963). In some cases, however, annulate lamellae are found, which connect directly with the parallel membranes of the typical endoplasmic reticulum (Fig. 36).

Another characteristic feature found in some oocytes of this stage is 'cytoplasmic segregation'. In the cytoplasm are found one or two bodies about 2 $\mu$ in dimension, which are composed of mitochondria and many vesicles, with an obscure limiting membrane. The mitochondria are small in size and round or oval in form, usually they are changed more or less in shape to polygon and in their inner structure to irregular and obscure cristae. Further their limiting membranes seem to be fused in most places to show a thick membrane. The vesicles, below 100 m$\mu$ in diameter, flock together around the mitochondria and form a spherical body (Fig. 31). In place of the cytoplasm there is found another body composed also of mitochondria and vesicles. It shows a deformed dumb-bell form and has a clear limiting membrane. The mitochondria become somewhat smaller and are more changed in structure than the previous ones. Their double-membraned wall is barely recognizable, their cristae almost disappear and their matrix becomes dark. Some vesicles

present in the body become large and measure about 200 m$\mu$. They consist of a core of moderate density and a surrounding membrane. Besides the mitochondria and vesicles, vacuoles of various sizes can be seen within the body. They are almost spherical but occasionally polygonal, clear and almost empty, the large one being about 1 $\mu$ in dimension (Fig. 32).

The interrelation between the oocyte and the follicular epithelial cells is about the same as in the last stage. The surfaces of both the oocyte and follicular epithelial cells are almost smooth and only a few ovular microvilli are recognizable. No marked changes are recognized in the follicular epithelial cells, basement membrane and theca cells.

**V. Late phase of the peri-nucleolus stage** (80–120 $\mu$ in diameter) (Pls. XIII to XVII)

In this stage the cytoplasm increases markedly in volume and the cytoplasmic organelles are distributed here and there forming large clusters. Large vacuoles, which seem to be the forerunner of yolk vesicles, begin to appear in the cytoplasm.

The nucleus decreases in relative volume to the cytoplasm, but still holds a round shape and smooth contour. The nuclear matrix becomes light due to the decrease in the number of distributed granules (Fig. 37). Nucleoli are found situated in the peripheral region of the nucleus. They are usually round or oval, sometimes dumb-bell shaped or polygonal and vary in size. Frequently two distinct morphological aspects are recognized in the nucleoli; one is made of fine granules densely packed and shows the homogeneous mass, while the other is made of fine granules loosely packed and shows a network form. High magnification of the two parts shows that both parts are also composed of about the same fibrous unit and an amorphous substance as in the young oocyte, and the difference in the appearance of the two parts seems to be attributed to the arrangement of the fibrous units and the amount of amorphous material (Figs. 22 to 24). The nuclear envelope runs rather smoothly, occasionally, small outpockets made of the outer nuclear membrane can be found. The mitochondrial rosettes and electron-opaque masses are encountered less frequently in the cytoplasm than the last stage.

Large clusters of mitochondria and Golgi complexes are found distributed here and there in the cytoplasm. The mitochondria are about the same in shape as those in the last stage, but they become elongated and large filamentous ones being 3.5 $\mu$ in length. The cristae develop well in some mitochondria, but poorly in others. Mitochondria seemingly in dividing or just after division are also recognizable. The intramitochondrial granules are always located within the mitochondria (Figs. 42 and 43).

Golgi complexes develop well and they consist of three components; flattened sac, vesicle and vacuole (Figs. 38 to 40). Now, Golgi vacuoles vary widely in

dimension, from 0.3 to 2 $\mu$. The larger the vacuoles are in size, the less the vacuoles present in the region are in number. This suggests that several small vacuoles become fused and mature as large ones. Small vacuoles are generally of low density and clear, while large ones include a substance of moderate density, which is deposited in one side or the periphery of the vacuole. Large vacuoles like this are found situated not only in the mitochondrion-Golgi complex region, but also in the peripheral region of the cytoplasm. Careful observations of the serial sections reveal that Golgi vesicles and flattened sacs are almost always located near the vacuole (Fig. 40). Judging from size, form, position and contents, it is most probable that the vacuole may be a precursor of yolk vesicles. Sometimes, very large Golgi complexes are found, about 3 $\mu$ in length, which have a break near the centre. In the Golgi complex shown in Figure 41, some of the flattened sacs have already been bisected and a few Golgi vesicles are located in the break area, while other sacs still remain as very long sacs.

Several small, dense bodies of about 0.5 $\mu$ were found in the periphery of some oocytes. They have no limiting membrane and look like small lipid droplets (Fig. 43). The matrix of the cytoplasm is very fine in texture and fully embedded with ribosomes.

In addition to these cytoplasmic organelles, the annulate lamellae described above are encountered in the cytoplasm of some oocytes (Fig. 44).

In this stage the surfaces of both the oocyte and follicular epithelial cells no longer run in smooth and in parallel. Between the two cells a clear interspace of various width is recognizable, measuring 0.5 $\mu$ at the widest part. A lot of ovular microvilli protrude into the interspace as tubular microprojections. They are usually curved irregularly and the large ones have a dimension of 1 $\mu$ in length and 0.25 $\mu$ in width. Follicular epithelial cells also produce microvilli which are smaller in number and size to the ovular microvilli. The ovular microvilli frequently are in close contact with the follicular microvilli, but no direct connection between the cytoplasm of the two cells has ever been observed. The so-called 'pinocytotic vesicle' is found in large numbers near the surface of the oocyte. The vesicles are below 100 m$\mu$ in diameter and moderate in density (Figs. 45 and 46). As shown in Figure 47, many ovular microvilli are found which have caveolae on their surface and contain many vesicles, while the caveolae or the vesicles situated just on the surface of the cytoplasm between the microvilli are few in number. These facts suggest that the microvilli are the main site where pinocytosis is taking place. The follicular epithelial cells increase in height somewhat but they are still flat, as a whole. The cytoplasm is usually filled with well developed endoplasmic reticulum showing long and slender profiles or round to oval ones studded with ribosomes. Mitochondria are comparatively few in number, but some are large in

size, about 1.5 $\mu$ in length and 1.0 $\mu$ in width. The cristae of the mitochondria develop well and run almost in parallel.

The follicular epithelial cells are covered throughly with a thick basement membrane, ranging from about 0.3 to 0.5 $\mu$ in thickness. Outside the basement membrane the theca cells of flat form are closely located. They generally stand in a row and have a flattened nucleus and the cytoplasm which contains a few small mitochondria, poorly developed endoplasmic reticulum, a small amount of vesicles, free ribosomes, and a trace of collagen fibers.

The boundary of theca cells runs smoothly, usually with a very narrow interspace, sometimes with a wide one. Here and there desmosomes are found between the cell membranes of neighbouring cells.

Blood capillaries are found on all oocytes. They are situated between theca cells, and the endothelial cells of the blood capillary are directly in contact with the basement membrane. Some capillaries are very fine and a little higher than the theca cells (Fig. 37), while others are comparatively thick and protruded strikingly beyond the theca cells (Fig. 48). Some kind of blood cells are usually seen among the capillaries.

## DISCUSSION

The growth and development of the goldfish oocyte during the first growth phase take place accompanying two important events, i.e., differentiation of the cytoplasmic organelles of the oocyte and the establishment of close relationship between the oocyte and the follicle. The following discussion, therefore, will be performed by focusing mainly upon these two events.

### 1. Nucleus

During this growth phase, no marked change in the form of the nucleus can be found except the gradual decrease in relative volume to the cytoplasm as already known by the previous work (Yamamoto and Yamazaki, 1961). The nucleoplasm shows a relatively low electron density and is sparsely embedded with numerous minute particles. Takamoto (1964) who studied the oogenesis of *Triturus*, demonstrated the chromosomal strands of 100 to 200 Å in thickness, which are studded with RNA particles approximately 300 to 400 Å in dimension. The present study also reveals small groups of particles showing a comparatively large size, which are found here and there in the nucleoplasm, but this work could not make clear their detailed structure.

Nucleoli are found in all oocytes throughout this growth phase and they increase in number according to the growth of the oocytes. Nucleoli in young oocytes are network-like masses and have no limiting membrane as in *Oryzias*

(M. Yamamoto, 1964). As already demonstrated in amphibian oocyte nucleoli (Brown and Ris, 1959; Takamoto, 1964), they are composed of a fibrous unit, approximate 80 Å in thickness, which seem to be arranged in bundles or in some rows and the fibrous unit contains a hollow centre of about 60 Å. The space between the fibrous units is occupied with an amorphous material of low density. Thus, the nucleoli of the goldfish are similar in fine structure as of the salamander oocytes, although the dimension of the fibrous unit differs somewhat in the two species.

A marked change in nucleoli is seen in oocytes larger than 80 $\mu$ in diameter. The nucleoli show two distinct morphological parts, one relatively dense and the other less compact. A close inspection of the nucleoli, however, shows that the two parts also consist of both the fibrous unit and the amorphous material which appear to be similar as those seen in younger oocytes, and the difference in the appearance of the two parts seems to depend on the arrangement of the fibrous units and the amount of the amorphous material.

In the oocyte of two amphibian species, Miller (1962) found the presence of nucleoli which are composed of two distinct parts, the dense, fibrous part and a loose, granular one, which correspond strictly to the two parts of goldfish nucleoli and he demonstrated that these two parts contain both RNA and protein, and further that the loose, granular part may be the site where both synthesis and accumulation of RNA happens and where protein synthesized extranucleolarly is accumulated.

The fine structure of the nuclear envelope is essentially similar to that of other animal oocytes studied (Sea urchin oocyte; Afzelius, 1955; Urechis oocyte; Sugiyama *et al.* 1963; Amphibian oocyte; Gall, 1954, 1956; Merriam, 1961; Takamoto 1964; Teleost, M. Yamamoto, 1964 etc). The two membranes of the nuclear envelope join each other at intervals to form both pores and perinuclear spaces respectively. The pores seem to be cylindrical in structure as demonstrated by Afzelius (1955). The cylindrical structure of the pore in the present material is not demonstrated clearly in transverse sections, while in tangential sections it appears as annuli, approximate 1,000 Å in inner diameter. This dimension of annuli is about the same as that seen in *Oryzias* oocytes (M. Yamamoto, 1964) and near to that of the amphibian oocytes. At about the centre of the annuli a minute vesicle-like granule, about 200 Å in dimension, is found. The central granule or globule within the annulus has already been found in the amphibian oocyte (Pollister et al. 1954, Gall, 1954; Merriam, 1961, 1962; Takamoto, 1964 etc) and sea urchin oocyte (Afzelius, 1955). Merrian (1962) stated that the globules within the annuli stick to the pore diaphragm located at the waist of the pores. At present Merriam's hypothesis, however, remains unsolved as a future problem.

Electron microscopic evidence on the nucleo-cytoplasmic exchange has already

been obtained in the oocyte of a frog (Pollister *et al.*, 1954), *Rhodnius* (Anderson and Beams, 1956) *Rana* (Ornstein, 1956; Miller, 1962; Lanzavecchia, 1962), *Xenopus* (Balinsky and Devis, 1963), *Triturus* (Takamoto, 1964) and the bird (*Schjeide et al.* 1964). They observed a transfer of a fine granular material from the nucleus to the cytoplasm through the nuclear pores. In the present study the figure suggestive of the outflow of a nuclear substance through the pores is also observed frequently in oocytes throughout the first growth phase. The outflowing substance, however, is not granular in form, but vesicular. Vesicles like this may also be found near the inside and outside of the nuclear envelope. Schjeide *et al.* (1964) advanced the hypothesis that, a common mode of transfer of nuclear material to the cytoplasm through the nuclear pores which are not open holes is by disassociation of vesicles arriving at the pore site on the nuclear side into very minute vesicles and reemergence of small vesicles on the cytoplasmic side which reassemble into large vesicles. The findings obtained in the present study may be explained quite well by the hypothesis of Schjeide *et al.*

**2. Cytoplasmic organelles**

*Mitochondria*

The rapid multiplication of the mitochondria is one of the most important events seen in the goldfish oocyte of the first growth phase. In this respect the present findings agree well with those obtained in other teleostean oocytes under a light microscope (Hibbard and Parat, 1928; Chaudhry, 1952; Ihnuma and Minamitani, 1952 etc), and an electron microscope (M. Yamamoto, 1964). The transitional changes of the topography and form of mitochondria along with the growth of the oocyte, however, are rather different between the present findings and those reported hitherto. Under a light microscope Ihnuma and Minamitani (1952) perceived the presence of two kinds of mitochondria, granular and thread-like, in the young oocyte of *Deiognathus*. However, the appearance and distribution of the two kinds of mitochondria in *Deiognathus* differ markedly from those obtained in the present study. M. Yamamoto (1964) studied the oocyte of the teleost, *Oryzias latipes*, by means of an electron microscope. According to him, in contrast to the goldfish oocyte in which both round to oval and elongated rod-shaped mitochondria can be revealed even in the youngest one, only round to oval mitochondria are present in the youngest oocyte of *Oryzias* and elongated rod-shaped or filamentous ones begin to appear when the oocyte grows up to 50 $\mu$. The distribution of mitochondria is also somewhat different in the two species. In the small oocytes of *Oryzias* mitochondria are mainly found in the peripheral region of the cytoplasm, while in the small oocyte of goldfish mitochondria are located mainly in the inner half of the cytoplasm.

As for the method of multiplication of mitocondria, Blanchette (1961) who studied

the rabbit oocyte and M. Yamamoto (1964) who studied the *Oryzias* oocyte surmised that the multiplication of mitochondria may be carried out mainly by their division. This conclusion is based on the occurrence of dumb-bell shaped mitochondria whose outer membranes are constricting at the point where the circular inner cristae appear to transverse the organelle. In the oocyte of the goldfish the mitochondria showing a similar feature and those with obscure limiting membranes at one end are encountered frequently at the time when mitochondria actively increase in number. Further, the decrease in size and the change in crista arrangement, which take place during the period of mitochondrial multiplication, seem to be powerful evidence favorable for the occurrence of the active division of mitochondria. The change in the internal organization of mitochondria during the period of mitochondrial multiplication has also been recognized in some mammalian oocytes (Yamada *et al.*, 1957; Anderson and Beams, 1960; Odor, 1960; Blanchette, 1961). Some workers (M. Yamamoto, 1964) regarded the change in crista arrangement as the reflexion of a physiological change in mitochondria. As mentioned above, however, it is more reliable to assume that the cristae may be changed in arrangement due to the mechanical pressure at the time of division. Thus, the present writers arrive at the conclusion similar to Blanchette's, that the multiplication of mitochondria may be carried out mainly by the division of pre-existing mitochondria.

The formation of mitochondrial rosettes is a characteristic feature seen in the goldfish oocyte of the first growth phase. The occurrence of mitochondrial rosettes has already been demonstrated in frog oocytes by Miller (1962) and Lanzavecchia (1962) Balinsky and Devis (1963), in the teleost oocyte by M. Yamamoto (1964) and in guinea pig oocytes by Adams and Hertig (1964). Balinsky and Devis (1963) and Adams and Hertig (1964) maintained that the mitochondrial rosettes appear to concern with proliferation of mitochondria. The basis of their conclusion lies in the fact that, 1) the formation of rosettes begins to start at the time when mitochondria are beginning to increase in number, 2) the mitochondrial rosettes are found even at a later stage of oogenesis, 3) the core is closely associated with primitive appearing mitochondria whose peripheral membrane or cristae look incomplete. The similar correlation between the occurrence of mitochondrial rosettes and the proliferation of mitochondria is recognized in the present study. Further, the presence of such mitochondria whose limiting membranes against the core look incomplete and whose matrix appears to have a direct communication with the core substance leads the present authors to the supposition that the formation of the mitochondrial rosette may be related to the proliferation of mitochondria due to taking core substance into mitochondria.

As for the core substance, Ornstein (1956), Lanzavecchia (1962), Miller (1962) and Balinsky and Davis (1963) insisted that the substance may be nuclear in

origin. They observed a fine granular material passing through nuclear pores into the cytoplasm, which afterward condenses into perinuclear masses and then associates with the mitochondria. On the contrary, M. Yamamoto (1964) and Adams and Hertig (1964) had doubts about this opinion, because they could not find the figures suggestive of the above facts. As mentioned above, the figures suggestive of the outflow of nuclear material are also observed commonly in the present material. Futher, the coalescence of the nuclear material to large electron-opaque masses situated in the perinuclear cytoplasm and disposing of mitochondria around the electron-opaque masses are also evident from the present findings. These facts support the nuclear origin of the core substance. As already demonstrated in *Xenopus* (Balinsky and Davis 1963), the core substance of the goldfish is also composed of the fibrous unit structure containing the light centre, which measures about 75 Å in entire thickness. The core substance, therefore, differs in structure from the substances flowing from the nucleus to the cytoplasm, which is vesicular in structure, and this suggests that there occurs some change in the nuclear vesicles at coalescence. Lanzavecchia (1962) and Miller (1962) postulated that the fine particles which flow out into the cytoplasm may be RNP in nature.

*Golgi complex*

According to M. Yamamoto (1964) the Golgi complex of *Oryzias* is seen as a cluster of a single type of small spherical vesicles until the oocyte grows up to 130 $\mu$ in diameter. In the present species, however, the Golgi complex which is clearly composed of flattened sacs and vesicles may be found even in the youngest oocyte below 20 $\mu$ in diameter. When the oocyte grows up to about 30 $\mu$, the Golgi complex not only increases in number, but also changes markedly in form. Besides flattened sacs and vesicles, Golgi vacuoles, various in number and shape, appear in this region. Adams and Hertig (1964) found a large concentration of smooth-membraned vesicles and short broad channels localized in the juxtanuclear region of the primordial oocyte of the guinea pig and interpreted it as an early stage of Golgi complex. Some of the Golgi complexes found in the goldfish oocyte apparently resemble this type of Golgi complex in guinea pig oocytes. In goldfish oocytes above 80 $\mu$ in diameter, the Golgi complexes increase in number and they, together with many mitochondria, make large clusters which are distributed here and there throughout the cytoplasm. The Golgi complex consists of three components as commonly known in somatic cells (Dalton, 1961), i.e. well developed flattened sacs, many vesicles and a few large vacuoles. In the oocyte of *Oryzias* (M. Yamamoto, 1964) and the guinea pig (Adams and Hertig, 1964) the Golgi complexes are composed of only flattened sacs and vesicles, Golgi vacuoles being totally lacking, while in the oocyte of the sea urchin (Afzelius, 1956), mouse (Yamada *et al.*, 1957), rat (Odor, 1960) and *Triturus* (Takamoto, 1964), the Golgi complexes consist of the three

components. In goldfish oocytes Golgi vacuoles are always present in the region of well developed Golgi complexes. They are usually spherical, occasionally polygonal, 0.3 to 2 $\mu$ in diameter, one to several in number. Small vacuoles are clear and empty, while large ones contain a substance of moderate density.

Golgi vacuoles of the growing sea urchin oocytes contain a granular component, and further most morphological stages from granular vacuoles to yolk granules can be found. Based on these facts, Afzelius (1956) surmised that yolk granules may originate in the Golgi complex. Balinsky and Davis (1963) also observed that one of the vesicles in the interior of the Golgi body is enlarged and it becomes to show the flasky structure which is characteristic of mature cortical granules, and they concluded that the cortical granules are actually produced by the Golgi body. About the same events are also recognized in the present study, that is, the coalescence of several small vacuoles into a large one, the appearance of a moderately dense substance within the Golgi vacuole. Further the large Golgi vacuoles show the features suggestive of a precursor of yolk vesicles which are mucoprotein in nature (cf. K. Yamamoto, 1958). Thus it is highly probable that the Golgi complex of the goldfish oocyte plays an important role in the formation of the yolk vesicle. Yasuzumi and Tanaka (1957) emphasized an important role of Golgi complexes at the time of yolk formation of a pond snail, and Kessel (1964) also the role of Golgi vesicles in the formation of proteineous yolk in tunicate oocytes.

Concerning the multiplication of the Golgi complex, Afzelius (1956) stated that the Golgi complex (dictyosome) in the sea urchin oocyte appears to increase in number by breaking into two at it's central part. In the present material the Golgi complexes which seemingly are in process of bisection or just after bisection are frequently encountered in the oocyte more than 80 micra in diameter (Fig. 39). So, the bisection of the well developed Golgi complex is supposed to be the mode of multiplication of the organelle in such a stage of the oocyte. On the contrary, the proliferation of the Golgi complex in younger oocytes seems to be attributed to the development of the primitive organelle, because the figures suggestive of the bisection of Golgi complexes are rarely found in younger oocytes. Hirsch (1963) advocated the following theory concerning the development of the Golgi complex (Lamellar-vacuolar-field), i.e. the X bodies budded off from the endoplasmic reticulum stretch themselves and arrange themselves in parallel, thus they give rise to new sacs. Small vacuoles at the end of the sacs are enlarged and separated and become Golgi vacuoles.

Judging from the findings described above, Hirsch's theory appears to be fit for the development of Golgi apparatus in the goldfish oocyte, though the authors could not get a definite idea concerning the origin of the vesicle corresponding to the X body.

*Endoplasmic reticulum*

The endoplasmic reticulum in the young oocytes of the goldfish as well as the frog (Kemp, 1956) and guinea pig (Adams and Hertig 1964) varies in form with the growth of oocytes. In the smallest oocyte the profiles of the endoplasmic reticulum appear as round to oval vacuoles or slender tubules with small, terminal inflated cisternae, which are distributed widely throughout the cytoplasm and are occasionally contiguous with the nuclear, cellular and mitochondrial membranes. Adams and Hertig (1964) observed a similar type of endoplasmic reticulum present in the simplest oocyte of the guinea pig, and they mentioned that the endoplasmic reticulum represents the major mechanism for the intake of materials required for a 'Maintenance metabolism'. In larger oocytes in which mitochondria are actively proliferating, the endoplasmic reticulum appears in the inner part of the cytoplasm as long, narrow and parallel tubules arranged in several rows concentric to the nuclear envelope. Between the tubules mitochondria are embedded making a regular row. Adams and Hertig (1964) observed the localized concentration of narrow cisternae of ergastoplasm associated with small vesicles and mitochondria, and regarded this structure as analogous to the basophilic element of the yolk nucleus described in the molluscan oocyte by Rebhun (1956) and to the cortex of the vitelline body of some spider oocytes described by Sotelo and Turujillo-Cenóz (1957). The yolk nucleus of fish oocytes usually is found located in the peri-nuclear region during the stage which shows 50 $\mu$ in diameter more or less (Yamamoto and Yamazaki, 1961; Dutt, 1964; Nayyer, 1964), and Nayyer (1964) demonstrated that the yolk nucleus of *Heteropneutes* contains mitochondria and lipid globules. Although the concentrated endoplasmic reticulum associated with mitochondria seemingly corresponds to the yolk nucleus described by Nayyer (1964) in the respect to the juxtanuclear position, crescent shape and the presence of mitochondria, the authors hesitate to interprete the structure as yolk nucleus, because many regions, as such, may be found here and there in the cytoplasm of larger oocytes of the present species.

In goldfish oocytes more than 80 $\mu$ in diameter, a well developed endoplasmic reticulum is found mainly in the region where mitochondria and Golgi complexes are flocked together and make a large cluster, and it appears to operate in the transport of substances necessary for the function and development of these organelles.

*Annulate lamella*

The annulate lamella has already been demonstrated in a variety of animal oocytes i.e., several genera of sea urchin (Afzelius, 1955), the snail, *Otala* and the clam, *Spisula* (Swift, 1956), the sea clam, *Spisula solidissinia* and the pulmonate snail, *Otala lactea* (Rebhun, 1956), the sand doller, *Dendraster excentricus* (Merriam,

1959), *Drosophila melanogaster* (Okada and Waddington, 1959; Waddington and Okada, 1960), the teleost, *Barbus conchonius* (Zahnd and Porte, 1963) the amphibian, *Necturus maculosus* (Kessel, 1963) and several genera of tunicates (Kessel, 1965). The 'system of pitted membranes' described by Balinsky and Davis (1963) in *Xenopus* appears also to correspond to the annulate lamellae observed in above species. The annulate lamellae found in goldfish oocytes closely resemble those described in the above species. They consist of two parallel membranes containing a series of annuli.

On the origin of this structure some workers (Afzelius, 1955; Swift, 1956; Rehbun, 1956) advanced the possibility that annulate lamellae may be formed by a delamination of the nuclear membrane of the oocyte or by another process. Kessel (1964, 1965) who studied in detail the annulate lamellae of amphibian's and Tunicate's oocytes arrived at the following conclusion: the vesicular elements made from the outer nuclear membrane of the oocyte arrange themselves as chains of vesicles which are located early beside the nuclear membrane and extend progressively into the surrounding cytoplasm, then the vesicles in a row may fuse, forming flattened cisternae, and finally an individual row of vesicles becomes more closely packed and exhibits the appearance of the annulate lamella. Okada and Waddington (1959) postulated the organization of the annulate lamellae from the material which contained fragments of the extensive nuclear envelope of the nurse cell. As mentioned above, however, the blebbing or delamination of the nuclear membrane was seldom encountered in the goldfish oocytes. Further, any favorable evidence supporting the opinion of Okada and Waddington (1959) could not be obtained in the present study. Whereas, the figures suggestive of the transformation of the endoplasmic reticulum into the annulate lamella are found occasionally in the present material. Thus, it seems rather reliable to regard the annulate lamellae found in the present material as a specialized type of endoplasmic reticulum.

Balinsky and Davis (1963) regarded the pitted membranes as equivalents of endoplasmic reticulum, and they surmised that the function of pitted membranes is to produce the masses of vesicular endoplasmic reticulum. Swift (1956) suggested that these lamellae may function in the transfer of genetic specificities from nucleus to the cytoplasm, Merriam (1959) stated that the necessary synthetic mechanism and specificity must reside in the structure of annulate lamellae, and Kessel (1965) gave the opinion that specific forces and activity may be selectively localized in the region of the annulate lamellae. On the contrary, Waddington and Okada (1960) observed the marked increase of double membranes and porous membranes in the cytoplasm of the degenerating *Drosophila* oocyte and such organization was considered not to represent the formation of functional organelles. The present authors observed also the abundant occurrence of annulate lamellae in degenerating oocytes of the goldfish. Thus, what functional roles lie in the annulate lamella

during the growth of oocytes is still obscure.

*Yolk nucleus*

The yolk nucleus is one of the noticeable organelles found in the cytoplasm of teleostean oocytes during the first growth phase. Many studies on the yolk nucleus of teleosts have been done by means of the routine cytological and cytochemical methods and good information on the organelle has been accumulated (cf. K. Yamamoto, 1958; Raven 1961; Dutt, 1964; Nayyer, 1964). However, M. Yamamoto's paper (1964) is the only one concerned with the fine structure of teleostean yolk nucleus. According to his observations, the yolk nucleus of *Oryzias latipes* shows a complicated network which is made from wavy threads approximate 100 m$\mu$ in width. Each thread is composed of electron-opaque particles about 100 Å in diameter embedded in an electron-opaque amorphous substance. And no limiting membrane is found around the organelle. Mitochondria, vesicles of endoplasmic reticula and occasionally Golgi complex are located within yolk nuclei. The structure of the yolk nucleus of *Oryzias*, therefore, is markedly different from that of any animal investigated hitherto (a calm by Rebhun, 1956; a frog by Kemp, 1956; a spider by André and Rouiller, 1957; two kinds of spiders by Sotelo and Cenóz, 1959; the guinea pig by Anderson and Beams, 1960; and Adams and Hertig, 1964; *Xenopus* by Balinsky and Davis, 1963; *Artemia* by Anteunis *et al.*, 1964).

The yolk nucleus of the goldfish is essentially identical with that of *Oryzias latipes*, but some differences are recognized between the two. The organelle of the goldfish is a large round body of 3 micra in diameter situated in about the the central region of the cytoplasm. It is composed of numerous fibrous units and an amorphous substance, and has no limiting membrane. The fibrous units is about 90 Å in thickness and contains a light centre of 50 Å in width. The units show a complicate arrangement and are embedded in the amorphous substance of low electron density. Any mitochondrion, an endoplasmic reticulum or Golgi complex was not found in this region in contrast to the yolk nucleus of *Oryzias*. As the present study could not make clear the structure of the yolk nucleus present in oocytes larger than 60 $\mu$ in diameter, therefore, detailed discussions remains for the future.

*Other cytoplasmic organelles*

Multivesicular bodies are rarely encountered in the cytoplasm of goldfish oocytes during the first growth phase. The bodies closely resemble in size and shape those described by M. Yamamoto (1964) in the mature oocytes of *Oryzias latipes*, except for the presence of a rod-like structure in the central part of the body. Sotelo and Porter (1959) found the multivesicular bodies distributed in the cytoplasm of the rat oocyte, relatively small in number in immature oocytes and numerous in full-grown ones. The multivesicular bodies of the rat are not only smaller in the

size of the whole unit and of contained vesicles than those of the goldfish, but also are different in the nucleoid which is located more or less at the centre and consits of densely packed vesicles. Sotelo and Porter (1959) postulated that the centrosphere masses originate from the accumulation of the vesicles freed by the disintegration and transformation of the multivesicular body, while M. Yamamoto (1964) regarded the multivesicular body of *Oryzias* as a kind of vitelline element.

The 'cytoplasmic segregation' is a phenomenon which has rarely been seen in oocytes of the first growth phase. At the early phase of this phenomenon mitochondria and many vesicles below 100 m$\mu$ seem to aggregate into a large spherical body with an obscure limiting membrane, and later the mitochondria appear to be deformed and changed into homogenous dark bodies, the vesicles become large, and vacuoles of various size appear in the region, simultaneously with the appearance of a clear limiting membrane. Markoff and Beutow (1964) reported a similar event which happens in the cytoplasm of *Euglena* at the time of starvation. According to their assumption this is an event related to the mechanisms supplying the energy necessary to the organism for their basal biochemical processes by sacrificing part of their cytoplasm. To clarify the significance of the 'cytoplasmic segregation', therefore, we look forward to a future experimental analysis.

**3. Relation between the oocyte and the follicle**

The formation of the follicle is one of the important events seen during this growth phase. Since Bühler's study (1902) was published, it has been well known that the follicle of teleosts consists of two distinct layers, i.e., an inner layer of follicular epithelial cells, which is composed of a single cell layer, and outer layer of theca cells, which usually consists of two layers of cells. Electron microscopic studies have demonstrated a basement membrane present between these two layers (Sterba and Müller, 1962; Müller and Sterba, 1963; M. Yamamoto, 1963; Jollie and Jollie, 1964). In young goldfish oocytes smaller than 30 $\mu$, follicular epithelial cells do not arrange themselves in a regular row, but they, together with one to three oocytes, form a group of cells, which take a somewhat irregular arrangement and appear to be surrounded with a basement membrane as a whole. No special relationship can be found between the oocyte and follicular epithelial cells and the apposing membranes of oocytes and follicular epithelial cells show a desmosome-like thickening in some places as commonly seen between neighboring cells.

When the oocyte grows up to about 45 $\mu$ in diameter, follicular epithelial cells appear as a single layer of cells, which completely surrounds each oocyte. Outside the follicular epithelial cells a thick basement membrane is located closely. Theca cells are arranged in nearly a single cell layer externally to this basement membrane. M. Yamamoto (1963) observed a similar composition of the follicle in *Oryzias* oocytes of about the same size as above. The surfaces of both the oocyte and

follicle cells are generally smooth and run almost in parallel with a narrow interspace. In some places, however, ovular microvilli, short and slender, can be seen. They stick into the cytoplasm of the follicle cells, but no direct connection between the cytoplasm of the oocyte and of follicle cells has ever been observed as revealed in *Cynolebias* by Müller and Sterba (1963), and in *Lebestis* by Jollie and Jollie (1964).

By the time the oocyte attains a diameter of 120 $\mu$, the interspace between the oocyte and follicular epithelial cells becomes wide and microvilli increase in number and size. Both the oocyte and follicular epithelial cells produce microvilli, and the microvilli of the follicular epithelial cells are smaller in size and less in number than the ovular microvilli, in contrast to the observations of M. Yamamoto (1963) and Jollie and Jollie (1964), who could find only the presence of the ovular microvilli. The presence of microvilli on the surface of oocytes has already been demonstrated in various kinds of teleosts; *Fundulus* (Kemp and Allen, 1956; Kemp and Hibbard, 1957), *Xiphophorus* and *Mollienisia* (Zahnd and Porte, 1962), *Lebestis* (Chambolle *et al.*, 1962; Jollie and Jollie, 1964), *Cynolebias* (Sterba and Müller, 1962; Müller and Sterba, 1963), *Brachidanio* (K. Yamamoto, 1962), *Oryzias* (M. Yamamoto, 1963), and also in many other vertebrates (Kemp, 1956; Yamada *et al.*, 1957; Sotelo and Porter, 1959; Press, 1959; Odor, 1960; Anderson and Beams, 1960; Waterberg and Stegner, 1960; Merker, 1961; Chiquoine, 1960; Adams and Hertig, 1964; Takamoto, 1964 etc.). The microvilli are cosidered to increase the absorptive or secretive surface (Kemp, 1956). And the mechanism for active transport of a nutrient into the oocyte is emphasized as depending on micropinocytosis (Anderson and Beams, 1960; Kessel and Kemp, 1962; Zahnd and Porte, 1962; Jollie and Jollie, 1964). In the present material this must be a true case, because the pinocytotic vesicles begin to appear numerously in the peripheral cytoplasm of this stage oocyte. According to Jollie and Jollie (1964), the pinocytotic vesicles in the *Lebestis* oocyte are found most numerously between microprojections and rarely within individual microprojections. On the contrary, the pinocytotic vesicles found in the goldfish oocyte are rarely situated on the surface of the cytoplasm between microvilli, whereas they are frequently present within individual microvillus or in the basal part of microvilli. This suggests that the microvilli may be the main sites where the pinocytosis is taken place. According to Bennett (1963a), pinocytosis is a kind of active transport mechanism induced by the movement of surface membrane. The microvilli are supposed to be cytoplasmic projections which may show active expansion and contraction in a living state. Thanks to Bennet's theory, therefore, the surface of microvilli is considered to be the most favorable site where pinocytosis is carried out. By this time the follicular epithelial cells become somewhat large in height but they are still flat in general. Most of the cells have the cytoplasm full of well-developed endoplasmic reticulum of rough surface. The rough form of endo-

plasmic reticulum is commonly accepted as functioning partly as the site of protein synthesis and partly as the channel of intracellular transport (Porter, 1961) Thus it is reasonable to suppose that the follicular epithelial cells play a role in the synthesis of specific protein and the transport of nutritive substance, which will be supplied for the oocyte. This supposition may be further supported by the presence of large mitochondria within the cytoplasm of the follicular epithelial cells and the production of microvilli on the surface of the cells.

A thick basement membrane completely covers the layer of follicular epithelial cells. The membrane is considered to be very rich in polysaccharides and acts as a filter and as a binder of ions (Bennett, 1963b). Thus, the substance which will be transported into the underlying follicular epithelial cells must be filtered first by the basement membrane. The layer of theca cells is very flat and the greater part of the layer still remains as a single layer. The theca cells connected with each other by distinct desmosomes contain several small mitochondria, poorly developed endoplasmic reticulum, sparsely distributed ribosomes, a few vesicles and a trace of collagen fibers in their cytoplasm. This cell layer is supposed to function first as a protecting and supporting one (Jollie and Jollie, 1964).

Now, well-developed capillaries are found located on all oocytes. They are seen situated between the theca cells, and the basement membrane directly underlies and supports their endothelium. Thus, the direct discharge of the nutritive substance from the capillaries to the basement membrane is possible, although the split of basement membrane detected in *Lebestis* (Jollie and Jollie, 1964) was not observed in the present study.

Conclusively speaking, during the first growth phase theca cells, follicular epithelial cells and the oocyte come to have an intimate relationship and build up a new unit of development, i.e. theca cells function as protecting and supporting elements, blood capillaries as suppliers of nutritive materials, the basement membrane as a selective filter of nutritive materials, follicular epithelial cells as producers of specific protein and transporters of nutritive substances and the oocyte itself has finished the preparation necessary for its development by producing the microvilli as the active absorption apparatus of nutritive substance and by developing the cytoplasmic organelles as apparates necessary for the synthesis of egg-proper substance and for the production of energy.

## SUMMARY

The growing oocyte of the goldfish during the first growth phase has been investigated with an electron microscope and the results obtained are as follows;

i) During the first growth phase the follicle layer which encloses the oocyte

has been built up. The layer consists of theca cells, basement membrane and follicular epithelial cells, which are arranged in turn from outside to inside. Blood capillaries are seen situated between the theca cells, directly supported by the basement membrane.

ii) The theca cells have been first recognized in oocytes of about 40 micra in diameter. They are very flat and stand in a row close to the outside of the basement membrane. The nucleus is flattened and the cytoplasm contains a few small mitochondria, poorly developed endoplasmic reticulum, and a small amount of vesicles and ribosomes. A trace of collagen fibers is recognizable.

iii) One layer of follicular epithelial cells has completely enclosed the oocyte by the time the oocyte grows up to 30 micra in diameter. The follicular epithelial cells are very flat in the early stage and become taller with the growth of oocyte, and they produce microvilli which are protruded into the intercellular space. The cytoplasm of the cells is full of endoplasmic reticulum of granular form. A few mitochondria, comparatively large in size, are also seen in the cytoplasm.

iv) During this growth phase a new, intimate relationship between the oocyte and the follicle arises due to the development of ovular microvilli. In small oocytes the surfaces of the oocyte and follicular epithelial cells are smooth and run almost in parallel with a narrow interspace. On the contrary, in large oocytes many microvilli develop on the surface of the oocyte, they are protruded into the widened interspace and are seen frequently in contact with the follicular microvilli. No direct connection between the cytoplasm of the oocyte and of the follicular epithelial cells has ever been observed. Many caveolae and many vesicles are seen on the surface or inside of the microvilli. This suggests that the microvilli are the main sites where pinocytosis is taking place.

v) The nucleus is changed in relative volume to the cytoplasm along with the growth of oocytes. It contains granular nucleoplasm, chromatin elements and nucleoli variable in number and shape, and it is enclosed with a double membraned envelope. The nucleoli are composed of both the minute fibrous unit with a hollow centre about 80 Å in entire thickness and amorphous material. The network pattern and dimorphism seen in the nucleoli seem to depend on the difference in the arrangement of the fibrous unit and the amount of amorphous substance. The double membraned envelope is perforated with pores of cylindrical structure. The annuli seen in tangential sections measure 1000 Å in inner diameter, and they are located with central vesicular granules, approximate 200 Å. Fine strands of vesicles, which pass through the nuclear pores, are frequently seen in the transverse section and this suggests the nucleo-cytoplasmic exchange.

vi) Mitochondria increase markedly in number during this growth phase. They are round or rod-shaped, variable in size and distribution, with the growth

of the oocytes. The multiplication of mitochondia seems to be attributed mainly to their division. The formation of mitochondrial rosettes is a common feature seen in the oocyte of this stage. The core substance is composed of the fibrous unit and amorphous substance, each fibrous unit measuring about 75 Å in entire thickness. This substance is supposed to be of nuclear origin and the rosette formation appears to be related to the taking of core substance into the mitochondria.

vii) Golgi complexes are seen in all oocytes throughout this growth phase. They are less in number in small oocytes and consist of flattened sacs and vesicles only, Golgi vacuoles being lacking. Numerous well developed Golgi complexes are found in large oocytes and are composed of three components. Golgi vacuoles are small in size and many in number in the organelle of an early stage, while they become large and less numerous in a well developed one. Enlarged Golgi vacuoles contain some substance of a moderate electron density and similar to yolk vesicles. Bisection of Golgi complexes seems to be a mode of multiplication of this organelle.

viii) Generally speaking, endoplasmic reticulum develops poorly, and its profiles appear as vacuoles or tubules. They are distributed sparsely all through the cytoplasm of the smallest oocyte, then they take a juxtaposition to mitochondria and are located mainly in the inner part of the cytoplasm. In the large oocyte the distribution of endoplasmic reticulum is concentrated mainly in the area of mitochondrion-Golgi complex clusters.

ix) The yolk nucleus is large in size and round in shape. It consists of a minute fibrous unit and an amorphous substance and has no limiting membrane. Other organelles are not seen within the body.

x) Annulate lamellae and multivesicular bodies are encountered in the cytoplasm of some oocytes of this stage. The 'cytoplasmic segregation' is observed once in a middle sized oocyte.

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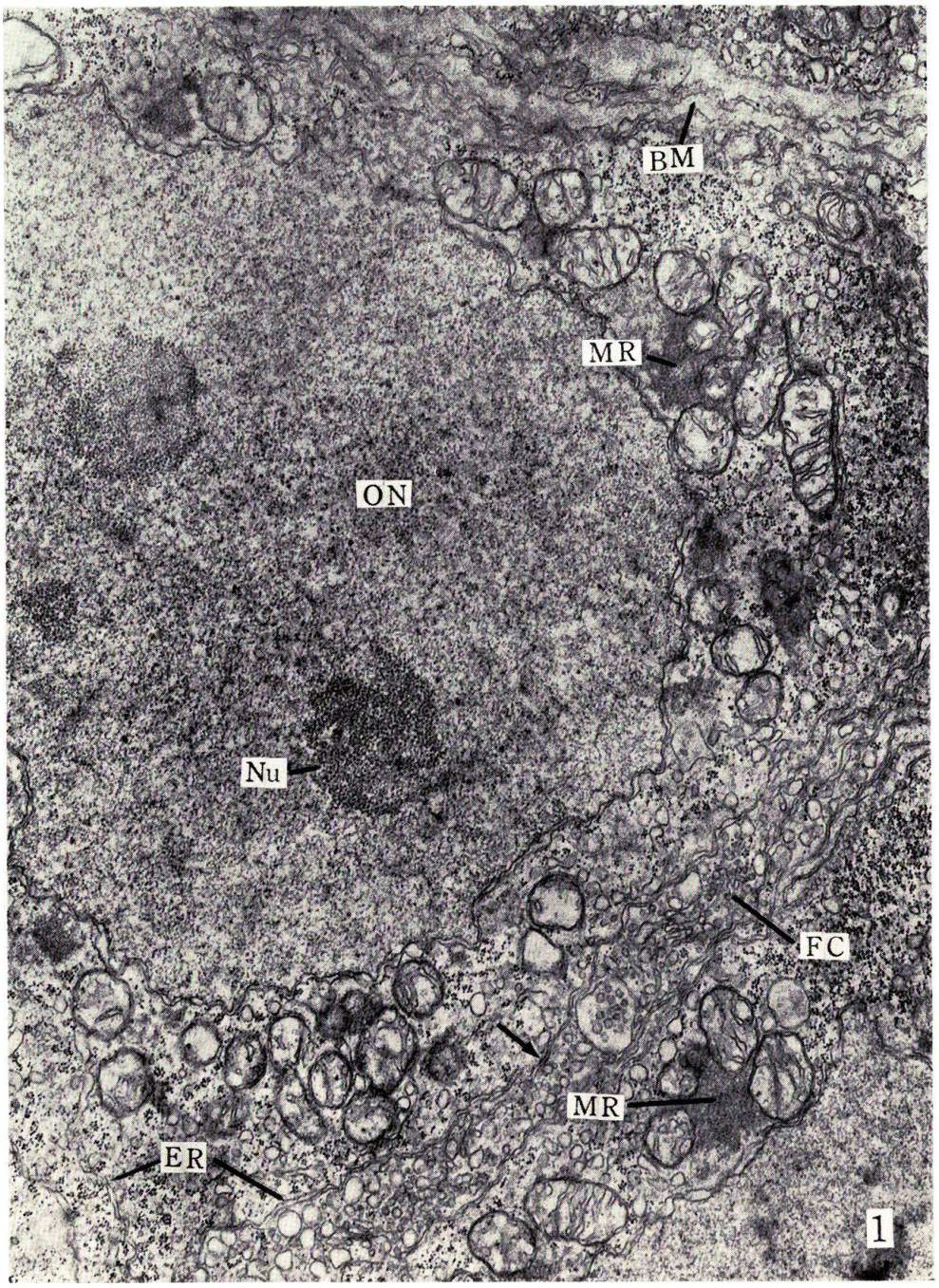
## Explanation of Plates

### ABBREVIATION

AL: Annulate lamella; BC: Blood cell; BM: Basement membrane; CC: Blood capillary cell; DM: Deformed mitochondria; ER: Endoplasmic reticulum; FC: Follicular epithelial cell; FN: Follicular epithelial cell nucleus; G: Golgi complex; GM: Golgi membrane (Golgi sac); GV: Golgi vacuole; GVe: Golgi vesicle; IG: Intramitochondrial granule; LD: Lipid droplet; M: Mitochondrion; MB: Multivesicular body; MR: Mitochondrial rosette; MV: Microvillus; NE: Nuclear envelope; Nu: Nucleolus; OM: Electron-opaque mass; ON: Oocyte nucleus; OP: Ooplasm; PV: Pinocytotic vesicle; TC: Theca cell; Ve: Vesicle; YN: Yolk nucleus

## PLATE I

Fig. 1. Low-power micrograph of the oocytes at the early phase of the chromatin-nucleolus stage. The oocytes are not yet enclosed completely by follicular epithelial cells. The arrow of the lower portion indicates the desmosome-like thickening seen at the apposing membranes of the oocyte and follicular epithelial cell. ×14,600




Yamamoto and Onozato: Electron microscope study on goldfish oocytes

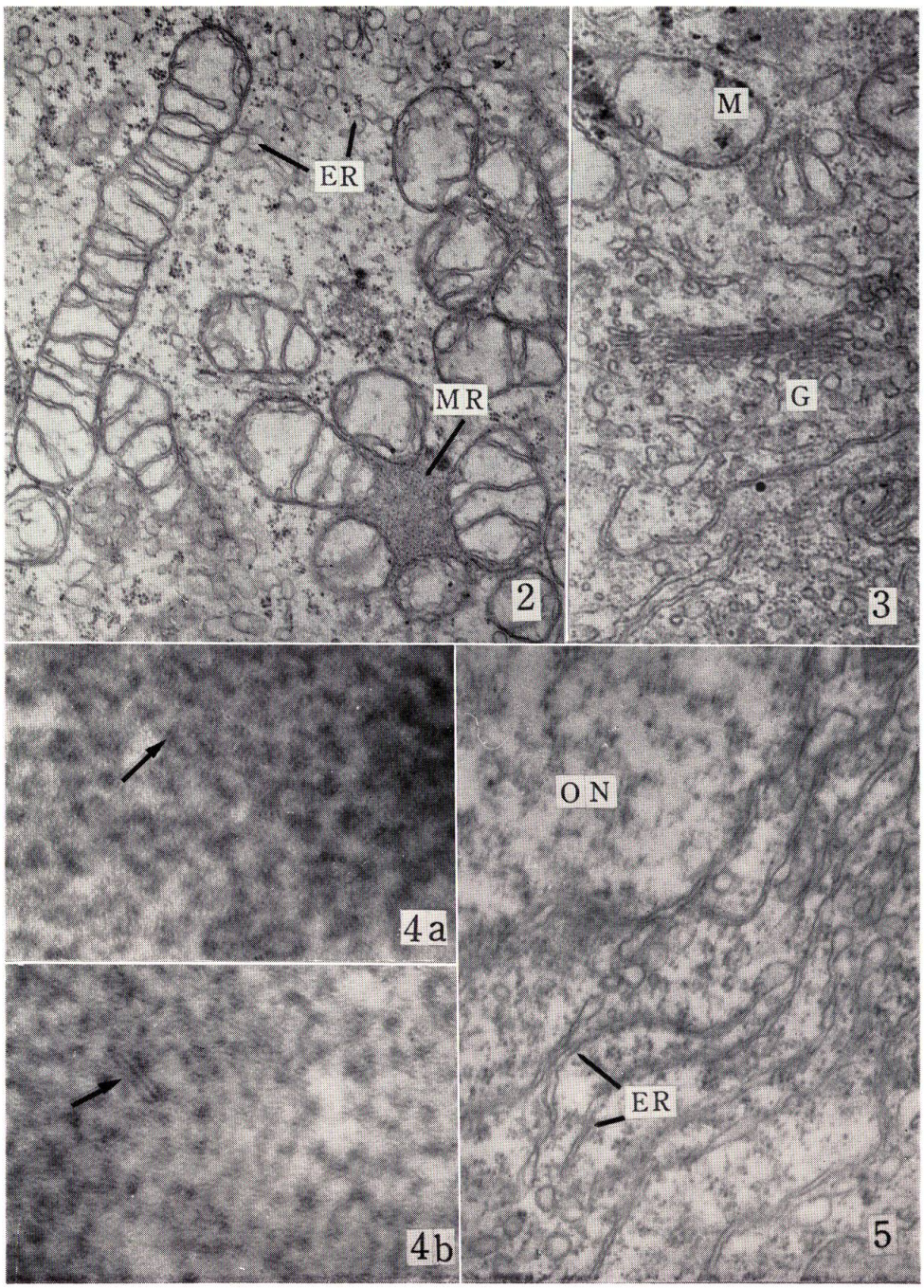
## PLATE II

Fig. 2. Mitochondria in an oocyte similar in stage to the above. A mitochondrial rosette is recognizable in the region at the lower right of the figure. ×20,000

Fig. 3. Golgi complex in an oocyte similar in stage to the above. It consists of flattened sacs and vesicles, Golgi vacuoles being absent in the region. ×21,000

Fig. 4. Highly magnified figure of a nucleolus. The arrow in 4b shows the fibrous structure of the unit, and that in 4a indicates a row of vesicles, showing the profiles of the fibrous units cut transversely. ×150,000

Fig. 5. Endoplasmic reticulum in an oocyte similar in stage to the above. The profiles of the E. R. appear as slender tubules with small, terminal inflated cisternae or small vacuoles. ×24,400

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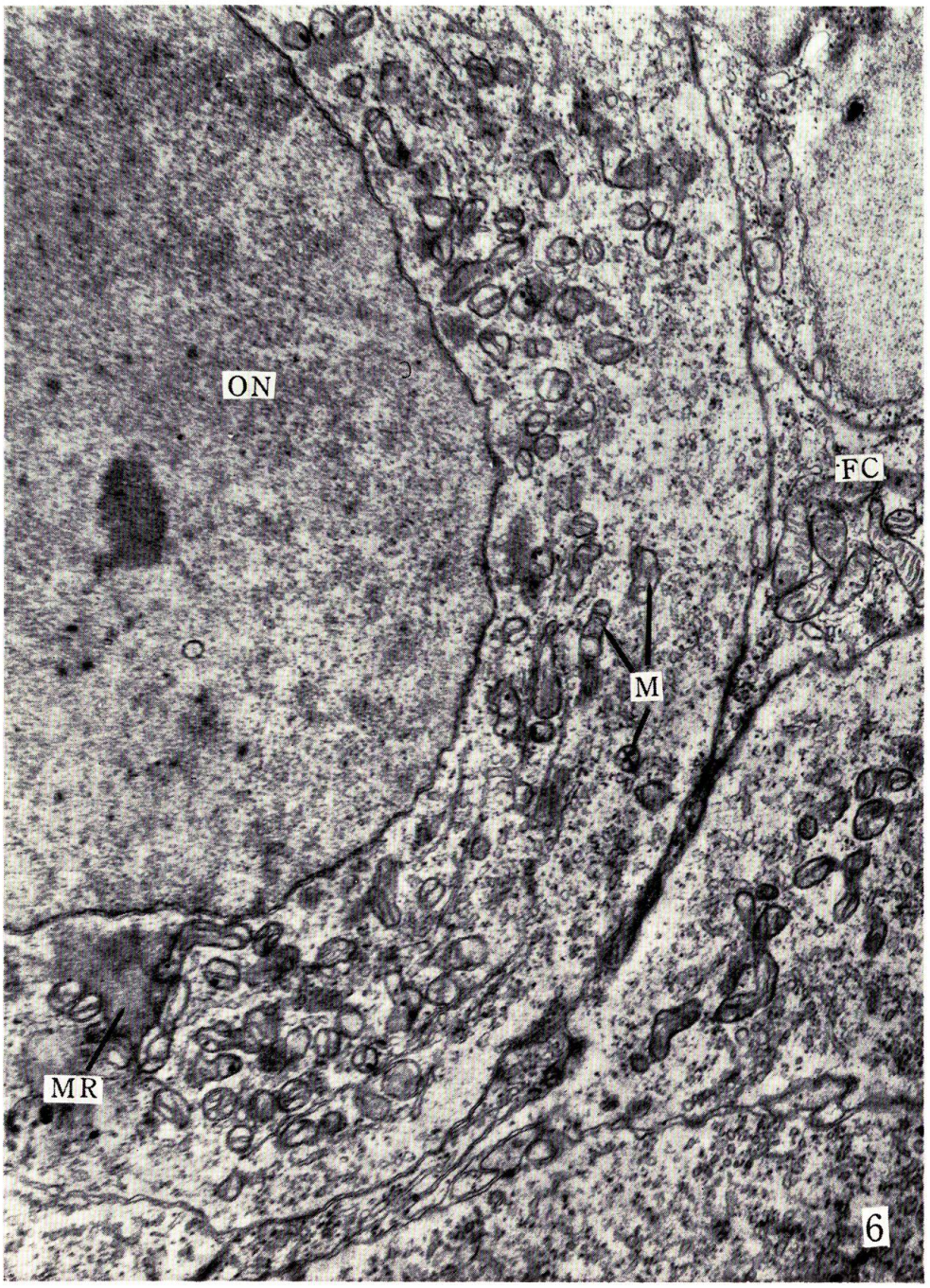
## PLATE III

Fig. 6. Low-power micrograph of an oocyte at the late phase of the chromatin-nucleolus stage. The follicular epithelial cells around the oocyte are not yet arranged regularly in a row. Note the fact that the mitochondria increase in number and decrease in size. ×11,700

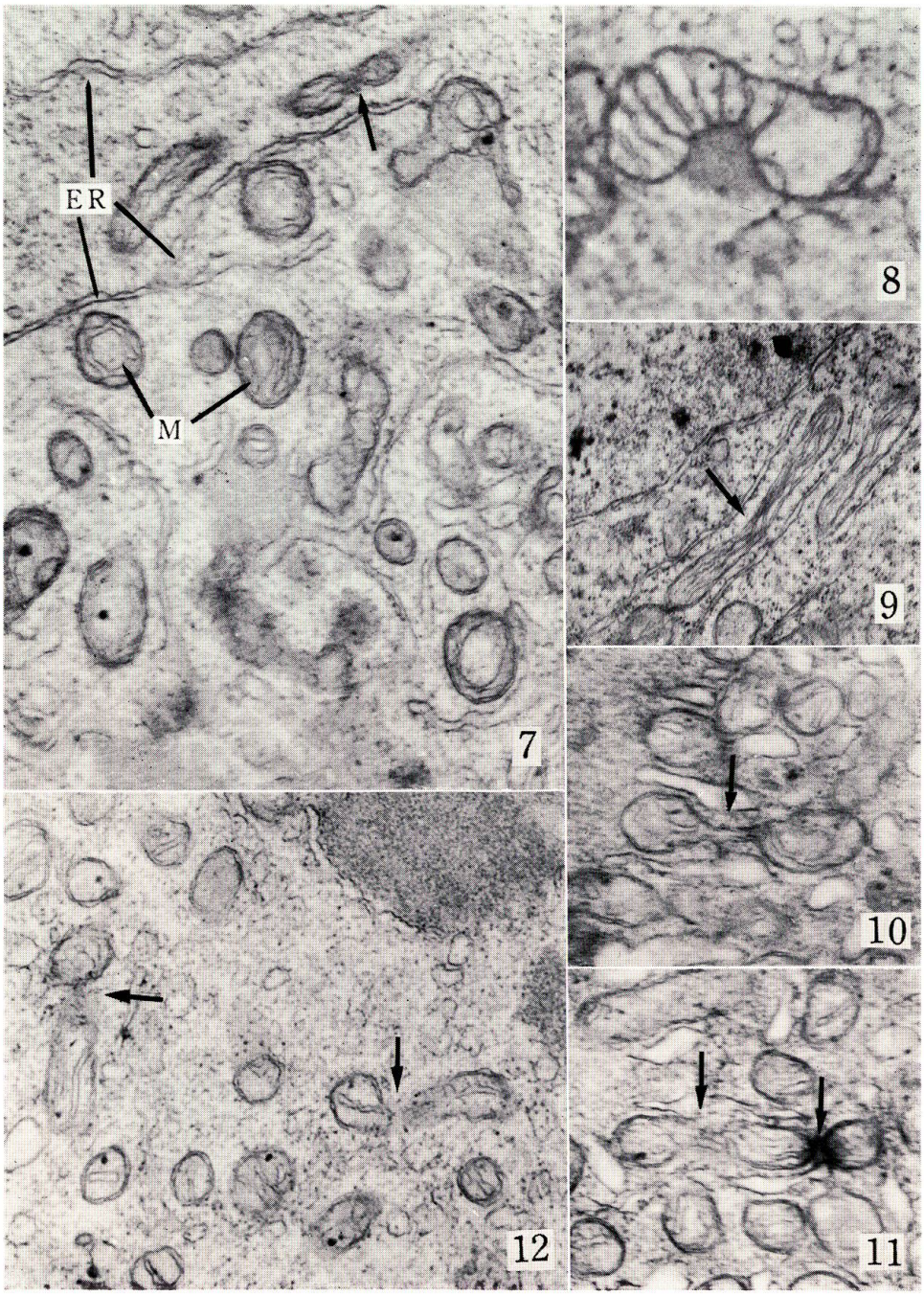



Yamamoto and Onozato: Electron microscope study on goldfish oocytes

## PLATE IV

Fig. 7. Mitochondria in the oocyte of the same stage as above. They show marked changes in size, shape and crista arrangement in comparison with those shown in Fig. 2. ×24,400

Figs. 8–12. Mitochondria seemingly in process of division. Note the change in the arrangement of cristae. Fig. 8. ×21,000; Figs. 9–12. ×24,500–25,500

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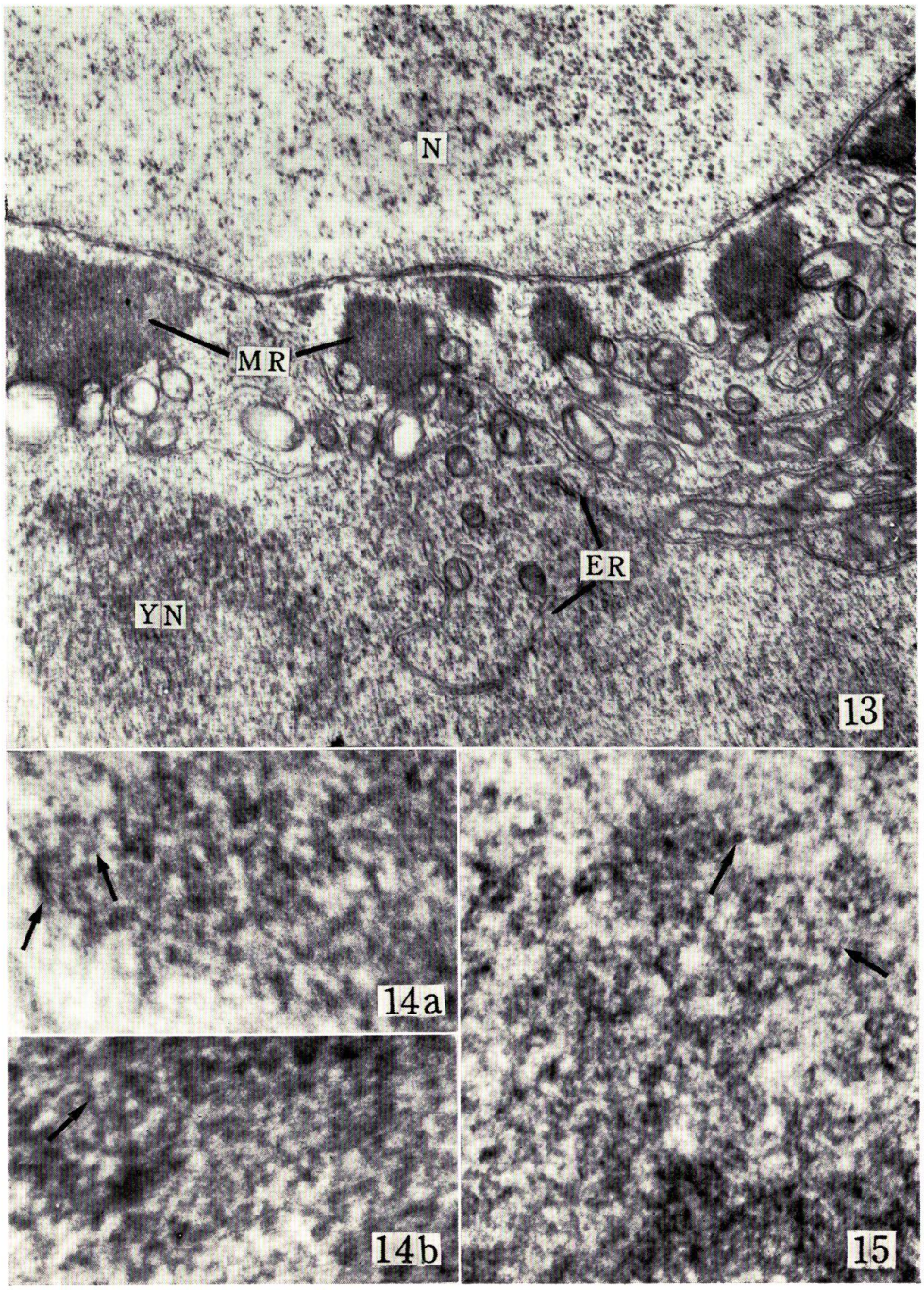
## PLATE V

Fig. 13. Juxtanuclear portion of an oocyte at the same stage as above. Mitochondrial rosettes are abundant in this region and the juxtaposition of mitochondria to the looped endoplasmic reticulum is also discernible. The yolk nucleus is seen in the lower left side of the figure. ×12,000

Fig. 14. High magnification of the core substance of mitochondrial rosettes. The arrow of Fig. 14a points to the fibrous form of the unit structure and that of 14b indicates vesicles showing the profiles of the fibrous units cut transversely. ×150,000

Fig. 15. Highly magnified figure of the yolk nucleus. The fibrous form of the unit structure is indicated by the arrow. ×150,000

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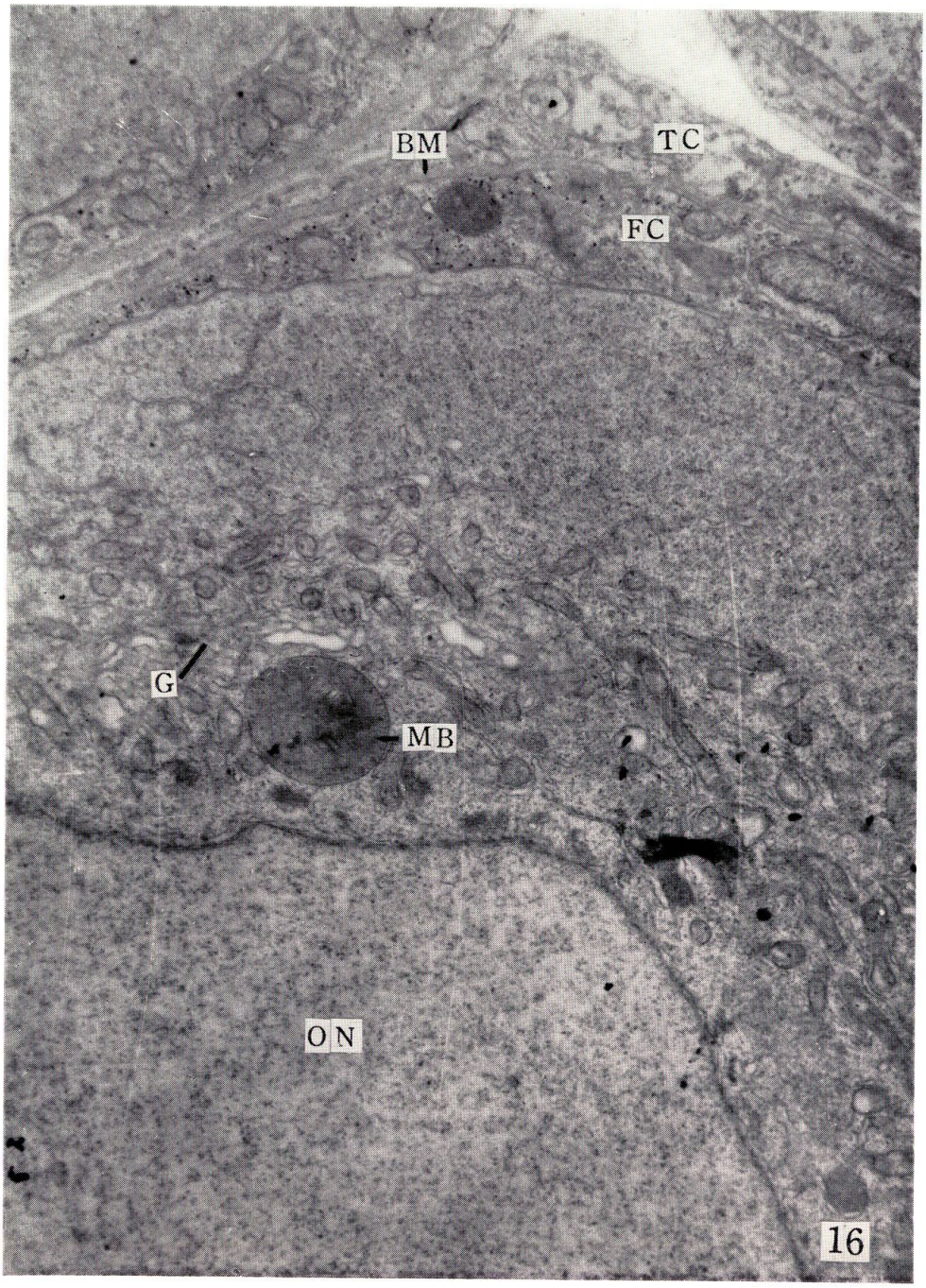
## PLATE VI

Fig. 16. Low-power micrograph of an oocyte at the early phase of the peri-nucleolus stage. The oocyte is enclosed completely by a single layer of follicular epithelial cells. External to the follicular epithelial layer, a basement membrane and a single layer of theca cells are seen. ×9,000

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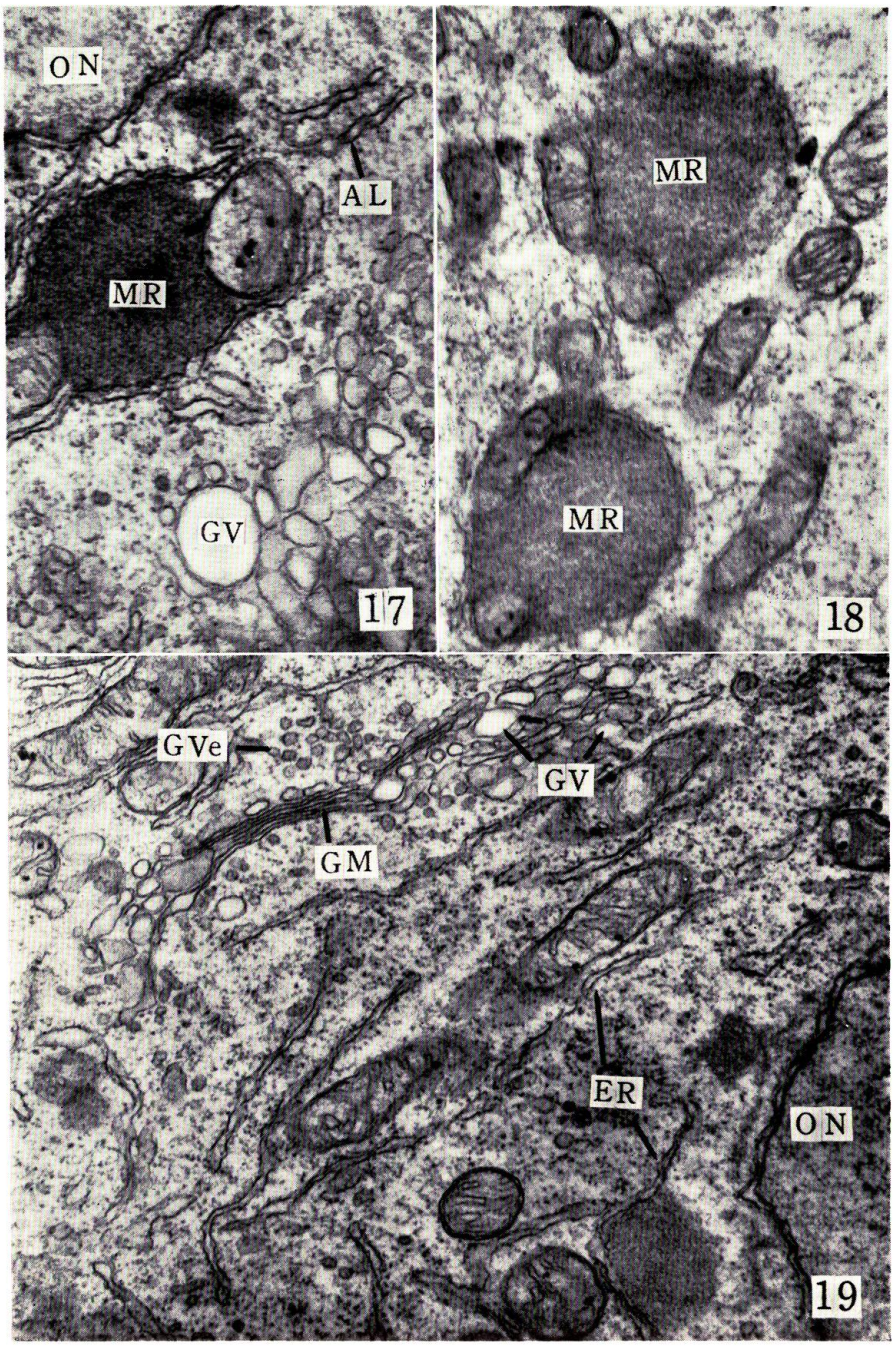
## PLATE VII

Figs. 17–18. Mitochondrial rosettes seen in oocytes similar in stage to the above. Attached mitochondria have poorly developed cristae and incomplete limiting membranes, and they appear to be primitive. ×24,000

Fig. 19. Golgi complexes seen in an oocyte similar in stage to the above. Beside the well developed Golgi complex (left side) the Golgi complex of the early developmental stage (right side) is found, which is composed of one flattened sac, numerous small vacuoles and a fair number of vesicles. ×25,000




Yamamoto and Onozato: Electron microscope study on goldfish oocytes

## PLATE VIII

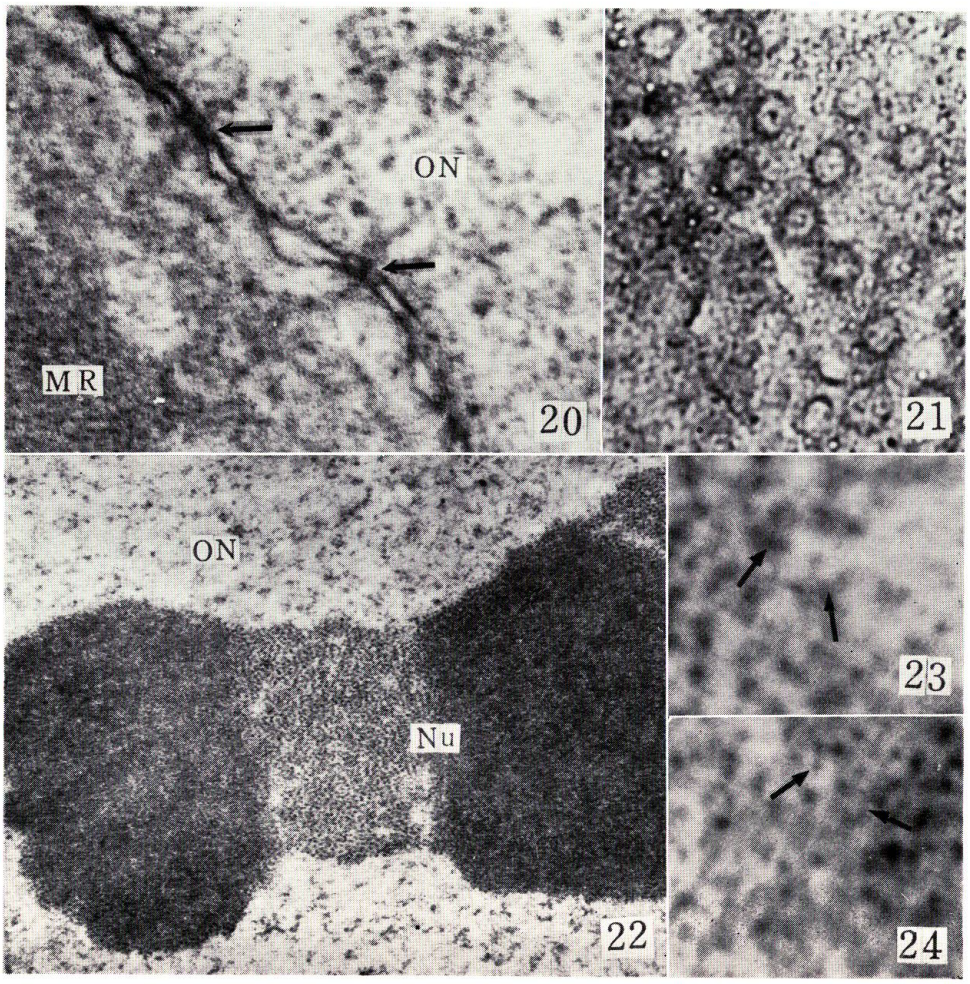
Fig. 20. Nuclear envelope sectioned transversely. The arrows indicates the outflow of the nuclear substance into the cytoplasm. ×48,000

Fig. 21. Annuli of the nuclear envelope. Note the annuli containing the central granule of vesicular form. ×48,000

Fig. 22. Dimorphous nucleolus seen in oocytes at the later phase of the peri-nucleolus stage. ×16,000

Fig. 23. Highly magnified figure of the loosely packed part of the nucleolus. The arrows indicate the profiles of fibrous units. ×150,000

Fig. 24. Highly magnified figure of the densely packed part of the nucleolus. ×150,000

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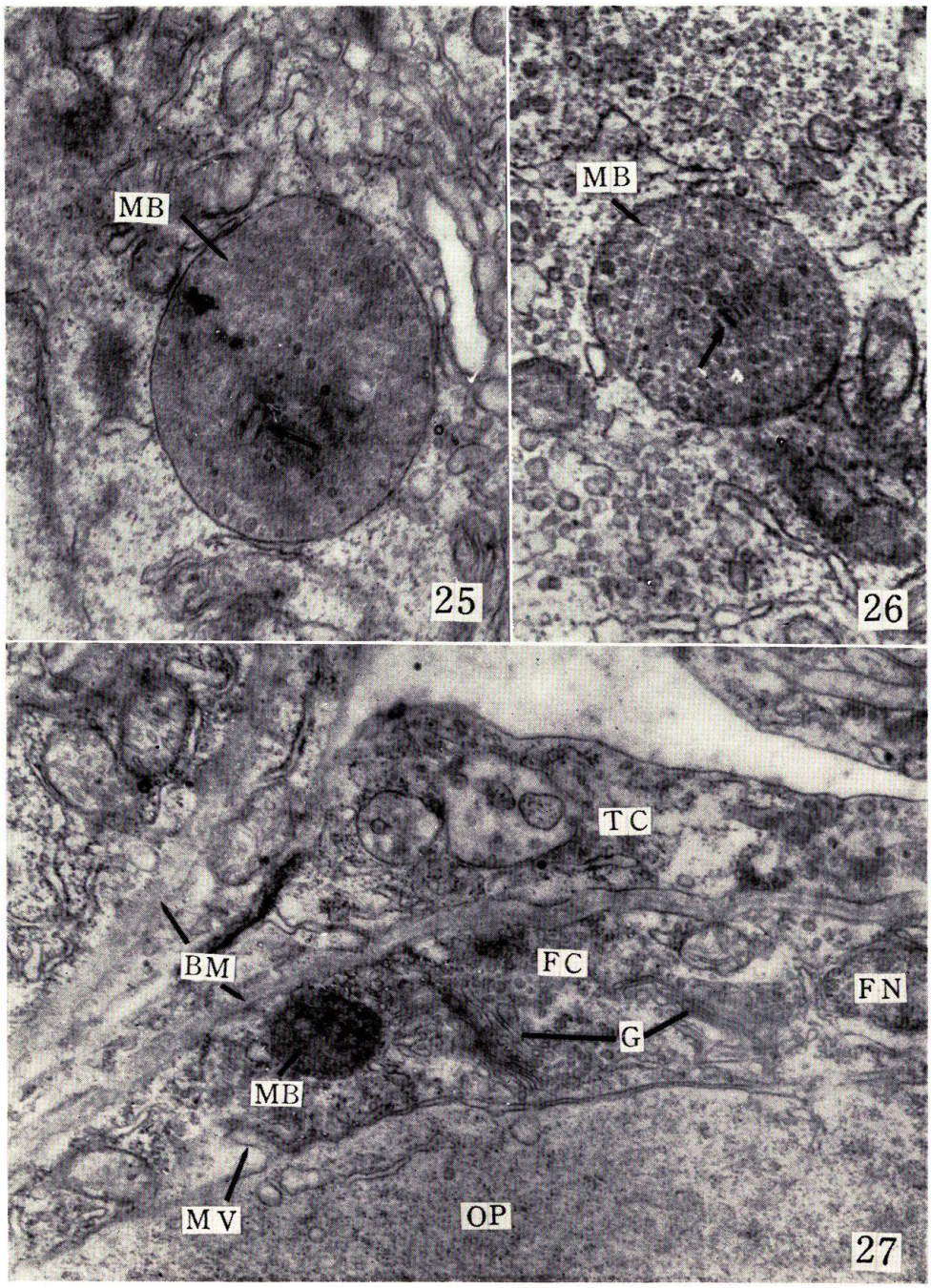
## PLATE IX

Figs. 25–26. Multivesicular bodies. Note the rod-like structure situated near the centre of the bodies (pointed by arrows). ×24,000

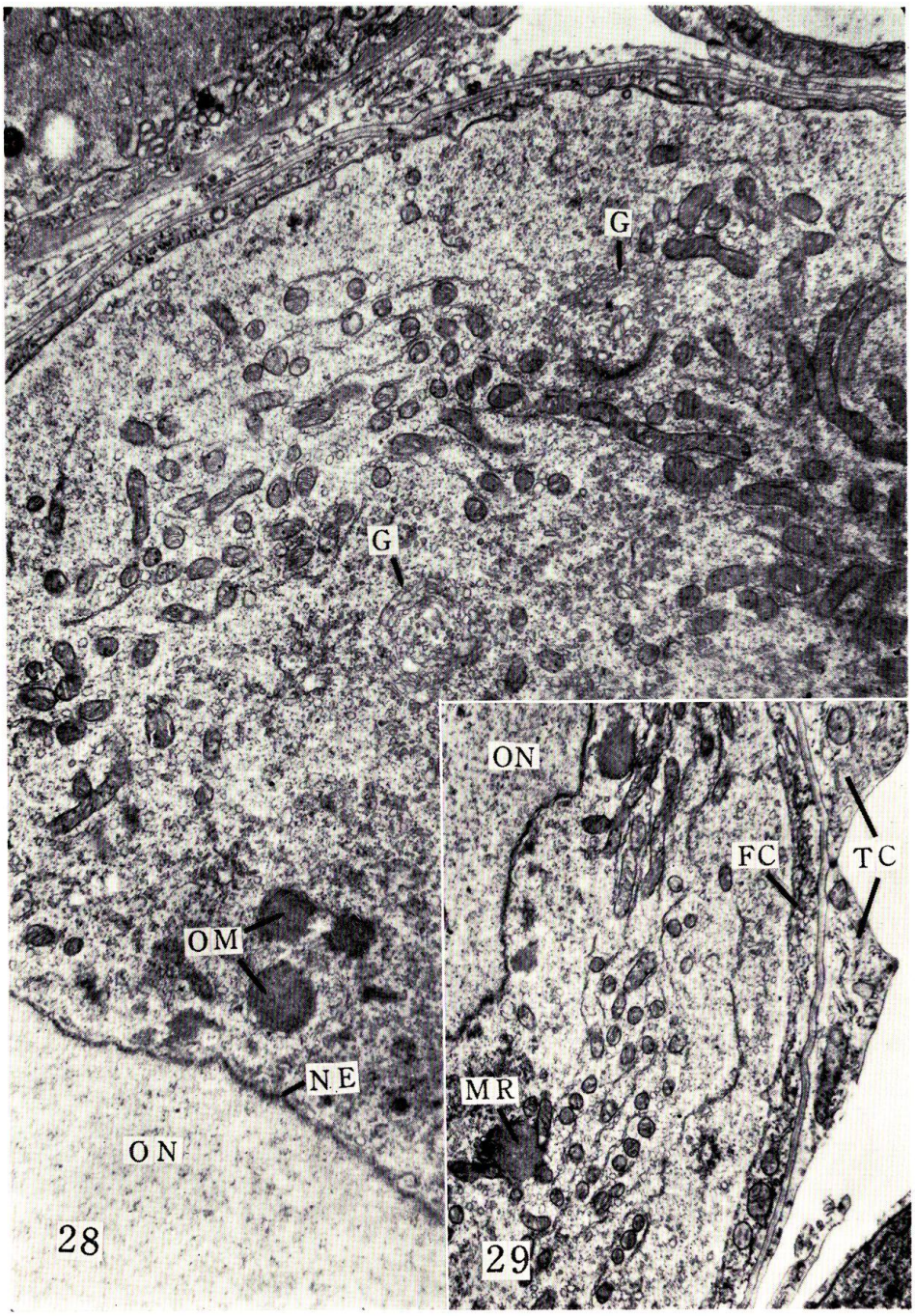
Fig. 27. Follicle layer of an oocyte at the early phase of the peri-nucleolus stage. The cytoplasm of the fiollicular epithelial cell is occupied with endoplasmic reticulum, Golgi complexes, ribosomes and a multivesicular body. A few microvilli can be found on the surface of the oocyte, but pinocytotic vesicles are rarely seen in the cytoplasm of the oocyte. ×17,200




Yamamoto and Onozato: Electron microscope stndy on goldfish oocytes

## PLATE X

Figs. 28–29. Low-power micrographs of an oocyte at the middle phase of the peri-nucleolus stage. The cytoplasmic organelles such as mitochondria, Golgi complexes and endoplasmic reticulum develop well and they are distributed widely in the cytoplasm. Fig. 28. ×7,500; Fig. 29. ×6,000

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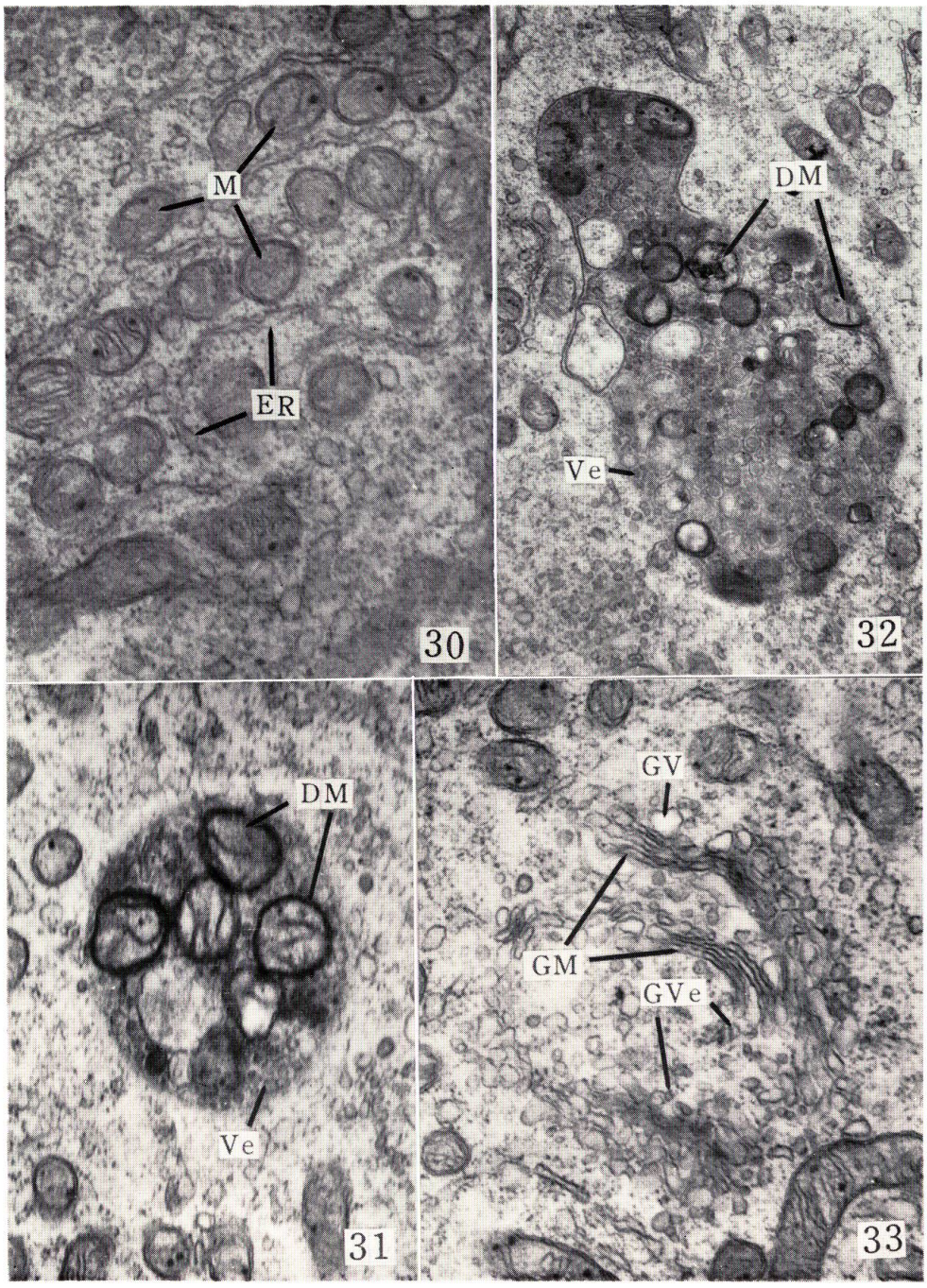
## PLATE XI

Fig. 30. Mitochondria in the same oocyte as above. The juxtaposition of mitochondria to the endoplasmic reticulum is noticeable. ×25,000

Figs. 31–32. "Cytoplasmic segregation" seen in the same oocyte as above. The region shown in Figure 31 exhibits no limiting membrane, while that in Figure 32 shows a clear limiting membrane. Fig 31. ×21,000; Fig. 32. ×13,500

Fig. 33. Golgi complexes in the same oocyte as above. They consist of well developed flattened sacs, many vesicles and vacuoles. ×22,500

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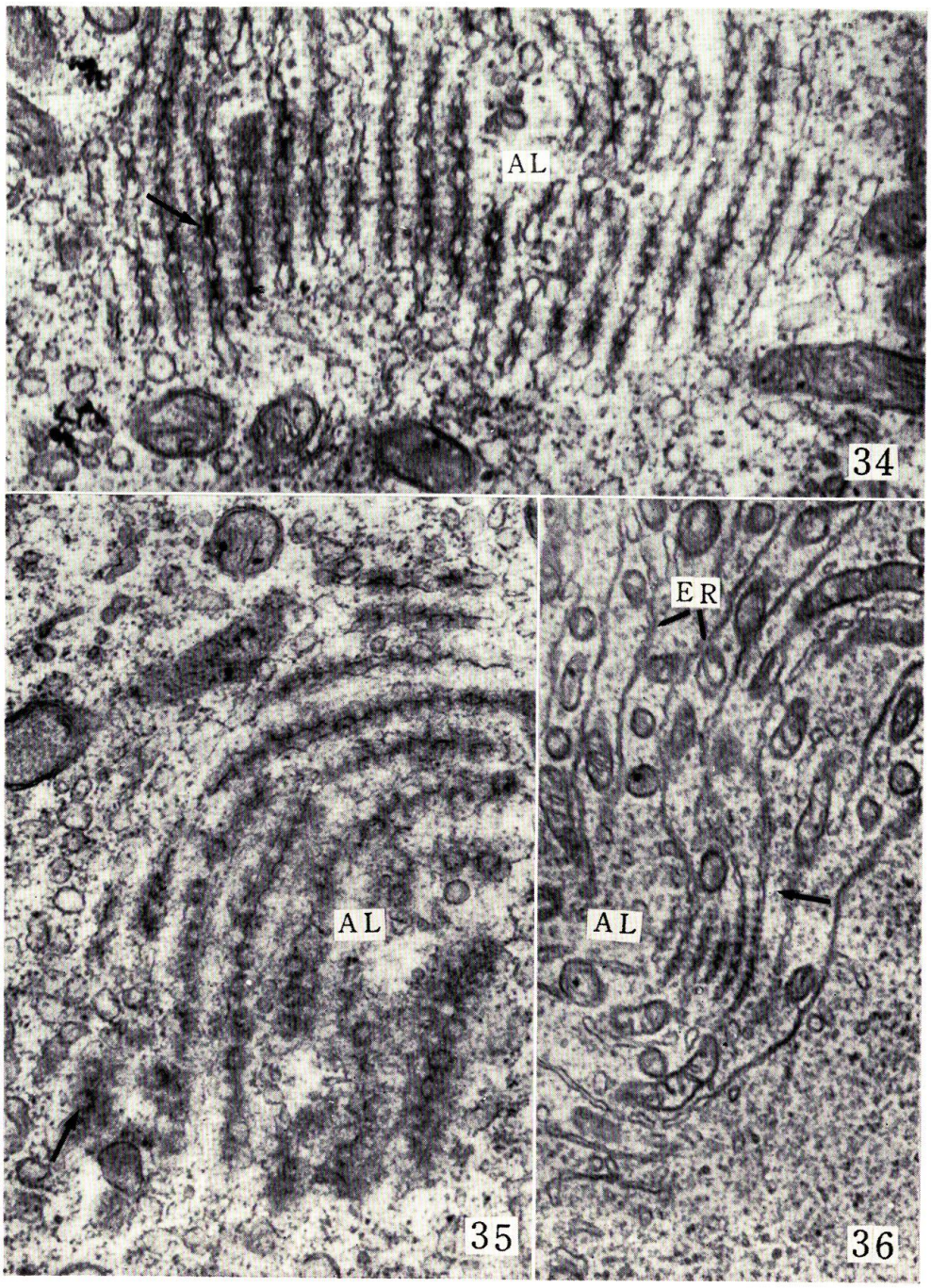
## PLATE XII

Fig. 34. Annulate lamellae sectioned transversely. Arrows indicate the site of pores. ×25,000

Fig. 35. Annuli of the annulate lamellae. The annulus is about the same in dimension as that of the nuclear envelope. ×25,000

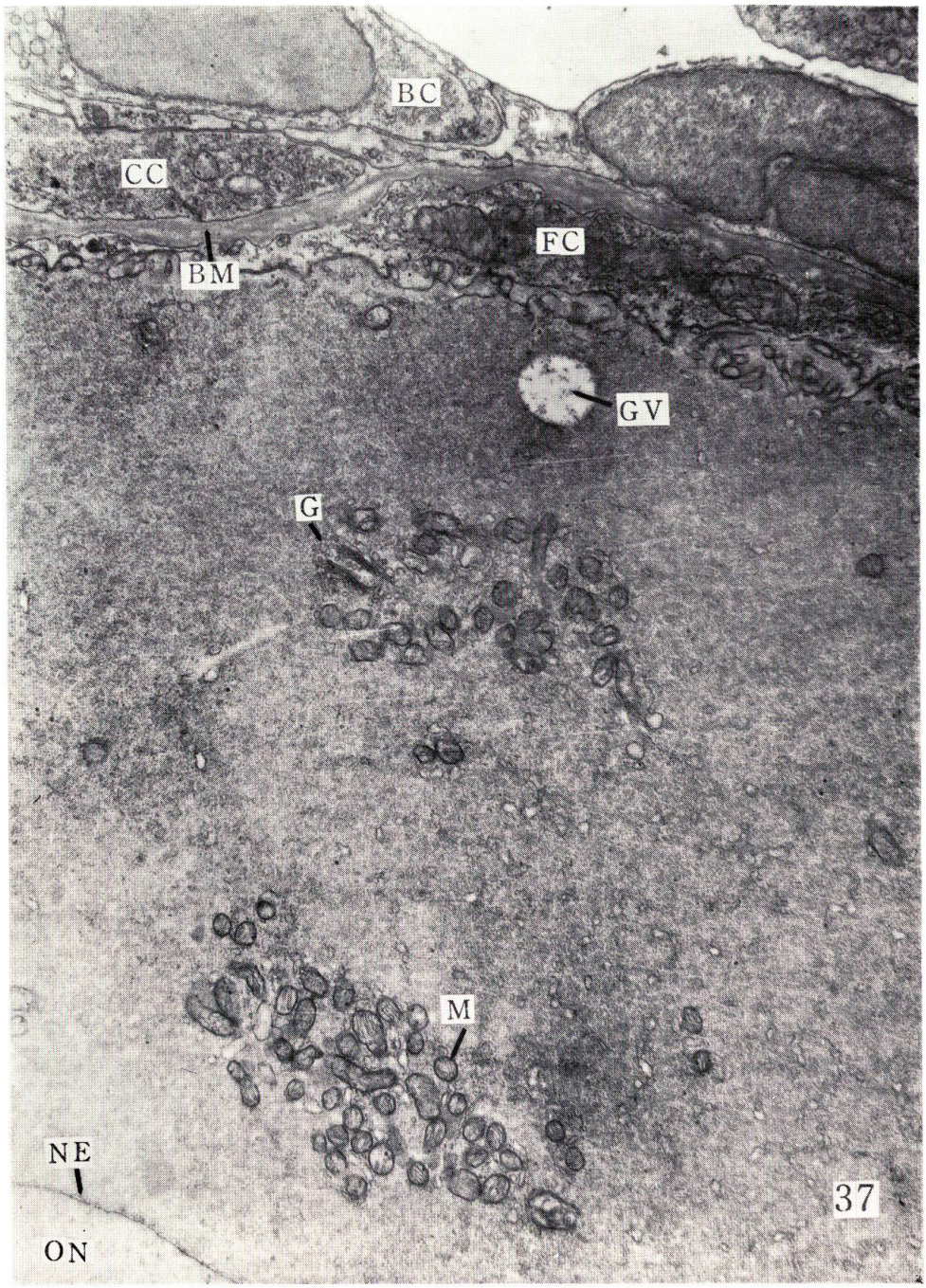
Fig. 36. Annulate lamellae and endoplasmic reticulum. The annulate lamellae are seen to be continuous with the parallel membranes of the endoplasmic reticulum (indicated by an arrow). ×15,000




Yamamoto and Onozato: Electron microscope study on goldfish oocytes

## PLATE XIII

Fig. 37. Low-power micrograph of an oocyte at the late phase of the peri-nucleolus stage. Mitochondria and Golgi complexes form large clusters and they are distributed here and there in the cytoplasm. In the widened intercellular space between the oocyte and follicular epithelial cells there are found many ovular microvilli and a few follicular microvilli. ×8,000

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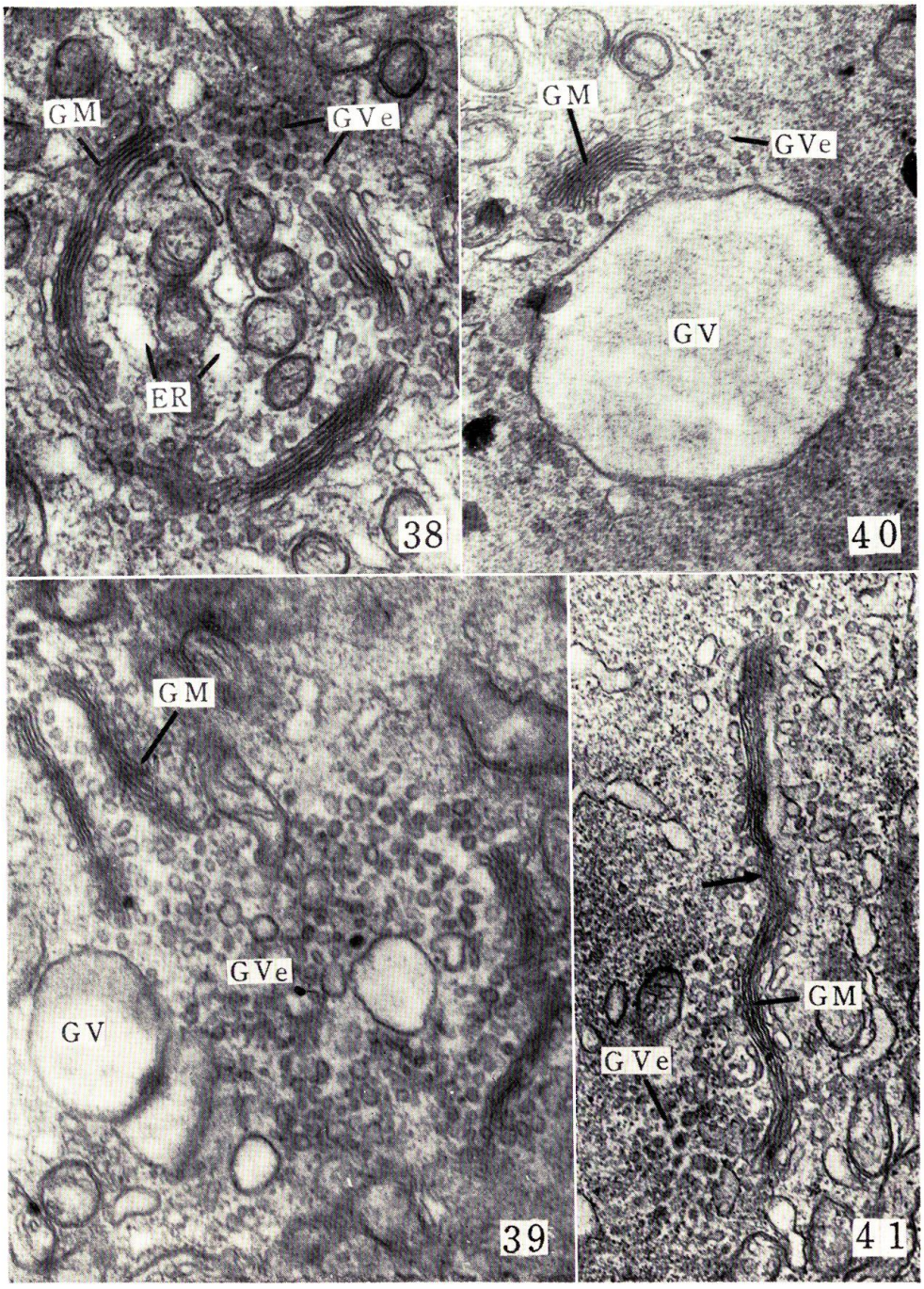
## PLATE XIV

Figs. 38–41. Golgi complexes in the oocytes similar in stage to the above. Golgi vacuoles appear to become large in size with the development of the Golgi complex (compare Fig. 40 to Figs. 33 and 39). The Golgi complex shown in Figure 41 seems to be in the process of bisection at about the central portion indicated by an arrow. Figs. 38, 40 and 41. ×24,000; Fig. 39. ×22,500

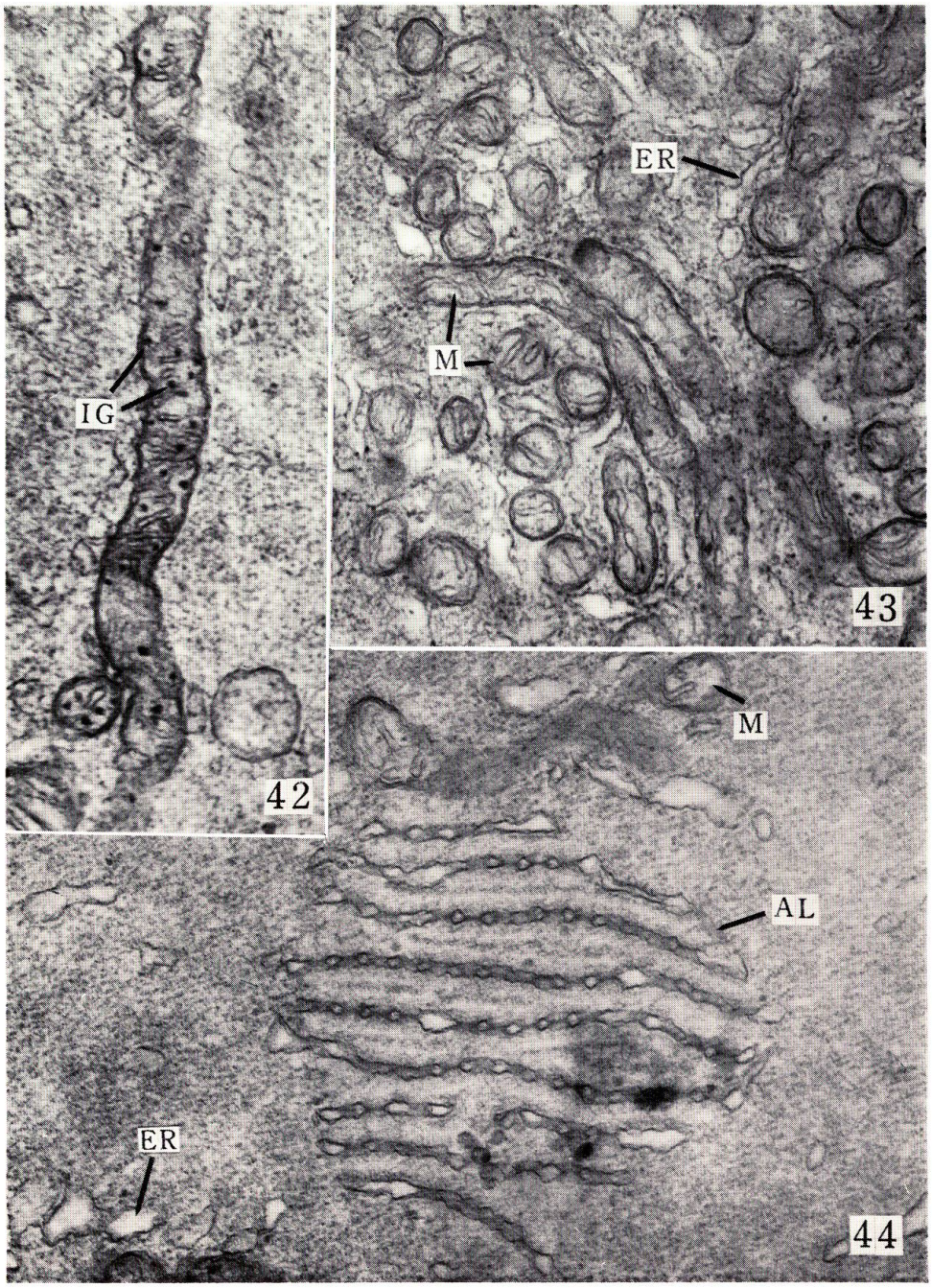



Yamamoto and Onozato: Electron microscope study on goldfish oocytes

## PLATE XV

Figs. 42–43. Mitochondria in oocytes at the same stage as above. In the region of Mitochondrion-Golgi complex clusters the abundant profiles of endoplasmic reticulum are always found. Mitochondria in this stage oocyte contain many intra-mitochondrial granules. Fig. 42. ×26,000; Fig. 43. ×24,000

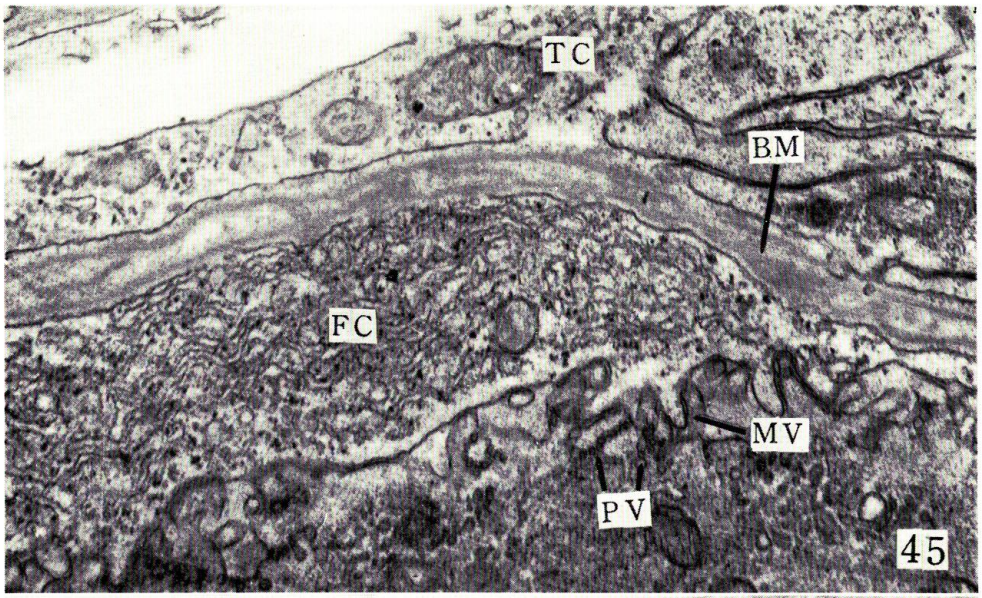
Fig. 44. Annulate lamellae present in an oocyte similar in stage to the above. ×25,200

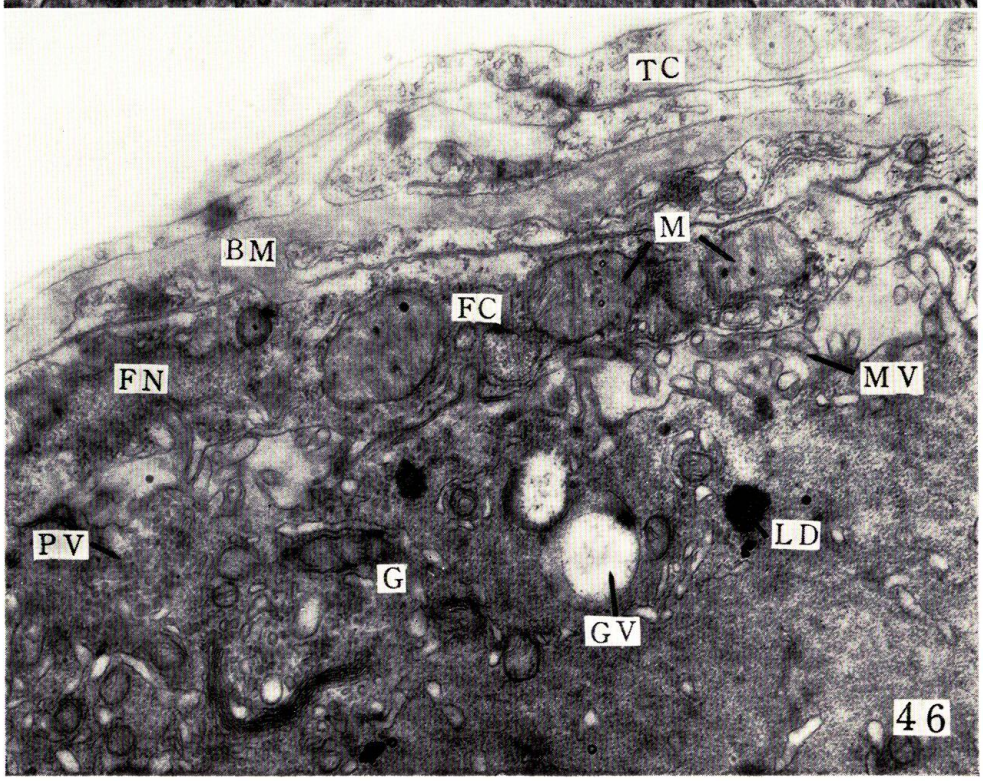



Yamamoto and Onozato: Electron microscope study on goldfish oocytes

## PLATE XVI

Figs. 45–46. Follicle layer and the surface of the oocyte at the late phase of the peri-nucleolus stage. The theca cells are meagre in cytoplasmic organelles, while the follicular epithelial cells contain well developed endoplasmic reticulum of rough form and a few large mitochondria in the cytoplasm. Besides many ovular microvilli and a few follicular microvilli, many pinocytotic vesicles are noticeable in the peripheral cytoplasm of the oocytes and within microvilli. Fig. 45. ×15,000; Fig. 46. ×13,500



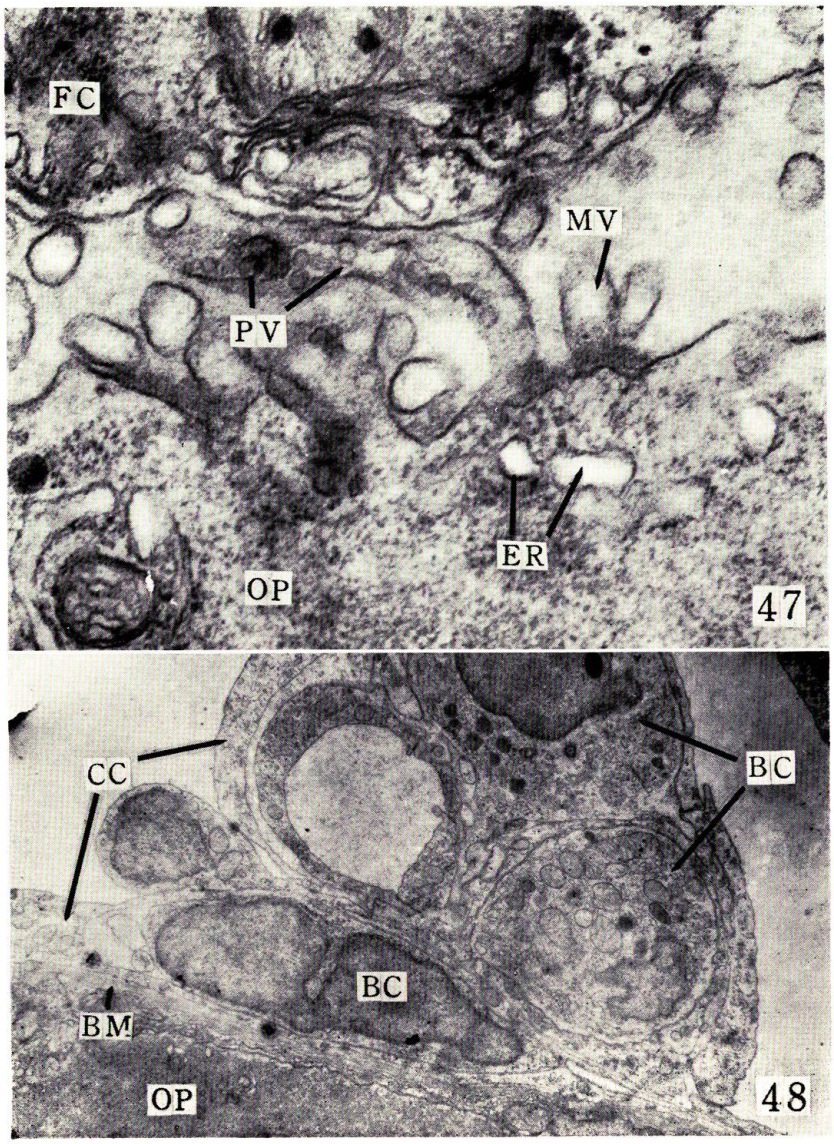




Yamamoto and Onozato: Electron microscope study on goldfish oocytes

## PLATE XVII

Fig. 47. High magnification of the ovular microvilli. Note the presence of pinocytotic vesicles within the microvilli and of the caveola on the surface. ×40,000

Fig. 48. Blood capillaries situated on an oocyte of this stage. The endothelium of one capillary is seen directly in contact with the basement membrane. ×4,500

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