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ELECTRON MICROSCOPE STUDY ON THE FINE STRUCTURAL
CHANGES IN THE OOCYTES OF GOLDFISH, *CARASSIUS*
AURATUS, DURING YOLK FORMATION STAGE

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Introduction

The detailed study of the fine structural changes, leading to the yolk formation, is rather essential before coming on to any conclusion regarding the process of the formation of yolk material in the oocyte.

With the advent of electron microscope, recently it has become possible to obtain higher resolution to clear several doubts and controversies in the cytological differentiation and basic pattern of the various organelles, which had arisen because of the limited resolution obtainable from the routine light microscopy.

Currently several authors have contributed vital information on the oocytes of various animals by using the electron microscope and several doubts have been clarified as regards to the change in the fine structure of cytoplasmic organelle.

As far as teleostean oocyte is concerned, several reports have been published to this day giving the useful information on the oogenesis; Kemp and Allen (1956) in *Fundulus*, Kemp and Hibbard (1957) in *Fundulus heteroclitus*, Zahnd and Porte (1962) in several teleosts, Chambolle *et al.* (1962) in *Lebistes reticulatus*, Sterba and Müller (1962) in *Cynolebias belotti*, K. Yamamoto (1962) in zebra fish, Müller and Sterba (1963) in *Cynolebias belotti*, M. Yamamoto (1963) in *Oryzias latipes*, Jollie and Jollie (1964) in *Lebistes reticulatus* and Anderson (1964) in certain teleost fish, have dealt mainly with the formation of egg membranes, Porte and Zahnd (1962) in teleosteans, Zahnd and Porte (1963) in *Barbus conchoniis*, have described the cytoplasmic organelle, Yamamoto and Onozato (1965) dealt with the changes occurring during the first growth phase in the oogenesis of goldfish, Droller and Roth (1966) in *Lebistes reticulatus*, Yamamoto and Oota (1967) have described the fine structure of yolk globule in zebra fish and Yamamoto and Oota (1967) have described the formation of yolk. A mention may also be made of a recent report of Kilariski and Grodzinsky (1969) dealing with the yolk of holostean fishes.

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Thus in the knowledge accumulated so far the report of M. Yamamoto deals with the complete oogenesis of the oocyte of teleosts but various stages in the development of oocyte and the fine structure have been dealt with rather briefly. The publication of the report of Yamamoto and Onozato (1965) no doubt deals in detail with the fine structural changes but that too only during the first growth phase. Thus as regards the knowledge pertaining to the fine structure and structural changes occurring in the oocytes of teleost fish during the second growth phase, this is too meagre to draw any firm conclusions regarding the complete oogenesis of teleost fishes.

In the present study the authors wish to clarify the fine structural changes in the oocyte of the gold fish during the second growth phase limiting to the yolk formation stage.

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Material and Method

The material for the present study comprises the maturing oocytes of goldfish, *Carassius auratus*, hatched and reared in the culture pond of the Faculty of Fisheries, Hokkaido University. The fish measuring between 6 to 11 cm, in body length were killed by de-cerebration in November 1968, February, May and July 1969 and the gonads were removed. The portion of ovary was cut into small pieces, while being submersed in the cold Millonig's solution (1961) and fixed for about two hours. Dehydration was done by the usual cold ethanol method providing two changes of propylene oxide and embedded in plastic epon-epoxy resin mixture (Luft, 1961). Some material was doubly fixed i.e. prefixed in 1.2% glutaraldehyde (Sabatini *et al.*, 1963) in phosphate buffer (pH 7.4) and postfixed in Millonig's solution. The material was sectioned on a Porter-Blum microtome with a glass knife at a thickness of about 400 Å to 700 Å and stained with uranyl acetate (Watson, 1958) and Reynold's (1963) staining solution. Before using, the Reynolds' staining solution was given 3000 cycles rotation to avoid the possible contamination due to the improper solubility of lead citrate. The supernatant solution was used for staining the sections picked on the carbon coated grids which gave satisfactory results. The electron micrographs were obtained with a Hitachi H.S. 7 electron microscope. The sections of 1 micron thickness were also obtained similarly and stained with azure II and methylene blue mixture (Richardson *et al.*, 1960).

Observations

1) Yolk vesicle stage:

The yolk vesicle stage in the oogenesis begins with the appearance of yolk vesicles and then the latter grow in size and number till they occupy most of the cytoplasm of the oocyte. Further these vesicles tend to migrate towards the periphery of the cytoplasm and arrange themselves in the outer two third portion of cytoplasm. During this stage the yolk vesicles measure between 3.8 micra to 13 micra in diameter. Morphologically their form is round to oval. The vesicles are enclosed by a quite prominent limiting membrane. The inner portion of these vesicles is filled with numerous fine particles and is quite low in electron density. Usually the central portion is surrounded by a moderate dense material in an indefinite manner (Fig. 2).

The oocyte is almost round in shape during this stage and is surrounded by prominent follicular epithelium cells and theca cells. The theca cells are arranged in two rows and are generally flat in shape having an elliptical nucleus. The cytoplasm of theca cells contains less developed endoplasmic reticulum, some vesicles, free ribosomes and very rarely the traces of fibrous material. The membrane of these cells is smooth in appearance having a narrow interspace between the two neighbouring cells but sometimes this interspace is wider also. The follicular epithelium, coated by a moderately thick and prominent basement membrane measuring between 0.3 to 0.4 micra in thickness, lies just beneath the row of theca cells (Fig. 1). The follicular cells have a prominent nucleus and the cytoplasm is filled with well developed endoplasmic reticulum of slender, long and sometimes round form having the clusters of ribosomes all around its margin. A few mitochondria of large size and well developed Golgi complexes are also encountered in the cytoplasm of the follicular cells. A developing zona radiata of highly electron dense nature is present in between the ooplasm and follicular epithelial layer and measures between 0.4 micra to 0.8 micra in width (Fig. 1). A clear interspace is observed in between the oocyte and follicular epithelium which varies in width widely measuring between 0.6 micra to 1.5 micra. Several ovular microvilli of the nature of tubular microprojections are protruded into this interspace. Their shape varies from straight to curved sometime irregular, the large ones measuring 1.9 micra in length and 0.2 micra in width. Some comparatively smaller microvilli are also projected from the follicular epithelium and are occasionally in contact with the ovular microprojections but the cytoplasmic connection between the oocyte and follicular cells is not observed as also reported by Yamamoto and Onozato (1965) in the late phase of peri-nucleolus stage in gold fish. A number of pinocytotic vesicles are also observed in the periphery of the ooplasm just below the zona radiata (Fig. 1) measuring less than 65 m micra.

They are located in clusters just at the base of the ovular microvilli which leads to a probability that the pinocytosis takes place inside the microvilli.

In the oocyte of this stage the contour of the nucleus is not regular and smooth but irregular and wavy (Fig. 3). The nuclear matrix is much lesser in electron density in comparison to cytoplasm. A number of nucleoli are observed to be situated in the peripheral region of nucleus beneath the nuclear membrane. The shape and size of the nucleolus varies widely but usually round to oval shape is observed. The double nuclear membrane runs rather irregularly throughout forming projections in the cytoplasm and is perforated at several places. The pores are quite numerous and measure between 70 m micra and 80 m micra in width (Fig. 3) and are suggestive of cylinder form. The presence of certain nucleolus-like electron opaque bodies might have a nuclear origin.

During this stage the occurrence of mitochondrial rosette is also observed rarely in the juxtannuclear position (Fig. 4). The mitochondrial rosette appears to be polygonal in shape, quite high in electron density measuring 3.2 micra in length and 1.6 micra in width and is devoid of any limiting membrane. Its contour is studded with several very young mitochondria of round, oval or irregular shape. Some of these mitochondria exhibit the presence of prominent double membrane and parallelly arranged cristae while some others exhibit poorly developed membrane and cristae, however, the electron density of the matrix of the developing mitochondria is in part much like that of the matrix of the core substance. Thus, the juxtannuclear situation of rosettes and other nucleolus-like bodies and the probable passing of nuclear material into the cytoplasm (Fig. 4), suggest that the core of the rosettes may be of nuclear origin and further the nuclear origin substance of the core is transferred into the mitochondria suggesting the existence of a possible relationship between the nucleus and the mitochondria.

Mitochondria are well developed and observed in several clusters in the cytoplasm (Figs. 1 & 5). Their shape varies from round to elongated but their double limiting membrane is irregular except when the mitochondria are in the advanced stage of development. The round one measures between 0.3 micra and 0.5 micra in diameter while large filament-like mitochondria measures approximately 1.4 micra in length and 0.3 micra in width. The cristae in some of the mitochondria are very prominent and are arranged in a parallel fashion in the moderate electron dense matrix. Certain mitochondria which are in the advanced stage of development exhibit the nearly disappearing state of cristae, obscure double nature of limiting membrane and the presence of certain circular vesicles measuring less than 66 m micra in diameter. The density of the matrix of these vesicle-like structures is the same as that of the mitochondrial matrix (Fig. 9). This developmental stage may be the forerunner of multivesicular bodies which will be dealt afterwards.

The endoplasmic reticulum is moderately developed. Its profile varies from

round or oval to narrow tubular with inflated cisternae (Figs. 1 & 5) and is thoroughly distributed in the cytoplasm. Frequently it forms parallel loops around the mitochondria. The limiting membrane of the endoplasmic reticulum is studded with ribosomes thus forming the rough surfaced endoplasmic reticulum. Free ribosomes are abundantly distributed in the cytoplasm.

Certain instances of the presence of annulated lamellae (Swift, 1956) are also encountered in the cytoplasm (Fig. 6). Several parallel arranged lamellae form a peculiar structure. The approximate distance between the two lamellae is 100 m micra. Morphologically an individual lamella is formed by the two parallel running membranes perforated by a series of pores or annuli, which leads to suggest a possible similarity with that of nuclear membrane. Figure 6 reveals that in transverse section the paired membranes of individual lamella are fused with each other at intervals thus forming pores measuring 60 m micra to 100 m micra and seem to be near similar to that of nuclear envelopes and pores. At certain places in the Figure 6, it may be observed that the ends of annulated lamellae are in direct contact with that of regular endoplasmic reticulum. At the ends of annulated lamellae certain vacuoles are also observed.

Well developed Golgi complexes are recognizable during this stage of the oogenesis. They usually are located in the vicinity of the yolk vesicles and are usually composed of three elements i.e. flattened sacs, Golgi vacuoles and Golgi vesicles (Fig. 7). The flattened sacs are well developed in some Golgi zones (Figs. 7 & 8) and their number ranges between 3 and 5, parallelly arranged, measuring between 1 micra and 3.5 micra in length. A good number of Golgi vacuoles, generally round in shape, measuring between 0.14 micra and 2.3 micra in diameter, are present in the Golgi zones during this stage. Usually in a particular area if the large vacuoles are present the number of small vacuoles seems to be much less. These Golgi vacuoles are moderately dense to electrons due to the presence of some substances inside them which is usually observed along the periphery of the vacuoles but sometimes is also observed in the central portion. Commonly the large vacuoles are found in or near the vicinity of Golgi zones but sometimes they just migrate to towards the periphery of the cytoplasm. The vacuoles migrating to the outer portion of the cytoplasm are growing in size and embedded with fine particles. Thus they come to resemble the yolk vesicles by and by. The location, structure and size based on the above observation suggest that the Golgi vacuoles are the forerunners of yolk vesicles.

Besides these organelles several multivesicular bodies were also observed in the cytoplasm (Fig. 9). Morphologically these bodies appear to be round to oval in contour with a prominent thick limiting membrane. At some places the limiting membrane exhibits the dual nature. The size of these bodies varies widely and ranges from 0.4 micra to 1.6 micra in diameter. Several circular vesicles

measuring under 100 m micra of comparatively more dense limiting membrane are found to be sparsely distributed in the matrix of these bodies. The electron density of these bodies is moderate. Circular vesicles present in these bodies seem to be much similar to that of observed in certain developing mitochondria (Fig. 4). Thus these observations suggest the possible origin of these multivesicular bodies from mitochondria.

2) Yolk globule stage:

When the well developed yolk vesicles have occupied the outer half zone of cytoplasm, the yolk globules begin to appear in the peripheral cytoplasm in between the yolk vesicles as minute granules. Later on they are accumulated in the inner and perinuclear cytoplasm of the oocyte.

In this stage the already developed follicular cells and theca cells grow more in height than the preceding stage (Fig. 10). The zona radiata becomes thick and measures about 1.8 micra in width. The theca layer is now prominently composed of two cell rows in thickness. The outer row of cells corresponds to the theca externa while the inner one to the theca interna. Rough surfaced endoplasmic reticulum and infrequently mitochondria are also observed in the cytoplasm of the theca cells. Certain small vesicles of moderate electron density are also encountered at some places in the cytoplasm. Very prominent desmosome junctions were also observed in between the theca interna and theca externa cells (Figs. 10 & 11). Several electron dense fibres of varying length are encountered in the cytoplasm of theca interna cells.

The basement membrane becomes slightly thicker than the preceding stage of oogenesis and ranges from 0.3 micra to 0.5 micra in width. The follicular cells become more in height with a prominent elliptical nucleus having a nucleolus in the perinuclear region (Fig. 12). Rough surfaced endoplasmic reticulum is quite well developed (Fig. 13). The number of mitochondria is a little more than was observed in the preceding stage. They are usually elongated with parallelly running cristae, intra-mitochondrial granules and intra-mitochondrial particles—the largest one measuring 1.5 micra in length and 0.36 micra in width which is approximately similar to preceding stage. Golgi complexes were not observed in the present study.

Besides these organelles several small rounded vesicles, measuring less than 83 m micra, are also observed in the cytoplasm of the follicular cells (Fig. 12). The interspace between the zona radiata and follicular cells is generally quite reduced in this stage in contrast to the preceding stage and may be due to the growth of the zona radiata. Certain faint desmosome junctions were also observed in between the two neighbouring follicular cells (Fig. 12). Figure 12 seems to illustrate the close association of ovular microvilli with follicular cells.

No marked change was observed in the nucleus of the oocyte during this stage and generally the condition of nucleus remains similar to that of the preceding stage.

During this stage the rough surfaced endoplasmic reticulum is usually not very well developed and is commonly tubular and elongated in form with inflated cisternae (Fig. 14), but still at some places round to oval forms were also detected. Free ribosomes are still abundantly distributed in the cytoplasm. Golgi complexes are also encountered in the cytoplasm but their number is very few.

As the oocyte advances in growth the multivesicular bodies slightly increase in number. The limiting membrane of these bodies becomes uniformly thick and in contrast to the preceding stage dual nature is not observed at all. Beside these bodies during this stage some different bodies also make their appearance in the peripheral cytoplasm, in between the developed yolk vesicles (Fig. 15) and are identified as yolk precursors. They are comparatively less electron dense and are devoid of vesicles. The yolk precursors are almost round or slightly oval in shape, measuring about 2.4 micra in diameter, having a prominent thin but uneven and discontinuous limiting membrane. The matrix of these bodies seems to be homogeneous except that of certain clear spaces without limiting membrane, forming possibly due to the distribution of fine particles in small clusters thus leaving clear spaces. The origin of these precursors may be traced back to the developing mitochondria whose cristae are obscure and are devoid of any vesicular-like structures (Fig. 9). These precursors are supposed to be transformed into primary yolk globules. During this process the limiting membrane of precursors gradually becomes smooth, continuous and thin along with the gradual increase in the electron density of the matrix. The clear spaces, however, lose their identity during the transformation, due to the re-arrangement of fine particles in the matrix in a uniform manner (Fig. 16).

The primary yolk globules (Figs. 17 & 18), are almost round or oval in form measuring about 2.2 micra in diameter. The electron density of the matrix increases possibly due to the dense and homogeneous distribution of fine particles yet the crystalline pattern of yolk do not make its appearance. These primary yolk globules grow enormously in number and size. The full grown yolk globule (Fig. 19) is composed of three components viz. one or two main bodies, the superficial layer and limiting membrane. The main body has a high electron density and has a crystalline form. This crystalline form of the main body is in the parallel band pattern (Fig. 20). In this pattern the straight parallel bands were observed with alternating dense and less dense bands. The centre distance of these bands is measured to be 170 Å approximately.

For the formation of yolk globules two kinds of pinocytosis vesicles seem to take part. The first ones make their appearance in the peripheral region of ooplasm which ultimately gets thoroughly distributed in the cytoplasm, being more

densely distributed in the peripheral region. The second ones are round to oval in form, measuring less than 200 m micra, dense in contents with a prominent limiting membrane. Their location suggests that similar to the primary pinocytotic vesicles, these vesicles are an extra-oocyte product formed during pinocytosis. Figure 18 illustrates the presence of dense substance in the microvilli particularly at the junction of cytoplasm which might have pinched off to form these secondary type of vesicles. Both type of these vesicles are observed to be clustered around and adjacent to mitochondria (Fig. 15) but, however, the actual fusion of pinocytotic vesicles and that of mitochondria is not observed in the present observations.

Discussion

The present observations, compiled as above, mainly deal with the differentiation, growth and transformation of various cytoplasmic organelle and follicular layer, in the oocytes of the goldfish, *Carassius auratus*, during the early phase of yolk formation.

The nucleus, however, exhibits no specific transformation in these stages except that the volume of nucleus decreases with the increase of the cytoplasmic volume. Yamamoto and Yamazaki (1961) have already described this fact in the case of goldfish. The ultra-structure reveals the dual nature of nuclear envelope, running irregularly forming projections in the cytoplasm and the two membranes join each other at intervals to form pores measuring between 70 m micra and 80 m micra which is similar to the various studies made on the nuclear envelope of the oocyte of different animals. Although the pore complexes of the nuclear envelope have been thoroughly studied, its fine structure becomes clearer day by day (Afzelius, 1955; Wischnitzer, 1958; Millonig *et al.*, 1968, in sea urchin oocytes; Sugiyama *et al.*, 1963, in the oocytes of urechis; Gall, 1954, 1956 and 1967; Merriam, 1961; Takamoto, 1964, in amphibian oocytes; M. Yamamoto, 1964; Yamamoto and Onozato, 1965, in teleosts etc.). Especially the excellent work of Gall (1967) in amphibian oocytes made clear the fine structure of nuclear membrane pores. The pores in the present study are suggestive of cylinder-like shape as reported by Afzelius (1955); Wischnitzer (1958) in sea urchins; Yamamoto and Onozato (1965) in gold fish.

The nuclear matrix is sparsely filled with numerous fine particles making it lower in electron density in comparison to the surrounding cytoplasm.

A number of nucleoli are found to be distributed under the nuclear envelope in the peripheral nucleoplasm of varying sizes, round to oval shape and with irregular contours, made up of two components viz. loosely packed fine granules. The present study seems to be similar to the two components observed by Yamamoto and Onozato (1965) in the small oocytes of the goldfish. According

to Miller (1962, 1966) these two parts contain RNA and protein, the synthesis and accumulation of RNA takes place in the loose and granular part along with the accumulation of extra-nucleolarly synthesized protein. But due to certain limitations the present study does not deal with the various nucleolar components, therefore does not need to discuss the detail of nucleolar components.

The nucleo-cytoplasm exchange has been reported widely by various authors i.e. Pollister *et al.* (1954) in frog; Anderson and Beams (1956) in *Rhodinus*; Ornstein (1956); Miller (1962); Lanzavecchia (1962) in *Rana*; Balinsky and Devis (1963) in *Xenopus*; Takamoto (1964) in *Triturus*. They have described the transfer of fine granular material from nucleus to the cytoplasm through the nuclear pores. While Schjeide *et al.* (1964) in the case of birds are of the opinion that there exists a common mode of transfer of nuclear material to the cytoplasm through the nuclear pores, which are not open holes, which is by dis-association of vesicles arranged at the pore site on the nuclear side into very minute vesicles and re-emergence of small vesicles on the cytoplasmic side which re-assemble into large vesicles. Kessel (1966 a & b); Lanzavecchia (1964); Miller (1967); Sotelo and Porter (1959); Szollosi (1965) are also concerned with the problem of nucleo-cytoplasmic exchange.

In the present study the nucleo-cytoplasmic exchange is observed very faintly in the form of very minute vesicles sometimes arranging in the form of a thread. But, however, the presence of certain vesicular-like structures at the pore site on the nuclear side as well as on the cytoplasmic side, suggests the transfer of nuclear material in the form of very minute vesicles similar to the opinion expressed by Schjeide *et al.* (1964) in birds and Yamamoto and Onozato (1965) in the goldfish.

1) Cytoplasmic organelles:

Annulated lamellae:

During the course of present study the authors came across certain isolated instances of the presence of annulated lamellae in the oocyte of the gold fish, resembling closely to that described in various animals by different authors; Afzelius (1955) in sea urchin; Swift (1956) in snail and clam; Rebhun (1956) in sea clam and pulmonate snail; Merriam (1959) in sand dollar; Okada and Waddington (1959) and Waddington and Okada (1960) in *Drosophila melanogaster*; Zahnd and Porte (1963) in a teleost, *Barbus conchoniis*; Yamamoto and Onozato (1965) in goldfish; Kessel (1963) in *Necturus*; Kessel (1965) in tunicates; Kessel and Beams (1969) in dragon fly and the "System of pitted membranes" described by Balinsky and Devis (1963) in *Xenopus* seems to correspond to the annulate lamellae described by the above authors. The annulate lamellae consist of two parallel running membranes perforated by a series of pores or annuli.

The origin of annulate lamellae from the nuclear envelope has been demonstrated by Afzelius (1955); Swift (1956); Rebhun (1965); Kessel (1963, 1965) and Okada and Waddington (1959) but in the present study, though the annulate lamellae is much similar to the nuclear envelope, the actual transformation of nuclear envelope into annulate lamellae has not been observed at all. A similar opinion was also expressed by Yamamoto and Onozato (1965) in the first growth phase of gold fish oocyte. Suggestive figures of the frequent presence of vacuoles at the end of lamellae and the prolongation of their ends into endoplasmic reticulum are observed in the present study. Regarding the possible formation of endoplasmic reticulum of a vesicular kind from annulate lamellae, the present authors agree with the views of Balinsky and Devis (1963) who have reported that the endoplasmic reticulum is equivalent to "pitted membrane" and the function of these pitted membranes is to produce the masses of vesicular endoplasmic reticulum. However, the present authors could not find any evidence of different functions assigned to annulate lamellae as the transfer of genetic specificities from nucleus to cytoplasm (Swift, 1956). The present authors partly agree with Merriam (1959) where he stated that the necessary synthetic mechanism and specificities must reside in the structure of annulate lamellae and Kessel (1965) for his statement that specific forces and activity may be selectively localized in the region of annulate lamellae. The present authors do not agree with Waddington and Okada (1960) where they considered that as the double and porous membrane markedly increases in the cytoplasm of degenerating *Drosophila* oocytes and as such these organelles are considered not to represent the functional organelles. The present authors did not study the degenerating oocytes of goldfish and thus we are not able to give any opinion regarding it. The present authors feel that as these organelles are very rarely occurring in these stages of oogenesis within the scope of the present study the functional role of these organelles in the growing oocytes needs further investigations.

Golgi complexes:

Well developed Golgi complexes were observed during this study. Although the structure almost remains the same in both the growing stage of goldfish oocyte described here except that the number of these organelles is more in yolk vesicle stage than the primary yolk globule stage. Golgi complexes consist of well developed flattened sacs, Golgi vesicles and Golgi vacuoles. M. Yamamoto (1964) in *Oryzias* and Adams and Hertig (1964) in guinea pig have reported that Golgi complexes consist of only flattened sacs and Golgi vesicles, while these complexes lack Golgi vacuoles which, however, is different from the present observations as far as Golgi vacuoles are concerned. The structure and components of Golgi complexes observed in this study have also been observed by Afzelius (1956) in sea urchin; Yamada *et al.* (1957) in mouse; Odor (1960) in rat; Takamoto (1964) in

Triturus; Yamamoto and Onozato (1965) in goldfish; Yamamoto and Oota (1967) in zebra fish. Golgi vacuoles in this study are always observed in the well developed Golgi complexes, spherical in shape with moderate density and from one to many in number. Suggestive figures for the possible formation of Golgi vesicles and Golgi vacuoles, formed in the Golgi complexes, being observed by the present authors. As far as the role of Golgi complexes is concerned, the present authors believe that the Golgi vacuoles, formed in the Golgi complexes, ultimately give rise to yolk vesicles. Further the large vacuoles show the structure suggestive of a precursor of yolk vesicle which are mucoprotein in nature (cf. K. Yamamoto, 1958). Thus it seems very probable that the Golgi complexes play an important role leading to the formation of yolk vesicles in the growing oocytes of goldfish and confirms the views of Afzelius (1956), in sea urchin oocytes; Balinsky and Devis (1963) in *Xenopus*; Yasuzumi and Tanaka (1957) in pond snail; Yamamoto and Onozato (1965) in the first growth phase of goldfish oocytes and Yamamoto and Oota (1976) in zebra fish, where they have expressed a similar opinion as regards the formation of yolk vesicles. While Droller and Roth (1966) in *Lebistes reticulatus* and Kessel (1964) in tunicates have suggested that Golgi complexes are associated with the formation of proteid yolk which seems not to be agreeable to us.

Mitochondria:

Mitochondrial rosettes are very rarely encountered in the yolk vesicle stage and not observed in the primary yolk globule stage during the present observations in the goldfish oocyte, suggesting that the presence of rosettes may be a characteristic of the first growth phase in the goldfish oocyte. Miller (1962); Lanzavecchia (1962); Balinsky and Devis (1963) in frog's oocytes; M. Yamamoto (1964) in teleost; Yamamoto and Onozato (1965) in the goldfish oocytes; Adams and Hertig (1964) in guinea pig oocyte have demonstrated the occurrence of mitochondrial rosettes.

Balinsky and Devis (1963); Adams and Hertig (1964) and Yamamoto and Onozato (1965) maintained that the mitochondrial rosettes appear to be concerned with the proliferation of mitochondria because certain developing mitochondria are found to be attached to the rosettes, possessing an incomplete limiting membrane at the point of contact and the communication of the matrix of both i.e. mitochondria and core substance of rosettes. The similar characteristics were also observed in the present study. This leads to the belief that the formation of mitochondrial rosettes may be related to the proliferation of mitochondria due to the transfer of core substance into developing mitochondria.

Ornstein (1956); Lanzavecchia (1962); Miller (1962) and Balinsky and Devis (1963) and Yamamoto and Onozato (1965) have insisted that the core substance may be nuclear in origin. However, M. Yamamoto (1964) and Adams and Hertig

(1964) do not think so as they did not find any suggestive figures. In the present study also we have very rarely observed the phenomenon of nuclear-cytoplasmic exchange through the porous nuclear envelope. So with a very limited number of figures it seems unlikely for us to arrive on same firm conclusions regarding this phenomenon and the formation of mitochondrial rosettes.

2) Formation of proteid yolk:

The already developed mitochondria, with a marked parallel running cristae, prominent double limiting membrane and containing intra-mitochondrial granules and particles in the first growth phase of goldfish oocyte (cf. Yamamoto and Onozato, 1965), undergo a marked change prior to the formation of yolk globule containing proteid yolk. The cristae and double limiting membrane becomes obscure. In the present investigation, during this stage, two sets of developing mitochondria are observed. The first type seems to contain some circular vesicles measuring less than 66 m micra in diameter and while the other set is devoid of these vesicular structures.

In between these two developing mitochondria and primary yolk globules, two morphological different bodies are referred to as intermediate stage. The first of these kinds is multivesicular bodies.

Various suggestive figures were observed in the present study leading towards the supposition that these bodies may be transformed into primary yolk globule by gradual disappearance of circular vesicles and formation of a large amount of fine particles. The other kind is yolk precursors which definitely lack the circular vesicles and its matrix is homogenously filled with numerous fine particles, sometimes with some limiting membrane and devoid of clear spaces. During the process of the transformation of precursors into primary yolk globules, numerous fine particles already present further increase in number. Thus our observations lead us to suppose that probably the transformation of mitochondria into primary yolk globule undergoes through two parallel but different channels i.e. in one process the intermediate product may be multivesicular bodies and in the second one it may be yolk precursors. The mitochondrial origin of proteid yolk has already been reported in amphibian oocyte by Lanzavecchia (1960, 1966); Ward (1962); Balinsky and Devis (1963) and Tokins (1964), in molluscan oocyte by Carraso and Favard (1958), in zebra fish by Yamamoto and Oota (1967). Though the main body of the mature yolk globule is crystalline in structural pattern in the above mentioned animals, but Lanzavecchia (1960, 1966); Ward (1962); Tokins (1964) and Carraso and Favard (1958) have stated that the crystalline bodies appear early in the mitochondria with double limiting membrane and a few intact cristae. However, in the goldfish the crystalline bodies are observed only in mature yolk globules. Even in the primary yolk globule

stage the crystalline structural pattern is not noticed at all. The primary yolk globule is homogenous in nature and packed densely with numerous fine particles which resembles the observations of Yamamoto and Oota (1967) in zebra fish oocyte. Balinsky and Devis (1963) have also demonstrated in *Xenopus* oocyte the presence of crystalline pattern in the proteid yolk formed through the non-crystalline primary yolk platelets with a limiting membrane. They further added that in *Xenopus* the cristae of mitochondria are changed into stacks of concentric membranes and then transformed into a more densely packed body similar to the primary yolk platelets while in our study on the goldfish and also in zebra fish (Yamamoto and Oota, 1967), the cristae of mitochondria becomes obscure and fine particles, which are a main component of primary yolk globule, appear in the matrix of mitochondria. Balinsky and Devis (1963) in *Xenopus* have suggested that the multivesicular bodies are possibly formed from mitochondria and ultimately get transformed into yolk platelets and pigment granules. Lanzavecchia (1966) has stated that in the amphibian oocyte the proteid yolk is originated from multivesicular bodies. Suggestive figures in our present investigations, make us to think that the multivesicular bodies and yolk precursors, probably have a common origin from mitochondria and a common fate awaits them viz. the transformation into primary yolk globules.

In the present investigations also the mature yolk globule is composed of three components i.e. crystalline main body, superficial layer and the limiting membrane.

3) Follicular layer and pinocytosis:

The follicle in the present investigations consists of usual one row of follicular cells surrounded by two rows of thecal cells. The thecal layer is separated from the underlying follicular epithelium by a prominent, rather regular basement membrane (Sterba and Müller, 1962; Müller and Sterba, 1963; M. Yamamoto, 1963; Jollie and Jollie, 1964; Yamamoto and Onozato, 1963; in different teleosts). Desmosome junctions are also observed in between the two neighbouring cells in both follicular and thecal layer. In our material we have observed markedly the presence of follicular microvilli though smaller in size than the ovular microvilli. The presence of these microvilli is also reported in so many teleosts by several authors but M. Yamamoto (1963) in *Oryzias* and Jollie and Jollie (1964) in *Lebistes* have denied the presence of follicular microvilli. According to Kemp (1956) the microvilli are considered to increase the absorptive or secretive surface. The transport of extra oocytic nutrient secretions into the ooplasm through the micropinocytosis has been emphatically reported by Anderson and Beams (1960) in vertebrates; Jollie and Jollie (1964); Yamamoto and Onozato (1965) and Yamamoto and Oota (1967) in teleosts. The morphological evidence also leads

us towards this interpretation in our investigations. In our material the pinocytotic vesicles are found in moderate number in the peripheral ooplasm beneath the zona radiata and in the proximity of microvilli. They seem to cluster around the developing mitochondria, sometimes very adjacent, and yolk precursors. Bennett (1963 a) stated that pinocytosis is a kind of active transport mechanism induced by the movement of surface membranes. During the synthesis of yolk the intake of extra oocytic nutrient substances through pinocytosis has been reported by many workers (Knight and Schechtman, 1954; Glass, 1959; Telfer, 1960 and 1961; Kessel and Beams, 1963; Anderson, 1964; Ramamurty, 1964; Roth and Porter, 1964; Stay, 1965 and Yamamoto and Oota, 1967). But with the exception of Yamamoto and Oota (1967) the remainder of the above authors have not mentioned the role of pinocytosis in relation to the proteid yolk synthesis. Droller and Roth (1966) and Yamamoto and Oota (1967) have also identified two kinds of pinocytotic vesicles, which is similar to our observations.

Summary

The electron microscopic investigation of the growing oocyte of gold fish during the second growth phase limiting to the yolk vesicle stage and primary yolk globule stage is summarized as follows:

1. Annulate lamellae are encountered very rarely and these organelles are supposed to be originated in the endoplasmic reticulum. The endoplasmic reticulum seems to be quite well developed and is usually of slender, tubular form with flattened cisternae, though oval to round forms are also observed but very rarely. The possible role of endoplasmic reticulum may be the transport of necessary functional material to Golgi complexes and mitochondria.

2. Well developed Golgi complexes are observed during the stages described in the present study and supposed to play the leading role in the formation of yolk vesicles containing carbohydrate yolk.

3. During the process of the development of mitochondria the cristae and double limiting membrane become obscure. The mitochondria develop and give rise to primary yolk globule containing proteid contents. However, the transformation of mitochondria into the primary yolk globules supposed to take place in two different channels. The intermediate product of the first process may be multivesicular bodies which are very frequently observed during these stages and of the other processes may be yolk precursors, filled with numerous fine particles. The structure of multivesicular bodies and yolk precursors differ widely.

4. The mature yolk globule seems to consist of three components viz. crystalline main body, superficial layer and a limiting membrane. The crystalline main body is composed of parallel running cristae.

5. The follicle consists of one row of follicular cells with well developed

mitochondria, endoplasmic reticulum and sometimes Golgi complexes. The theca layer consists of two rows- the external row of theca externa and the internal row of theca interna. The basement membrane, which is quite prominent in appearance, lies in between the theca cells and follicular cells. Sometimes desmosome junctions are also observed in between the two neighbouring cells of theca as well as follicular layer. Several micro-tubular projections of ovular as well as follicular nature, are observed in the interspace in between the zona radiata and zona granulosa, however, no cytoplasmic communication between the ovular microvilli and follicular microvilli is observed.

6. A sufficient number of primary and secondary pinocytotic vesicles are observed in the peripheral cytoplasm near the microvilli also. These pinocytotic vesicles sometimes are also observed to surround the developing mitochondria and yolk precursors. These vesicles are supposed to play some role in the formation of proteid yolk.

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Explanation of Plates

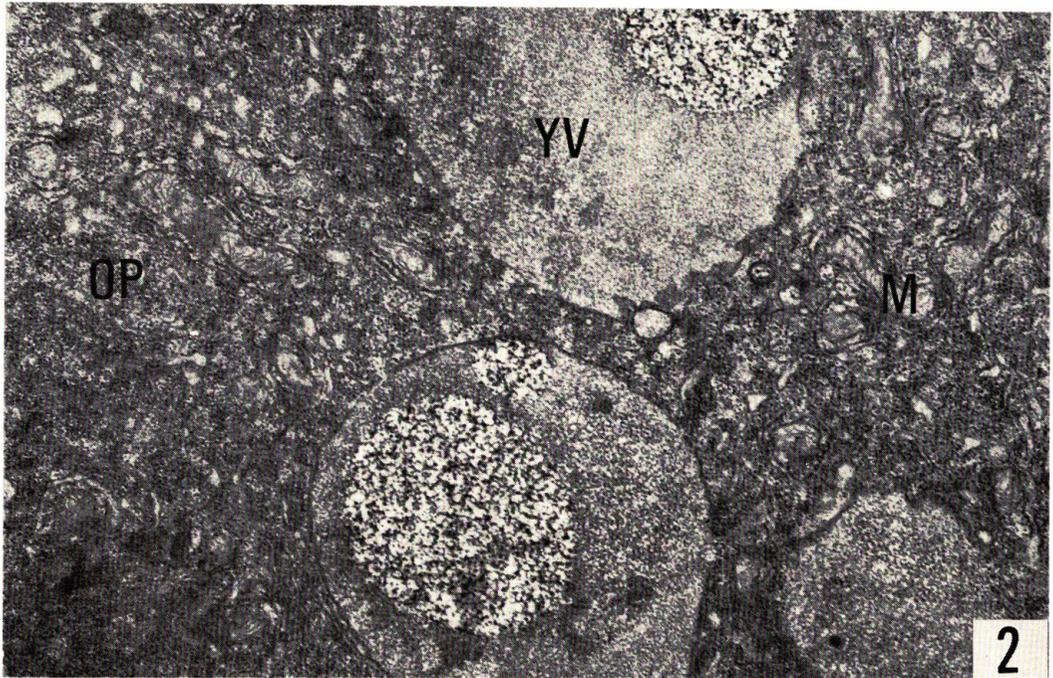
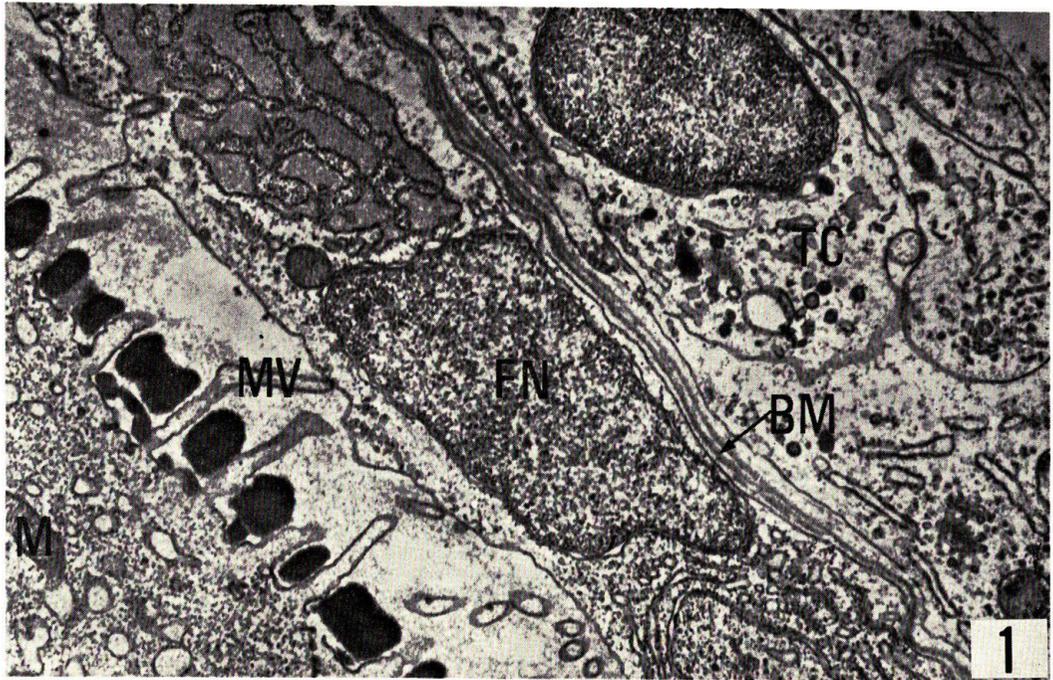
Abbreviations

AL, annulate lamella;	BM, basement membrane;
DJ, desmosome junction;	FN, follicular epithelia cell nucleus;
G, Golgi complex;	GS, Golgi sac;
GV, Golgi vacuole;	GVe, Golgi vesicle;
IG, intra-mitochondrial granule;	IP, intra-mitochondrial particle;
L, lysosome;	LM, limiting membrane;
M, mitochondrion;	MB, multivesicular body;
MBy, main body;	MR, mitochondrial rosette;
MV, microvillus;	MYG, mature yolk globule;
NE, nuclear envelope;	NU, nucleolus;
ON, oocyte nucleus;	OP, ooplasm;
PeYG, precursor of yolk globule;	PPV, primary pinocytotic vesicle;
PYG, primary yolk globule;	PV, pinocytotic vesicle;
SPV, secondary pinocytotic vesicle;	SL, superficial layer;
TC, theca cell;	
YV, yolk vesicle;	ZR, zona radiata.

PLATE I

Fig. 1. Low-power micrograph of the oocyte at the early phase of yolk vesicle stage. The oocyte is thoroughly enveloped by the follicular layer. Theca cells are well developed. $\times 22,000$

Fig. 2. A low power micrograph showing the structure of yolk vesicles. $\times 19,200$



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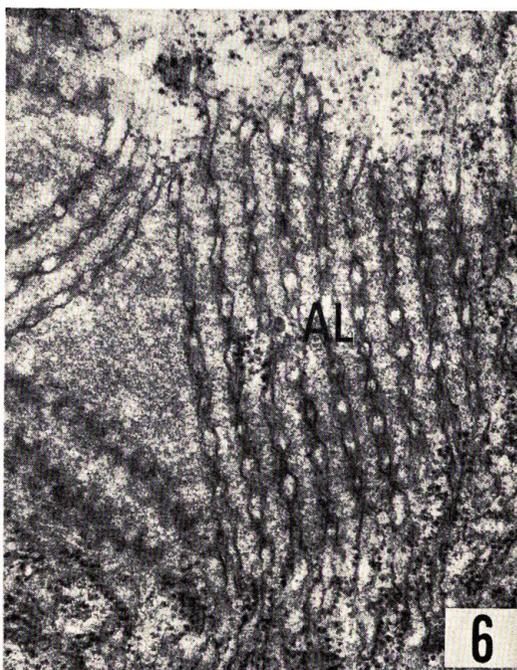
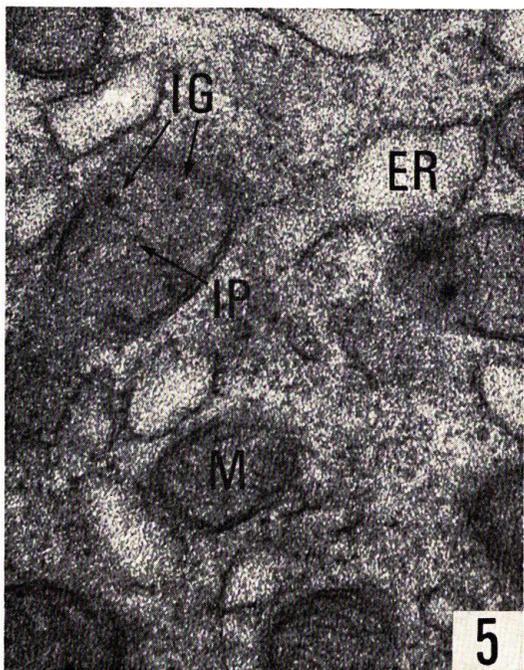
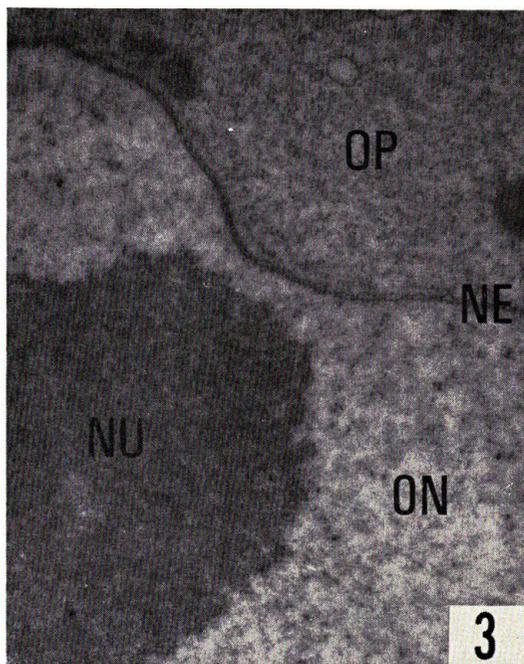
PLATE II

Fig. 3. The irregular contour of nuclear envelope which is double membraneous and is perforated by cylinder-like pores. $\times 48,000$

Fig. 4. Mitochondrial rosette near the perinuclear region of cytoplasm. Several mitochondria are observed to be attached with the core substance. It seems that the matrix of one of the mitochondria is in communication with the matrix of the core. $\times 22,000$

Fig. 5. During the similar stage, mitochondria with intra-mitochondrial granules and intra-mitochondrial particles. $\times 84,000$

Fig. 6. Isolated instances of annulate lammellae are also observed during this stage of the oogenesis. These seems to be no marked change in this organelle than the preceding stage. $\times 20,000$



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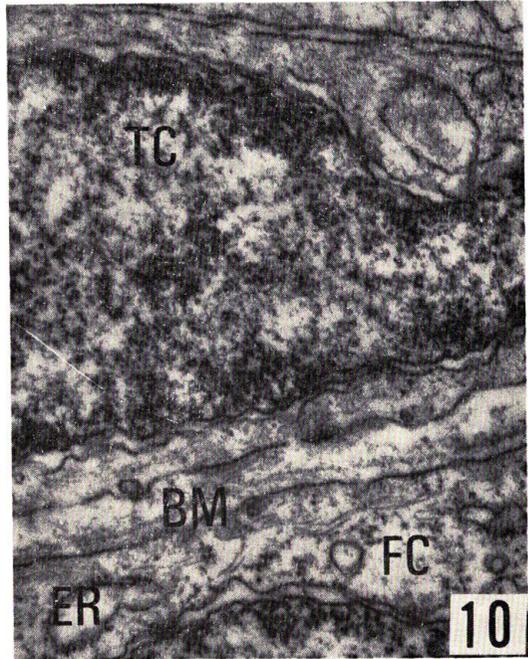
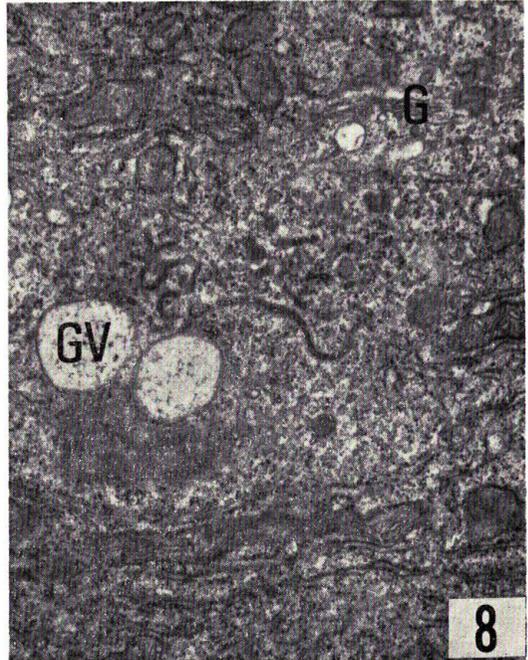
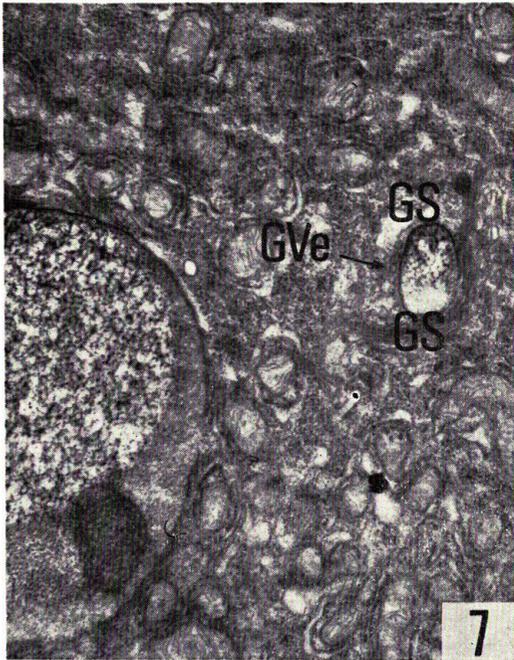
PLATE III

Fig. 7. Golgi complex with developed flattened sacs with quite prominent Golgi vacuoles. $\times 20,400$

Fig. 8. Golgi complex, though less in number but quite well developed. $\times 30,000$

Fig. 9. This micrograph exhibits the developing stages of mitochondria and multi-vesicular body. $\times 24,800$

Fig. 10. Follicular envelope, during the primary yolk globule stage, showing well developed theca cell and follicular cell with prominent basement membrane in between. $\times 16,400$

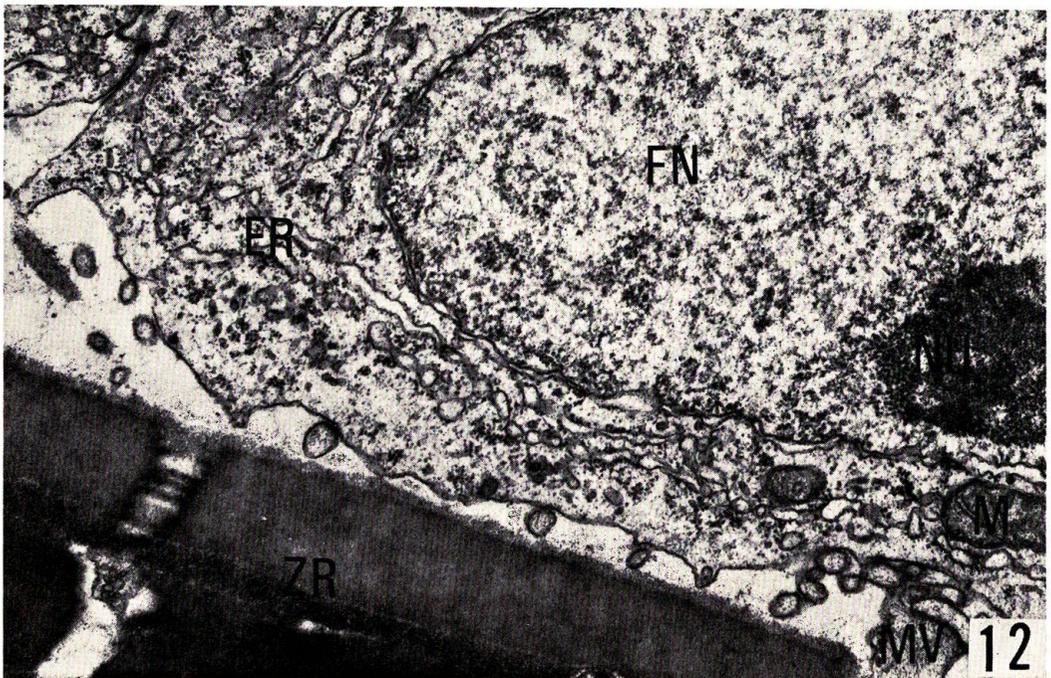
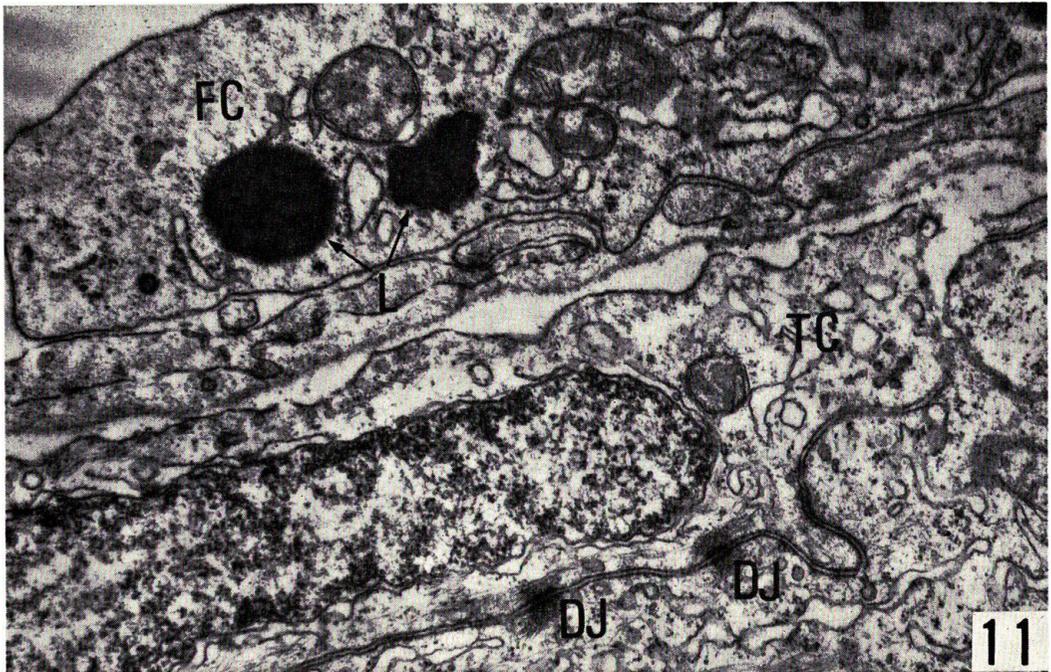


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PLATE IV

Fig. 11. This figure illustrates at least the presence of three prominent desomesome junctions between the two neighbouring cells of theca. Lysosomes are also observed in the follicular cell. $\times 20,000$

Fig. 12. Follicular microvilli. Some small vesicles are observed in the ovular microvilli, probably pinocytotic in nature. The nucleus of follicular cell possesses a nucleolus. $\times 20,000$



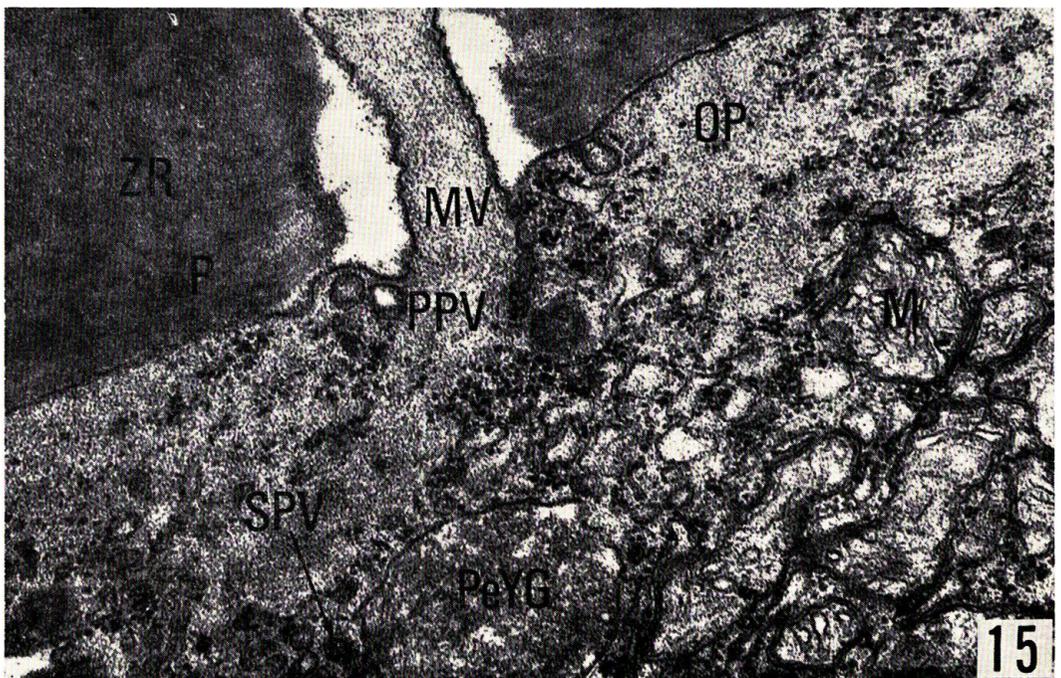
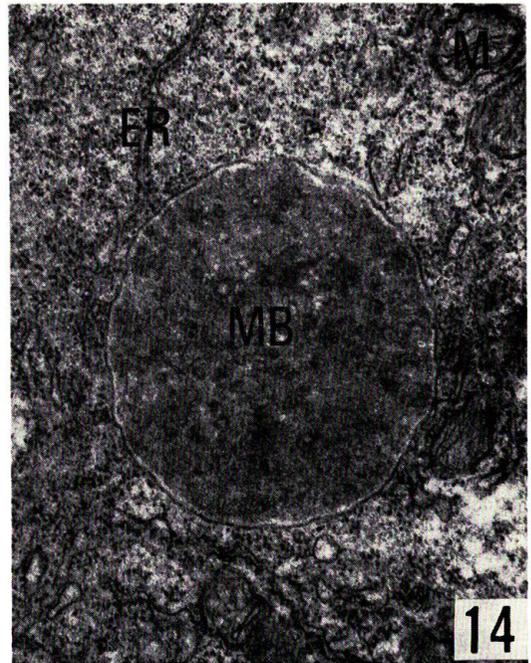
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PLATE V

Fig. 13. The well developed rough-surfaced endoplasmic reticulum in the cytoplasm of the follicular cell. $\times 32,000$

Fig. 14. The developing stages of mitochondria and multivesicular body. $\times 37,000$

Fig. 15. Primary and secondary pinocytotic vesicles are illustrated by this micrograph during the primary yolk globule stage, particularly present in the peripheral ooplasm. Note the irregular contour and diminishing cristae of the mitochondria. $\times 24,000$



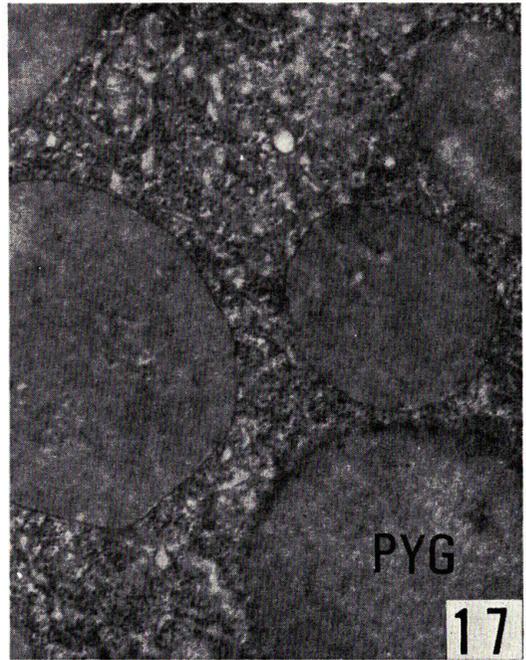
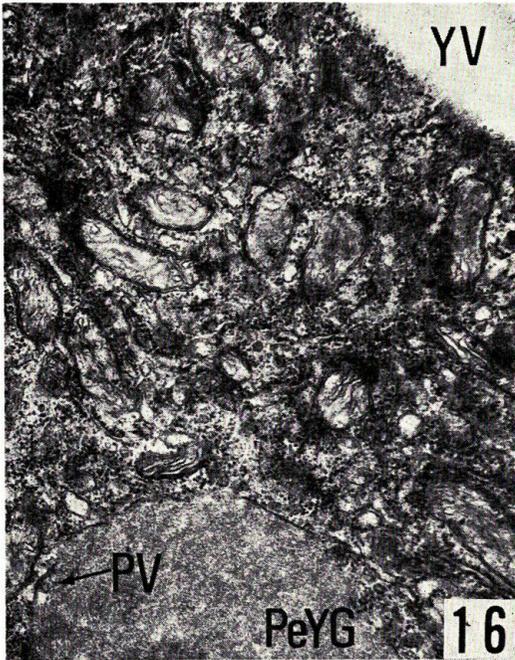
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PLATE VI

Fig. 16. The ingestion of pinocytotic vesicles by the yolk precursors may be seen in this micrograph. $\times 34,400$

Fig. 17. Some primary yolk globules with homogeneous matrix dispersed with numerous dense fine particles. $\times 23,000$

Fig. 18. The figure illustrates the presence of yolk precursors in the similar as above. Several pinocytotic vesicles are found to be distributed near zona radiata and yolk precursor. $\times 12,000$

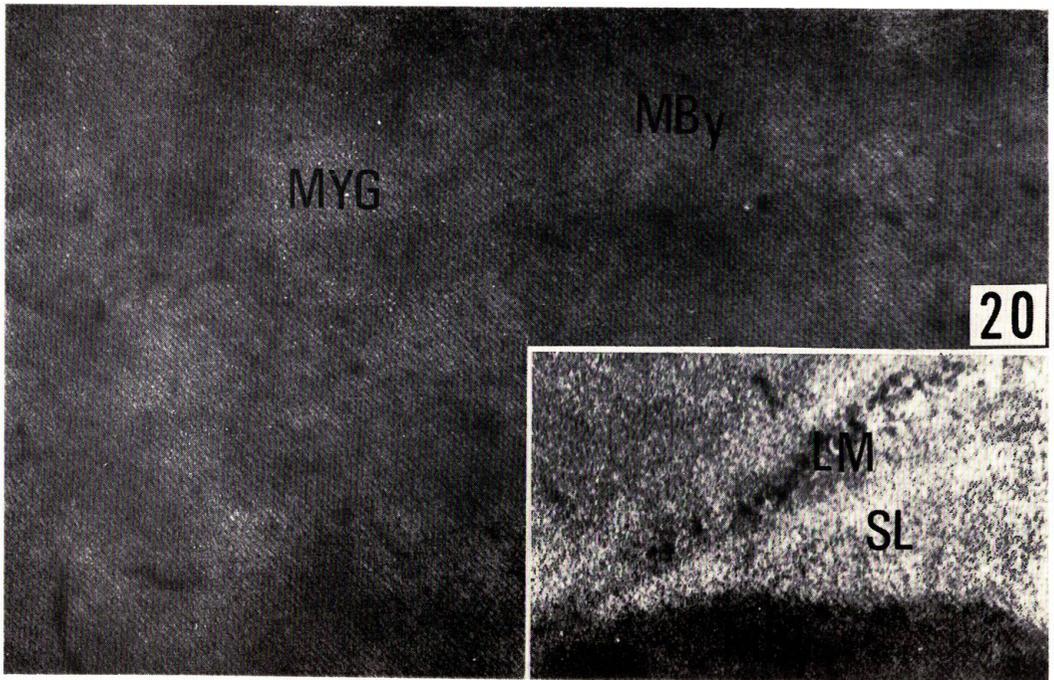
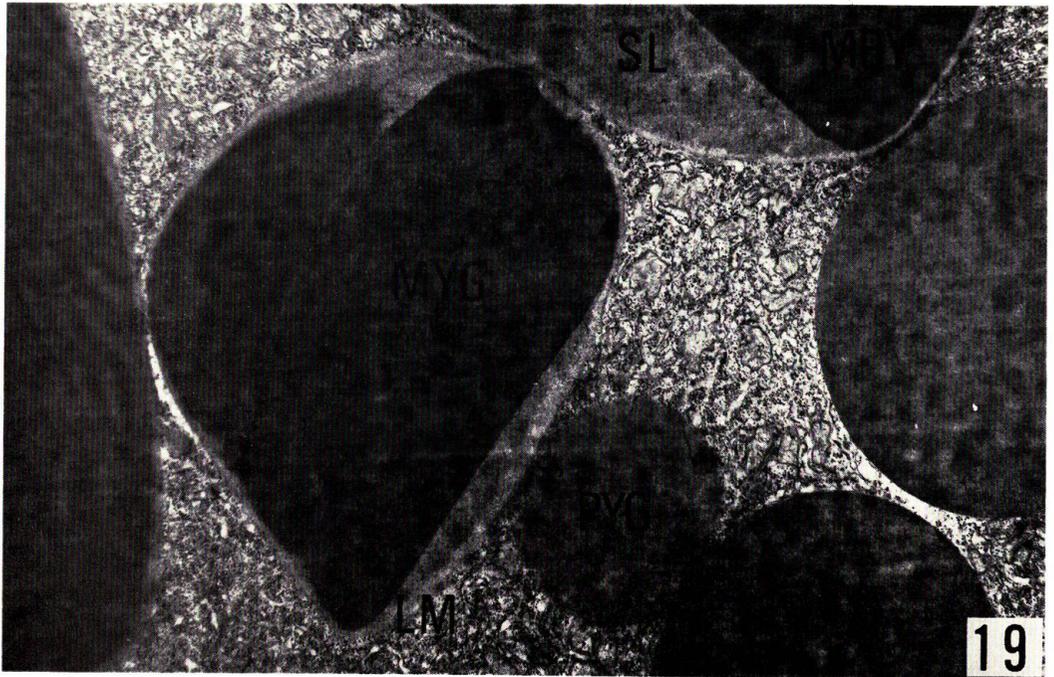


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PLATE VII

Fig. 19. The mature yolk globule with one to two main bodies, superficial layer and limiting membrane. $\times 8,400$

Fig. 20. This micrograph, magnified very highly, exhibits the parallel, uniform pattern of crystalline proteid yolk. $\times 140,000$. Inset micrograph illustrates the superficial layer and limiting membrane. $\times 140,000$



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