A FINE STRUCTURAL INVESTIGATION OF OOGENESIS IN THE BROWN SHRIMP *PENAEUS AZTECUS*

A Thesis Presented to the Faculty
of the Department of Biology
University of Houston

In Partial Fulfillment of the
Requirements for the Degree
Master of Science

by

Frederick L. Hill

May 1974

ACKNOWLEDGEMENTS

The author wishes to thank Dr. W. H. Clark for his efforts toward the completion of this study. Thanks are also extended to Dr. R. A. Neal for supplying animals for this study.

Technical assistance from K. R. Wilson and M. Kleve was greatly appreciated.

Special appreciation is expressed for the invaluable assistance of Dr. L. E. Franklin in overcoming technical pitfalls.

The author wishes to thank both Judy Hill and Lucy Wilmeth for their efforts at the typewriter.

A FINE STRUCTURAL INVESTIGATION OF OOGENESIS IN THE BROWN SHRIMP *PENAEUS AZTECUS*

An Abstract of A Thesis Presented to the Faculty
of the Department of Biology
University of Houston

In Partial Fulfillment of the
Requirements for the Degree
Master of Science

by

Frederick L. Hill

May 1974

ABSTRACT

The ovary of Penaeus aztecus is located in the dorsal cephalothorax and consists of anterior, lateral, and posterior lobes. Follicular egg development is exhibited, with the follicular cell layer present until spawning. Vitellogenesis may be divided into early and late production of yolk materials. The swelling of short discontinuous elements of rough endoplasmic reticulum is responsible for the early (intracellular) aspect of yolk production. Micropinocytotic uptake of materials at the oolemma and the subsequent fusion of the micropinocytotic vesicles contribute to the late (extraoocytic) phase of yolk platelet production. The most striking morphological attribute of the mature oocyte is the presence of large cortical rods. These rods demonstrate a partial cortical reaction prior to spawning and a subsequent "explosive" ejection of their contents at spawning.

TABLE OF CONTENTS

INTRODUCTION

Oogenesis in the phylum Arthropoda has been the subject of several excellent histological and fine structural investigations in recent years (see Hinsch, 1969; Kessel, 1968; and Cone and Scalzi, 1967 for review). The majority of this work has been concerned with the vitellogenic stages of oogenesis from members of the Insecta (see Anderson, 1969 for review), Crustacea (see Hinsch, 1969 for review), Xiphosura (Dumont and Anderson, 1967), and Pycnogonidae (King and Jarvis, 1970). These studies have documented interesting variations in the mechanisms of yolk production among members of this phylum.

The insects tend to rely upon extracellular manufacture of yolk proteins and subsequent uptake of these proteins by the developing ova through the process of micropinocytosis; while, the crustaceans rely principally upon intracellular yolk synthesis. Some workers have noted pinocytosis in crustaceans at the time of vitellogenesis (Hinsch, 1969; Kessel, 1968; and Beams and Kessel, 1963); however, a direct correlation between this micropinocytosis and yolk sphere formation has not been established. In the class Xiphosura both intracellular synthesis and micropinocytotic accumulation of preformed elements contribute to the total yolk content of the mature ova (Dumont and Anderson, 1967).

It is interesting that little emphasis has been placed on the cortical cytoplasm in the above studies. Nor, for that matter have

organelles distinct to the cortical cytoplasm (for example, cortical granules) ever been described in any of these studies.

Several light microscopic studies have been done on oogenesis in the marine prawns (Penaeids) (see Caillouet, 1972; and Burukovski, 1969 for review), however, to our knowledge no fine structural studies are found in the literature. This is surprising since mature ova from species of the Penaeidae exhibit extremely large cortical inclusions which are "explosively" ejected from the eggs at spawning (Hudinaga, 1942). It is also interesting that these animals have received little attention when one considers their economic importance.

We have initiated fine structural studies of oogenesis in the brown shrimp, Penaeus aztecus. This report deals specifically with the cellular mechanisms of vitellogenesis and cortical rod formation in the developing ova of P. aztecus.

MATERIALS AND METHODS

Female brown shrimp, Penaeus aztecus, which were gravid, immature, or in varying stages of ovarian maturation were collected from the Gulf of Mexico near Galveston, Texas, by trawling and were subsequently maintained in aerated aquaria. Ovaries were removed through the dorsal area of the carapace and further dissection of the ovarian tissues was carried out while these tissues were submerged in a paraformaldehyde-glutaraldehyde fixative (Karnovsky, 1965) at room temperature. After complete distention of the ovarian contents, the tissue was transferred to fresh fixative for one hour. The tissue was subsequently washed with a phosphate buffer (Millonig, 1961) for one hour (three, twenty minute changes) and post fixed in 1.0% osmium tetroxide buffered with 0.1 M phosphate for one hour at 1-2 °C. The tissues were dehydrated in a graded acetone series, embedded in a low viscosity epoxy resin (Spurr, 1969), and sectioned on either a Sorvall MT-2 ultramicrotome or an L.K.B. 4800 ultramicrotome with glass knives. Thin sections were mounted on uncoated copper grids, stained with methanolic uranyl acetate and lead citrate (Venable and Coggeshall, 1965), and examined with a Hitachi HS-8 electron microscope. Thick plastic sections (0.5 u), for light microscopy, were stained with 0.25% aqueous toluidine blue in 0.15% sodium borate (Dewel and Clark, 1974).

RESULTS

The ovary of the brown shrimp P. aztecus is composed of a common body and several lobes. The common body of the ovary is located beneath the carapace of the cephalothorax. It is posterior to the digestive gland and dorsal to the heart. Two anterior lobes extend into the head region, and fourteen lateral lobes (seven on each side) extend ventrally to the region of the gills. Two short posterior lobes are present, and two posterior lobes extend to the telson (fig. 2).

To facilitate description we have divided oogenesis into four main stages which can be easily determined at the light microscopic level (fig. 1).* The first stage (prefollicular) consists primarily of late oogonia and early oocytes just entering the first meiotic prophase. The second stage (early follicular) is characterized by the presence of a distinct layer of large, cuboidal follicular cells. The third stage (late follicular) is divided into two distinct phases; an early phase (yolk sphere) where large yolk spheres become evident with the subsequent attenuation of the follicular layer, and a late phase (cortical rod) where cortical specializations become prominent. The fourth stage (mature oocyte) has the appearance characteristic of oocytes just prior

* Other investigators have proposed various schemes for the staging of penaeid oogenesis (Hudinaga, 1942; King, 1948; Oka, 1967; Burukovskii, 1970). Our stages differ from those proposed in the past. This difference does not represent disagreement in results, but, rather reflects the use of advanced techniques.

to spawning (fig. 1). Prefollicular oogonia are present within the ovary at any time during maturation, however, oocytes of only one of the remaining stages are present at any particular time. Oogenesis beyond the prefollicular stage is synchronous.

Prefollicular Stage

Prefollicular oogonia are oval cells, approximately 65u in diameter, containing large centrally located nuclei and a relatively small amount of cytoplasm. The nuclei contain several round nucleoli dispersed around the peripheral karyoplasm (fig. 3).

At the fine structural level the cytoplasm of the prefollicular oogonium is granular in nature and conspicuously lacks organelles with the exception of a few mitochondria and aggregates of electron dense material closely apposed to the nuclear membrane. These aggregates are associated with nuclear pores, and aggregates of similar electron density are observed in the adjacent karyoplasm. Closely associated with the nucleoli are several electron dense nucleolar emmissions (fig. 4).

As growth continues short segments of rough endoplasmic reticulum are observed within the cytoplasm. These reticular elements become dialated with an electron dense material (fig. 5).

Early Follicular Stage

The oocyte has now reached a diameter of approximately 90 u and a distinct layer of follicular cells is present. The follicular cells are cuboidal in shape, approximately 18 u in diameter, and have cen-

trally located nuclei (fig. 6). At the fine structural level the peripheral karyoplasm contains dense heterochromatic material (fig. 7), and the cytoplasm contains numerous mitochondria and large amounts of rough endoplasmic reticulum. A basement lamina is present at the periphery of the follicle cell layer. However, no such lamina is observed between the lateral surfaces of adjacent follicle cells. While there is little membrane interdigitation between adjacent follicle cells, interdigitation is pronounced between follicular cell membranes and the oolemma of an adjacent oocyte (fig. 7).

At the light microscopic level the early follicular oocytes have centrally located nuclei which contain large, irregularly shaped nucleoli closely associated with the nuclear envelope. The cytoplasm appears homogeneous with the exception of light staining patches which are frequently observed.

At the fine structural level the ooplasm contains numerous dialated reticular elements. Dialation of these elements continues with the concomittant appearance of small (47.5 mu in diameter) electron dense intracisternal granules (fig. 8). At this stage of development the cytoplasm is densely packed with ribosome-like particles, mitochondria are abundant in the perinuclear cytoplasm, and the nuclear envelope is "honeycombed" with pores (fig. 10).

Late Follicular Stage

Yolk sphere: at the light microscope level the follicle cells appear attenuated. They contain centrally located elliptical nuclei

which display darkly stained heterochromatic material in their peripheral karyoplasm. The cytoplasm appears quite dense at this time (fig. 9). At the fine structural level electron dense heterochromatic material is evident in the peripheral karyoplasm. No nucleoli are present. The cytoplasm contains great quantities of rough endoplasmic reticulum. Many vesicular elements are present with which ribosome-like particles are associated. Mitochondria are present in fair numbers and are randomly spaced throughout the cytoplasm. A basement lamina is present at the periphery of the follicle cell layer and a distinct intercellular space, containing a flocculent material, is apparent between the follicle cells and oocyte (fig. 11).

At the light microscope level the oocyte is a "pear shaped" cell approximately 250 u in diameter which contains a centrally located nucleus. This nucleus contains several nucleoli located in the peripheral karyoplasm. In contrast to the early follicular stage oocyte, these nucleoli are round and vary in size (compare fig. 6 to fig. 9). Several dark staining inclusions, believed to be yolk spheres, are present in the cytoplasm (fig. 9).

At the fine structural level numerous changes in the oocyte architecture are noted during this period. The oolemma contains many caveolae at this time (fig. 14) and microvilli (0.1 u in diameter) are found at regular intervals about the perimeter of the oocyte (figs. 11, 12, 13, and 14). These microvilli project to a region of close proximity to the follicle cell membranes (figs. 11, 12, and 13) and in some

instances are seen projecting into the cytoplasm of a follicle cell (fig. 12). In such instances the oolemma appears continuous with the plasmalemma of the follicle cell, suggesting cytoplasmic continuity. Granular material and microfilaments are apparent within the microvilli.

Numerous micropinocytotic vesicles are seen "pinching off" the oolemma (fig. 13) and are frequently observed in the cortical ooplasm (fig. 13). Their contents closely resemble the electron dense material found in the intercellular space and caveolae. Aggregates of pinasomes are randomly distributed throughout the ooplasm. These aggregates appear to represent fusion centers which give rise to the yolk spheres. Continuity between the contents of the pinasomes and the matrix of the maturating spheres is evident (fig. 15). The periphery of the growing spheres is electron dense while fusion continues (fig. 15). Once the spheres reach a diameter of approximately 4 u their peripheral density is lost and fusing pinasomes are no longer evident (fig. 11). The matrix of a mature sphere has a somewhat mottled appearance (fig. 11).

The perinuclear cytoplasm lacks yolk platelets but contains elements of swollen rough endoplasmic reticulum. The dialated elements of endoplasmic reticulum (which contain intracisternal granules) are randomly dispersed throughout the cytoplasm (fig. 11). Also present in the cytoplasm at this stage of development are membrane bound bodies approximately 0.6 u in diameter (fig. 16). Surrounding these bodies is an electron dense bristled coat with which many ribosome-like particles are associated. The matrix of the bodies contains regularly spaced

tubular elements which are 46 mu in diameter and demonstrate a distinct polarity (fig. 17). Also present in the matrix of the bodies are a few electron dense particles which resemble the intracisternal granules found in the swollen elements of rough endoplasmic reticulum.

The nucleus at this time is much the same as described for the early follicular stage with the exception that nuclear pores are fewer in number.

Cortical rod: as in the yolk sphere phase, follicle cells appear greatly attenuated. However, there is a marked diversity of follicle cell morphology which is especially noticeable at the fine structural level. While many of the follicular cells are as previously described (yolk sphere phase) (fig. 19), others demonstrate great disruption of their cytoplasmic contents. These contain discontinuous fragments of rough endoplasmic reticulum, only a few mitochondria, and the cytoplasm lacks the granularity seen in adjacent follicular cells (fig. 18). A basement lamina is still present at the periphery of the follicular cell layer (figs. 18 and 19).

The intercellular space separating the oocyte from the follicular cell layer is still evident as previously described (yolk sphere phase). Oocyte microvilli are present which extend to contact the follicular cell membrane (figs. 18 and 19). The ooplasm of the oocyte is densely packed with yolk spheres which are quite obvious at the light microscope level. The nucleus is as previously described in the yolk sphere phase (fig. 20).

At the fine structural level regularly spaced regions of amorphous material are observed in the cortical cytoplasm. These regions are irregular in shape, flocculent in nature, and rapidly increase in volume. Numerous electron dense inclusions (0.43 u in diameter) are present around each region, and membrane fragments are also around the periphery of these developing centers (fig. 21). These bodies rapidly develop into the characteristic cortical rods of the mature oocyte (Hudinaga, 1942).

Mature cortical rods are approximately 6 u X 9 u, and the matrix of each rod is packed with small "feathery" structures. Each of these structures has an electron dense axis 0.36 u long and 24 mu in diameter. Projecting from this axis are electron dense fibrillar elements 48 mu long and 4 mu in diameter. These structures closely resemble a laboratory bottle brush (figs. 22 and 24).

Mature Oocyte Stage

The mature oocyte and associated follicular layer are as previously described for the cortical rod phase of the late follicular stage with one striking exception. Shortly before ovulation the cortical rods move to the periphery of the oocyte and open (fig. 23 and 24). The internal matrix of each becomes continuous with a flocculent material present in the oocyte-follicle intercellular space. The "bottle brush" substructure is present in the intercellular material in the area immediately surrounding the point of continuity between the cortical rod and the intercellular material (fig. 25).

DISCUSSION

Just prior to vitellogenesis the early oocytes (prefollicular stage) of P. aztecus exhibit nucleolar emissions with the subsequent passage of nucleolar material into the ooplasm. Similar events are well documented in other Arthropods (Kessel, 1968; Cone and Scalzi, 1967; Anderson, 1964; Beams and Kessel, 1963).

However, unlike these early stages of growth a considerable degree of divergence is found among various members of the Arthropoda during the other stages of oogenesis. Vitellogenesis in the Arthopods has been shown to follow two patterns. The Crustacea rely upon intracellular yolk synthesis while the Insecta are diametrically opposed in relying upon extracellular synthesis of yolk materials.

Intracellular yolk synthesis has been demonstrated in the lobster oocyte by Kessel (1968), in the oocytes of the fresh water crustacean, Daphina, by Kessel (1968), in spider crab oocytes by Hinsch and Cone (1969), and in crayfish oocytes by Beams and Kessel (1963). These workers have observed a distention of the cisternae of rough endoplasmic reticulum which was present in the oocyte at the onset of vitellogenesis. Frequently these reticular elements have been shown to be numerous and to form interconnecting branches throughout the cytoplasm (Kessel, 1968; and Beams and Kessel, 1963). Concomittant with the swelling of the endoplasmic reticulum was the appearance of electron

dense intracisternal granules (Hinsch, 1969; Kessel, 1968; and Beams and Kessel, 1963). In some instances these intracisternal granules increased in number and subsequently fused into discrete yolk spheres (Kessel, 1968; and Beams and Kessel, 1963). Micropinocytosis has been noted in the crustaceans at the time of vitellogenesis but has not been associated with yolk sphere production (Kessel, 1968). Beams and Kessel (1963) suggested that pinocytosis is involved in the formation of a chorion in crayfish oocytes.

Extracellular yolk synthesis, characteristic to the Insecta is accomplished by the micropinocytotic uptake of extracellular yolk proteins, with the subsequent fusion of this material into discrete yolk spheres. This process has been demonstrated in silverfish oocytes by Cone and Scalzi (1967), in roach oocytes by Anderson (1964), in mosquito oocytes by Roth and Porter (1964), in cecropia moth oocytes by Stay (1965), and in saturniid moth oocytes by Telfer (1961). Anderson (1964), for example, observed an electron dense material in the intercellular space separating the oocyte from the follicle cell. This dense material is transported into the ooplasm via micropinocytotic vesicles (pinasomes) which subsequently fuse to form yolk spheres. Oocytes producing yolk in this fashion characteristically lack endoplasmic reticulum and Golgi apparati.

The interpretations of these morphological observations have been supported by various labeling techniques. Stay (1965), through the use of fluorescein labeled antibodies, demonstrated the uptake of extra-

cellular proteins and their subsequent incorporation into yolk spheres. Telfer and Melius (1963), through the use of autoradiographic techniques, also demonstrated the incorporation of extracellular proteins into yolk.

The Xiphosurans, another class of Arthropods, demonstrates both intracellular and extracellular yolk synthesis (Dumont and Anderson, 1967). Dumont and Anderson (1967) noted a distinction between early vitellogenesis (intraoocytic) and late vitellogenesis (extraoocytic). The intraoocytic synthesis relies upon rough endoplasmic reticulum and Golgi apparati. This process closely resembles the vitellogenic process described for crustaceans (for example Kessel, 1968). Conversely the extraoocytic synthesis relies upon micropinocytotic uptake of extracellular materials in a manner similar to the insects (for example Cone and Scalzi, 1967).

Vitellogenesis in P. aztecus, as in Xiphosurans, involves both intracellular and extracellular synthesis of yolk. As Penaeid oogonia progress from the prefollicular to the early follicular stage, elements of the rough endoplasmic reticulum become dialated in a manner reminiscent of crustaceans (Hinsch, 1969; Kessel, 1968; Beams and Kessel, 1963) and the Pycnogonid, Nymphon gracile, (King and Jarvis, 1970). In P. aztecus, however, the elements of endoplasmic reticulum are not profusely branched but appear as discontinuous fragments. The cisternae of Penaeid reticular elements do contain intracisternal granules which are similar in size and morphology to those described

in other crustacean oocytes. However, in the other systems described there is a proliferation and fusion of the intracisternal granules resulting in the formation of yolk spheres. This is not the case in P. aztecus. The swollen reticular elements containing sparse numbers of intracisternal granules are present long after large yolk spheres have developed. These yolk spheres are produced by the fusion of numerous micropinocytotic vesicles. In this respect Penaeid vitellogenesis is very similar to vitellogenesis in the insects.

It is interesting that the vitellogenic process in P. aztecus, a crustacean, is similar to vitellogenesis in a Xiphosuran and not other crustaceans as described to date. However, in contrast to the Xiphosurans, which demonstrate solitary egg development, (Dumont and Anderson, 1967), a distinct layer of follicular cells is present in P. aztecus from the early follicular stage until the oocyte is mature. Follicle cells have been observed in other crustaceans during oogenesis; lobsters by Kessel (1968), crayfish by Beams and Kessel (1963), and in spider crabs by Hinsch (1969). A follicular cell layer has also been shown in various insects (cone and Scalzi, 1967; Stay, 1965; Telfer, 1965; Anderson, 1964; Roth and Porter, 1964; Telfer and Melius, 1963; Telfer, 1961). The follicle cells of P. aztecus contain numerous mitochondria and prolific rough endoplasmic reticulum. Similar follicle cell morphology has been shown in the silverfish (Cone and Scalzi, 1967) and in the lobster (Kessel, 1968).

With the exception of the Penaeid shrimp, organelles peculiar to

the cortical cytoplasm (e.g. cortical granules) have not been described in previous Arthropod studies (Burukovskii, 1970; Oka, 1965; King, 1948; Hudinaga, 1942). This is interesting since cortical granules are commonly found in the eggs from other invertebrate phyla (see Dewel and Clark, 1974; and Anderson, 1968 for review). The analogous granules in P. aztecus oocytes are more appropriately termed cortical rods owing to their cylindrical shape and large size. They are approximately ten orders of magnitude larger (6u X 9 u) than the cortical granules of the sea urchin, Arbacia punctulata, (Anderson, 1968) or the anemone, Bunodosoma cavernata, (Dewel and Clark, 1974).

A distinct region of cortical cytoplasm is not apparent in P. aztecus although a distinct cortical region has been observed in a Pycnogonid by King and Jarvis (1970). This cortical region contains few cellular organelles and yolk platelets are excluded from it. A similar oocyte cortex has been observed in other species (Dewel and Clark, 1974; Anderson, 1968; and Raven, 1961).

Cortical granule formation has been associated with the Golgi apparatus in previous studies (see Anderson, 1968 for review). However, P. aztecus oocytes demonstrate an obvious lack of Golgi bodies and the origin of the cortical rods is unknown at this time. The first evidence for the production of rods is the appearance of the flocculent amorphous regions in the peripheral cytoplasm of the oocyte.

A cortical reaction has been described for the ova of a wide variety of organisms (see Anderson, 1968; and Dewel and Clark, 1974 for

review). P. aztecus oocytes demonstrate a partial cortical reaction while still in the ovary, and a subsequent "explosive" cortical reaction upon entering sea water. Dewel and Clark (1974) have also shown a cortical reaction before spawning in B. cavernata. To our knowledge these are the only two instances where a cortical reaction has been described prior to spawning. Other studies have described cortical reactions at the time of spawning, just subsequent to spawning, at fertilization, or at various stages of development (see Anderson, 1968 for review). Though somewhat controversial most investigators believe that the cortical reaction is responsible for investing layers which aid in a block to polyspermy or provide protection from the environment for the developing zygote.

The cortical reaction in penaeid oocytes may be divided into two distinct events. The first, prior to spawning, being the slow release of material which forms a primary investing coat around the oocyte; the second event being the ejection of cortical rods at spawning. Since fertilization takes place during the interim between these two events (Hudinaga, 1942; Clark, W. H., et al, 1973) it is difficult to believe that the cortical rods play a role in a block to polyspermy. Thus, the cortical material probably provides the late oocyte and early zygote with a protective investment.

Penaeid oogenesis cannot be classified under distinct regime presently in the literature for the Arthropods. While penaeid vitellogenesis closely resembles that in the Xiphosura, the penaeid oocytes

contain a distinct follicular layer which is lacking in the Xiphosurans. Unlike any of the Arthropods described to date the penaeid oocytes possess distinct cortical structures probably analogous to cortical granules as described in other eggs. Because of these interesting variations and the economic importance of the Penaeids they present a very attractive subject for further investigation.

SUMMARY

(1) The ovary of Penaeus aztecus is located in the dorsal cephalothorax and consists of anterior, lateral, and posterior lobes.

(2) Follicular egg development is exhibited, with the follicular cell layer present until spawning.

(3) Vitellogenesis may be divided into early and late production of yolk materials. The swelling of short discontinuous elements of rough endoplasmic reticulum is responsible for the early (intracellular) aspect of yolk production. Micropinocytotic uptake of materials at the oolemma and the subsequent fusion of the micropinocytotic vesicles contribute to the late (extraoocytic) phase of yolk platelet production.

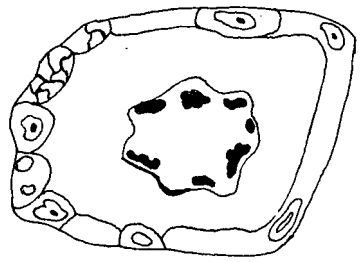
(4) Large cortical rods demonstrate a partial cortical reaction prior to spawning and a subsequent "explosive" ejection of their contents at spawning.

FIGURES and LEGENDS

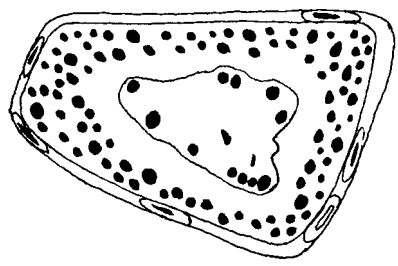
Figure 1 A schematic representation showing classification of the stages of oogenesis in P. aztecus.

PREFOLLICULAR STAGE

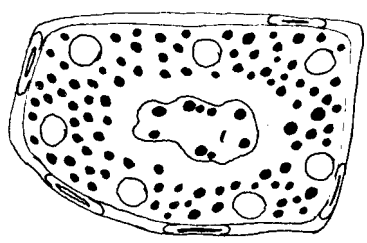
EARLY FOLLICULAR STAGE



LATE FOLLICULAR STAGE
YOLK SPHERE PHASE



CORTICAL ROD PHASE



MATURE OOCYTE

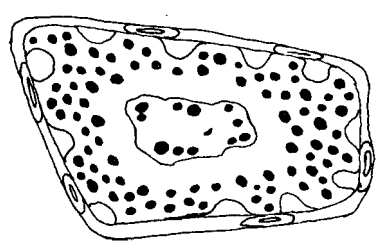
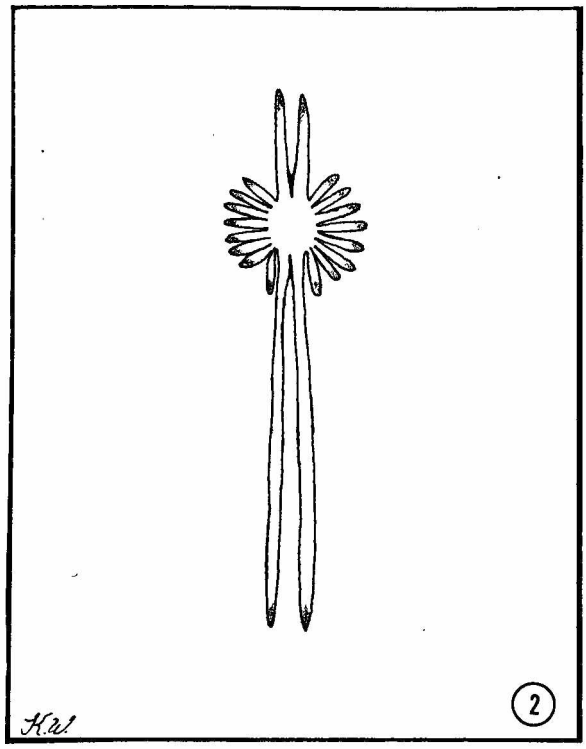


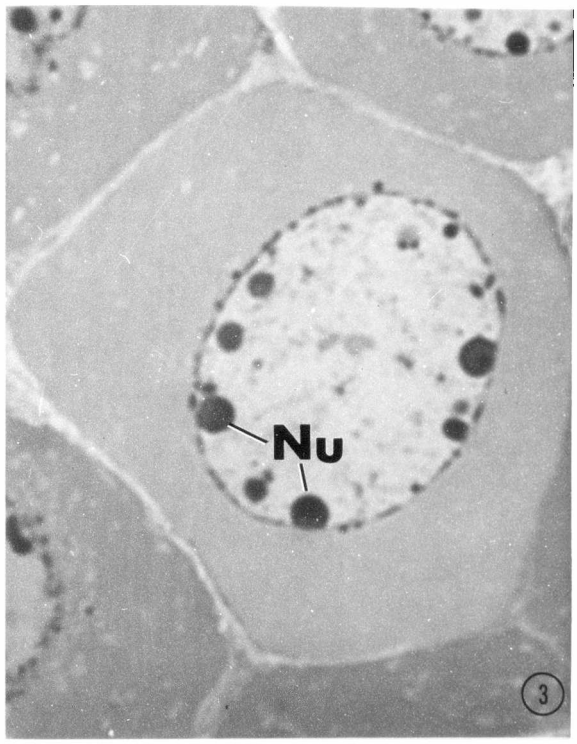
Figure 2 A drawing of the ovary of P. aztecus.

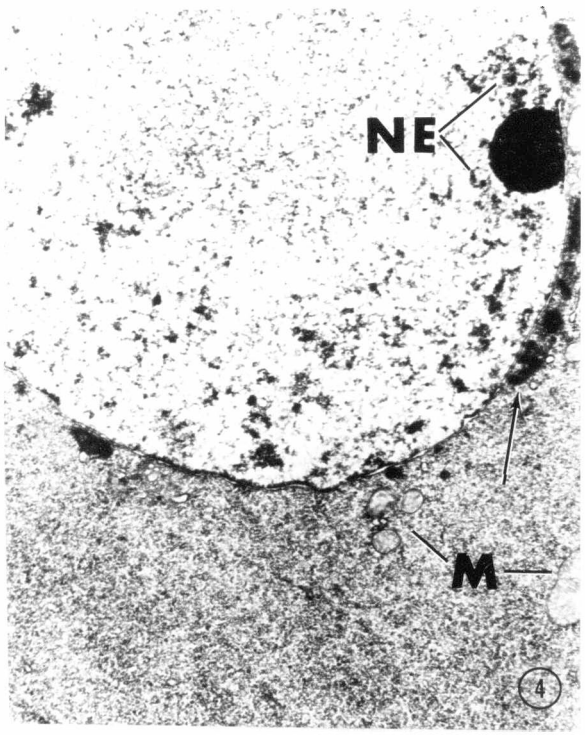
Figure 3 A photomicrograph of a prefollicular stage oogonia. Nu, nucleoli. X 1,300.

Figure 4 An electron micrograph of a portion of a prefollicular stage oogonia. NE, nucleolar emissions; M, mitochondria; electron dense aggregates associated with the nuclear membrane, arrow. X 8,640.

Figure 5 An electron micrograph showing the dialation of the reticular elements (arrow) of a prefollicular stage oogonia. X 33,600.


K.v.
2


Nu
3


NE
M
4

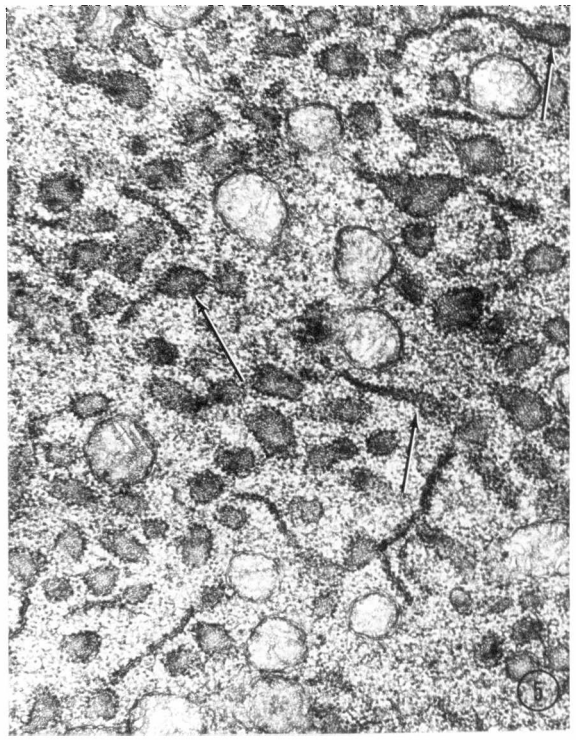
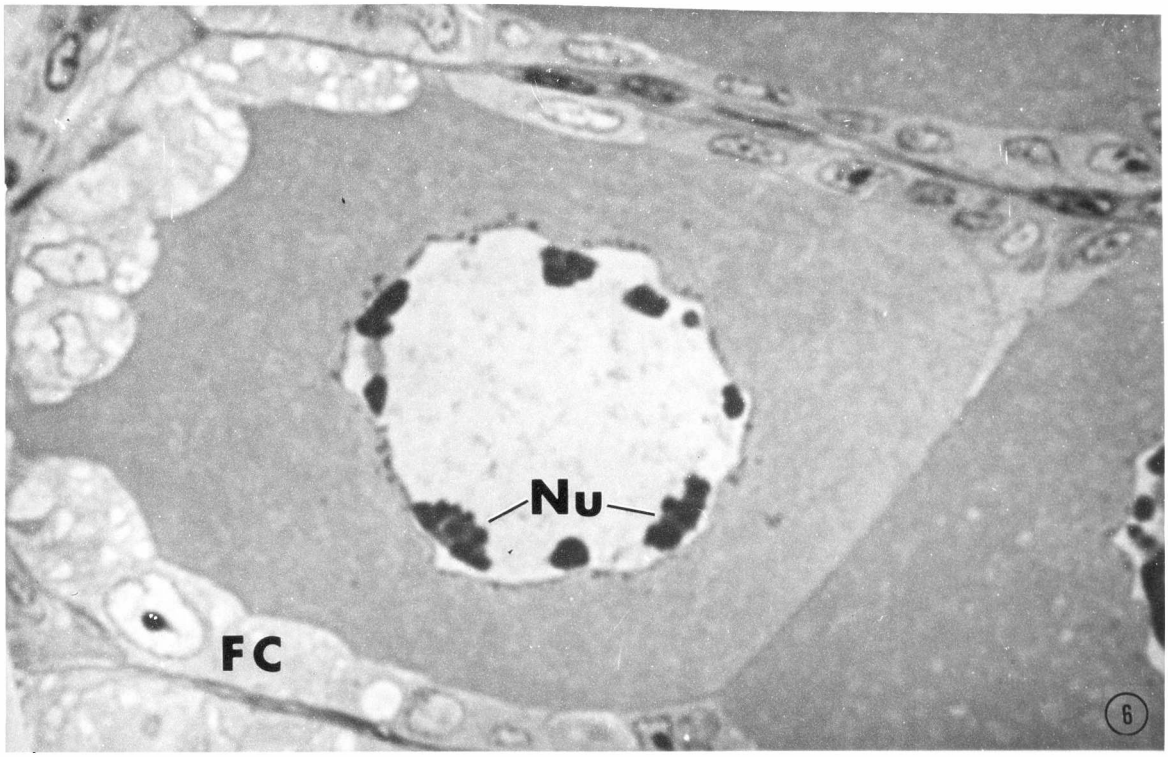
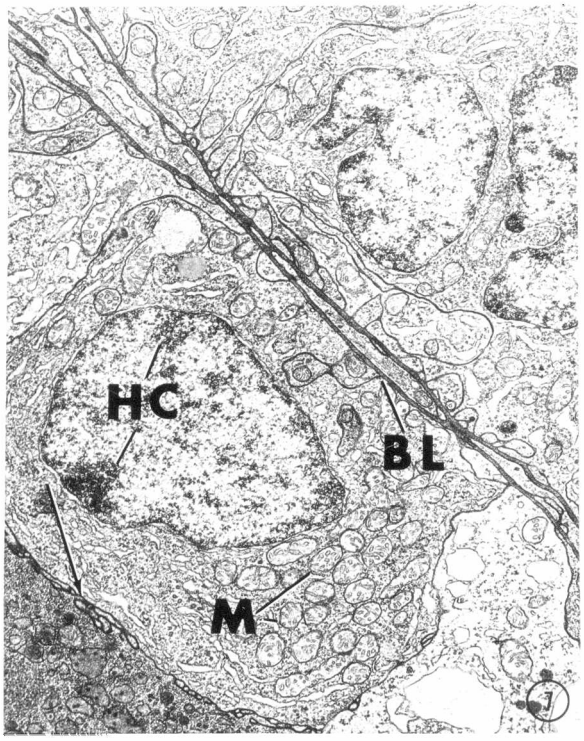

5

Figure 6 A photomicrograph of an early follicular stage oocyte showing a follicle cell layer and irregularly shaped nucleoli. FC, follicular cells; Nu, oocyte nucleoli. X 1,200.

Figure 7 An electron micrograph of a follicle cell (early follicular stage). HC, heterochromatin; BL, basement lamina; M, mitochondria; arrow, membrane interdigitation. X 7,150.

Figure 8 An electron micrograph of the swollen reticular elements which contain intracisternal granules (early follicular stage). RE, swollen reticular elements; icg, intracisternal granules. X 27,300.


Nu
FC
6


HC
BL
M
7

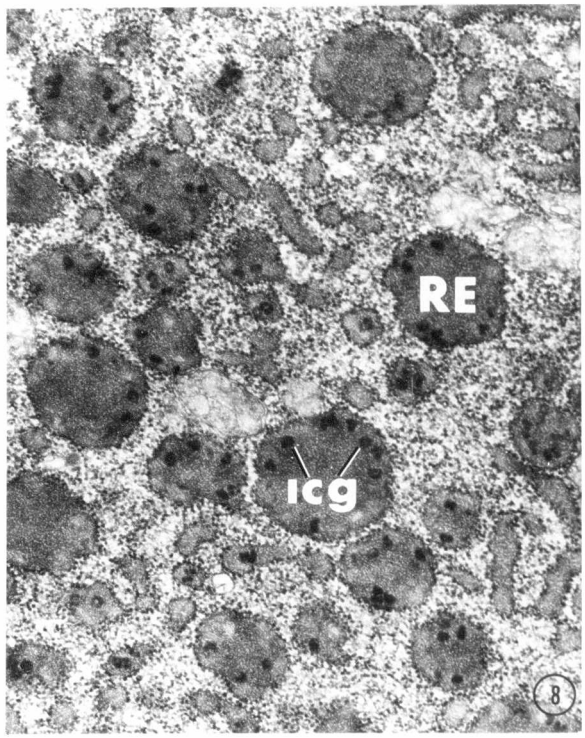

RE
icg
8

Figure 9 A photomicrograph of a late follicular stage (yolk sphere phase) oocyte. Y, yolk platelets; Nu, nucleoli; FC, follicular cell layer; FCN, follicle cell nucleus. X 640.

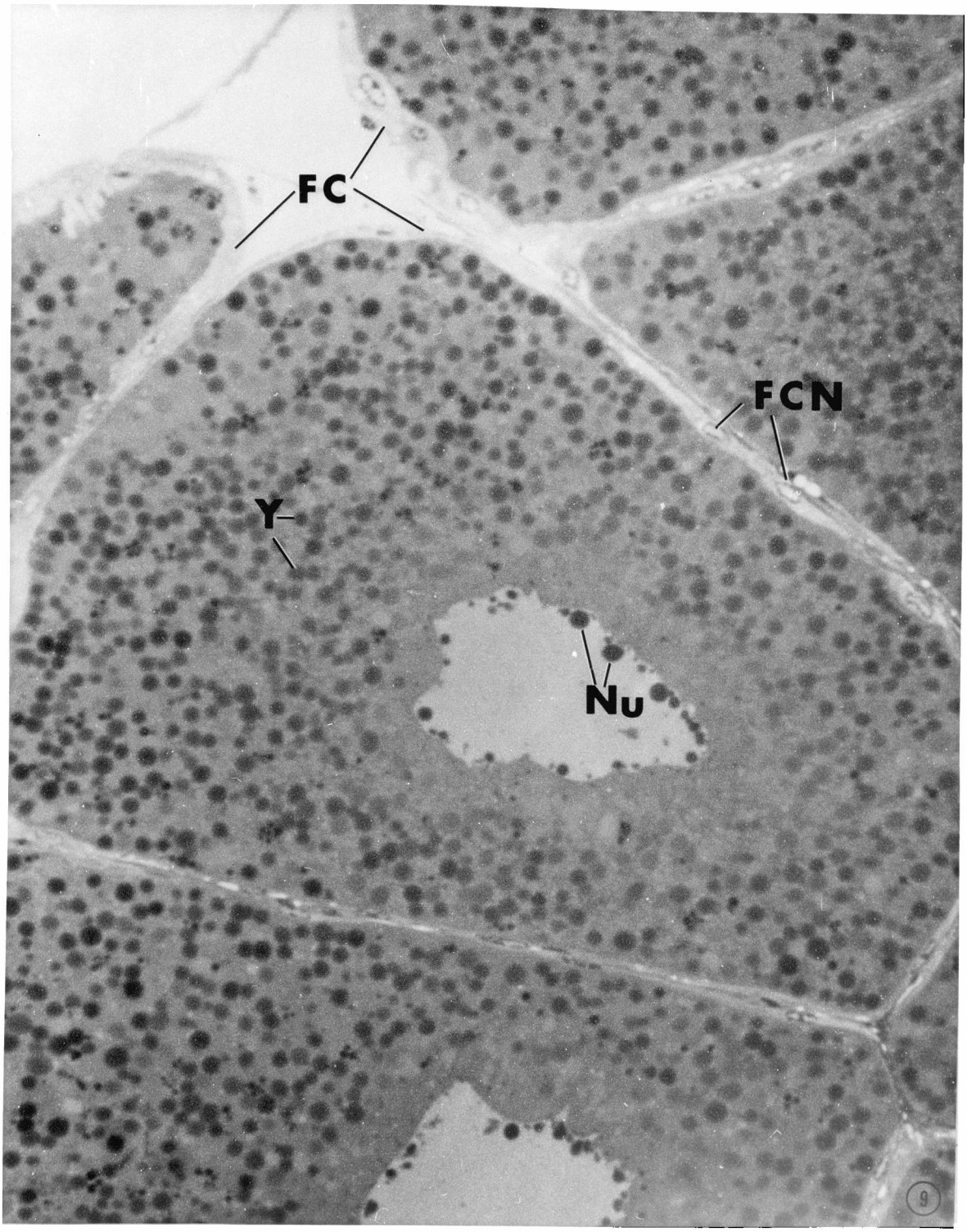
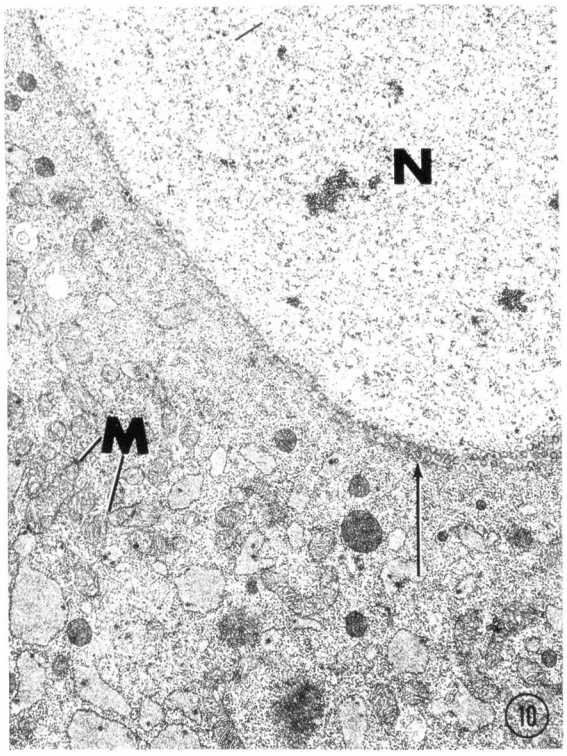

FC
FCN
Y
Nu
9

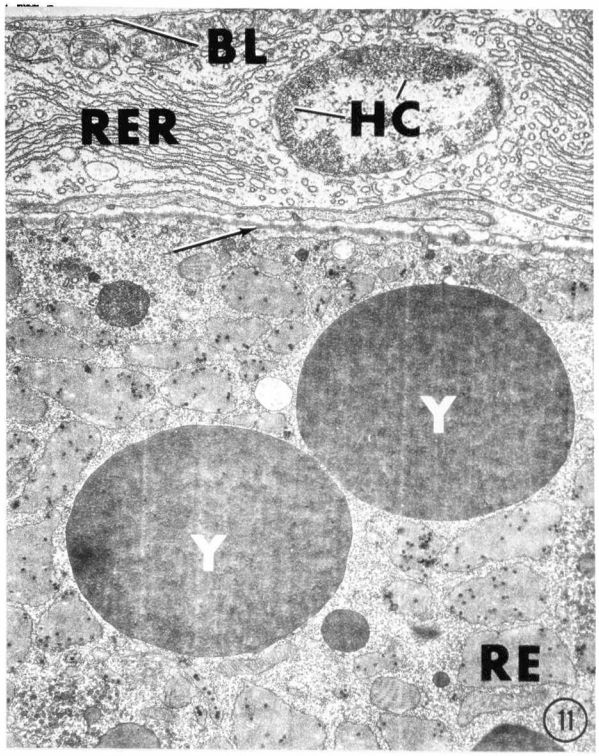
Figure 10 An electron micrograph showing the perinuclear region of an early follicular stage oocyte. N, nucleus; M, mitochondria; arrow, "honeycombed" nuclear membrane. X 9,350.

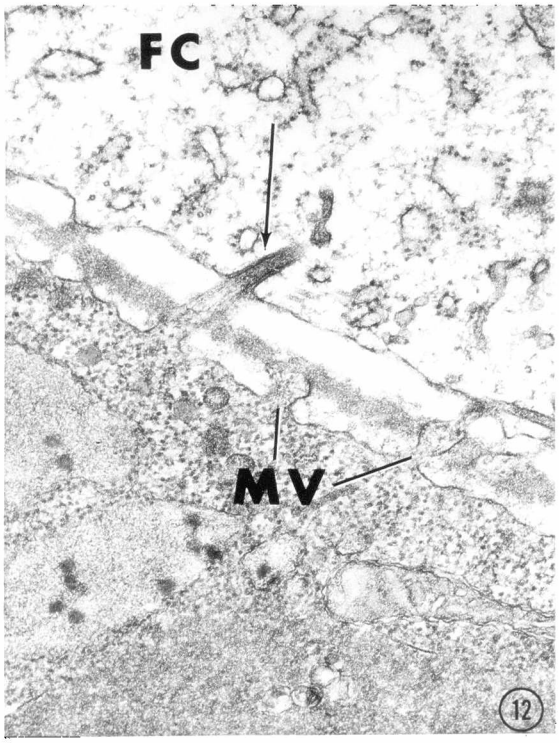
Figure 11 An electron micrograph showing the follicular cell layer and cortical cytoplasm of a yolk sphere phase oocyte. BL, basement lamina; HC, heterochromatin; RER, rough endoplasmic reticulum. The adjacent oocyte contains Y, yolk; RE, swollen reticular elements; arrow, flocculent intercellular material. X 9,720.

Figure 12 A high magnification electron micrograph showing the follicle cell - oocyte (yolk sphere phase) interaction. FC, follicle cell; MV, oocyte microvilli; arrow, oocyte microvillus projecting into follicle cell cytoplasm. X 37,800.

Figure 13 An electron micrograph showing the cortex of an oocyte (yolk sphere phase). P, pinasomes; arrow, micropinocytotic vesicle "pinching off" the oolemma; FC, follicle cell. X 35,700.


N
M
10


BL
RER
HC
Y
Y
RE
11


FC
MV
12

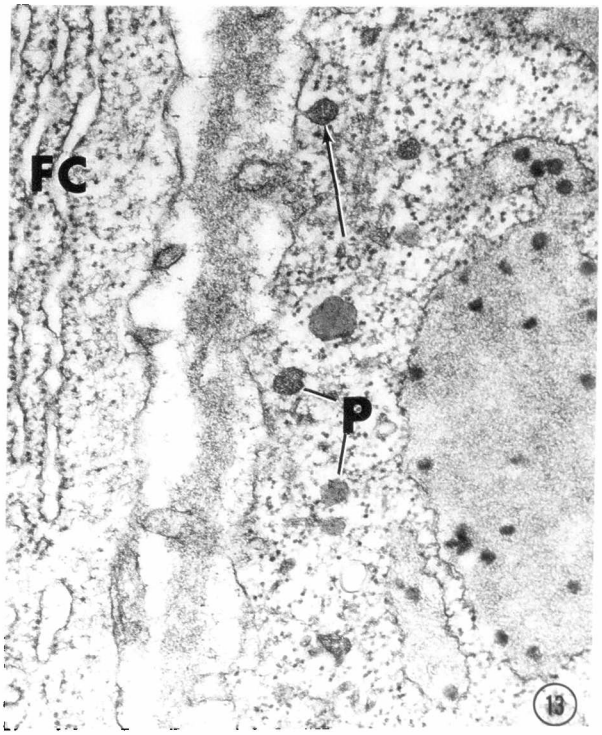

FC
P
13

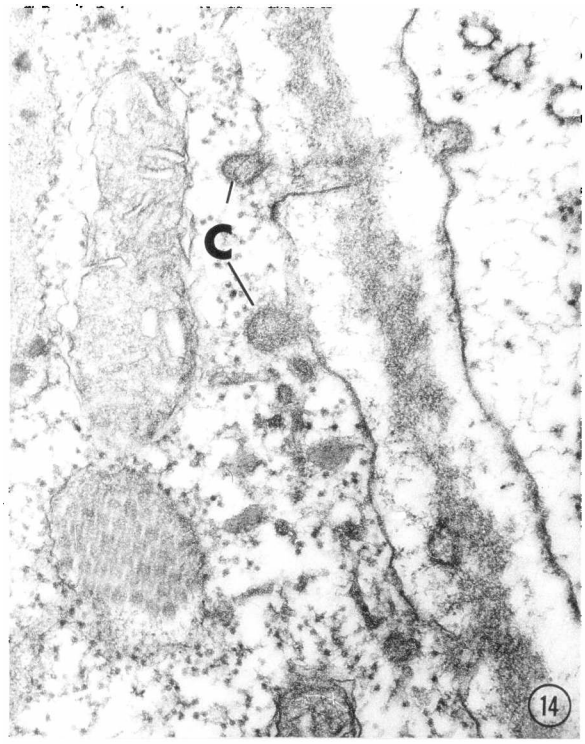
Figure 14 An electron micrograph showing the cortex of a yolk sphere phase oocyte. C, caveolae. X 42,000.

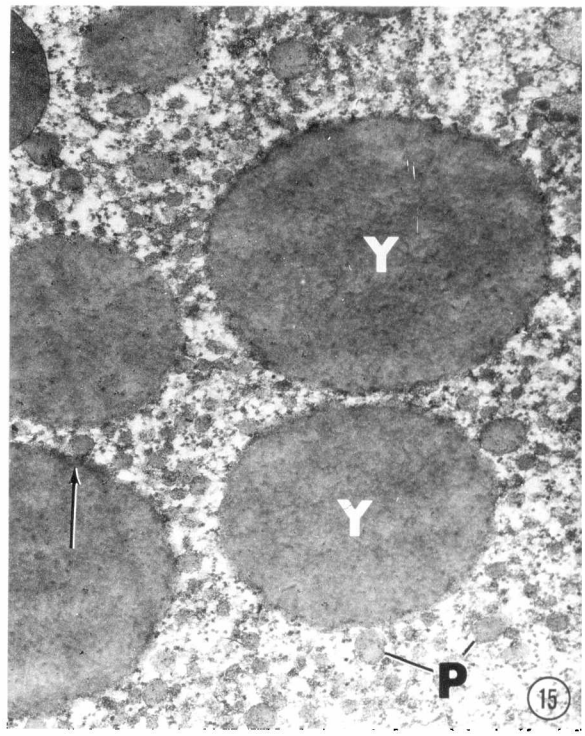
Figure 15 An electron micrograph of the cytoplasm of an oocyte (yolk sphere phase). Y, developing yolk platelets; P, pinasomes; arrow, pinasome fusing with growing yolk platelet. X 32,970.

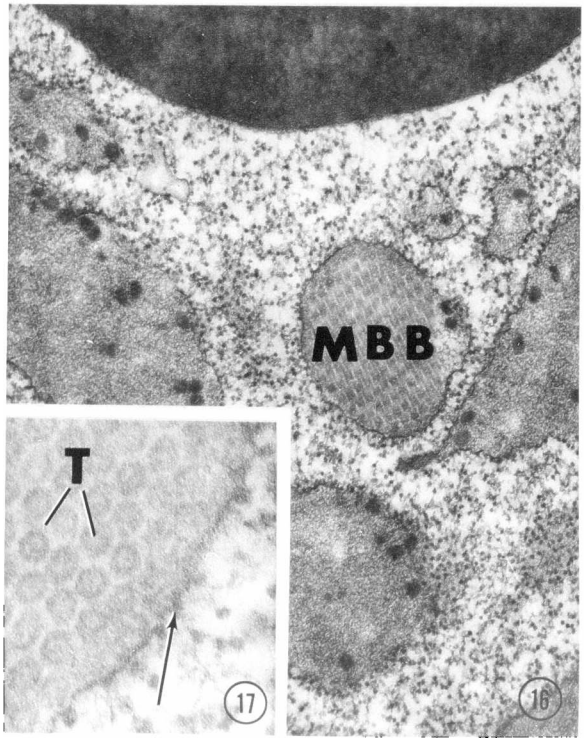
Figure 16 An electron micrograph of a membrane bound (tubule containing) body in the oocyte cytoplasm. MBB, membrane bound body. X 31,500.

Figure 17 A high magnification electron micrograph of the surface of a membrane bound body. T, tubules; arrow, electron dense bristled coat. X 89,700.

Figure 18 An electron micrograph of the follicular layer and the cortical region of an oocyte (yolk sphere phase). BL, basement lamina; FC, follicle cell; M, mitochondria; arrow, oocyte microvillus contacting follicle cell membrane. X 18,900.


C
14


Y
Y
P
15


MBB
16

T
17

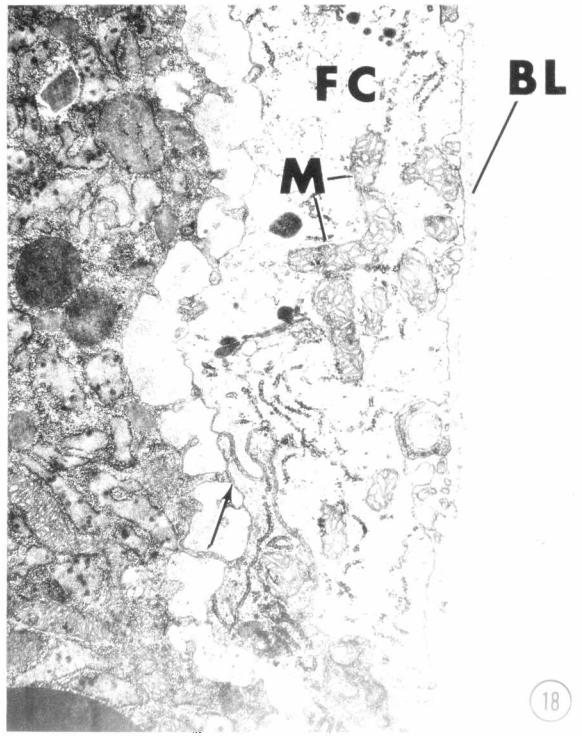
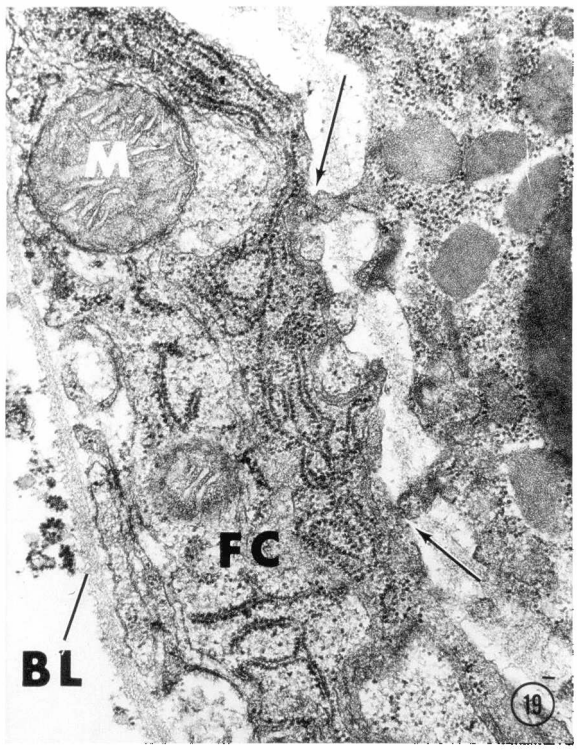

FC
BL
M
18

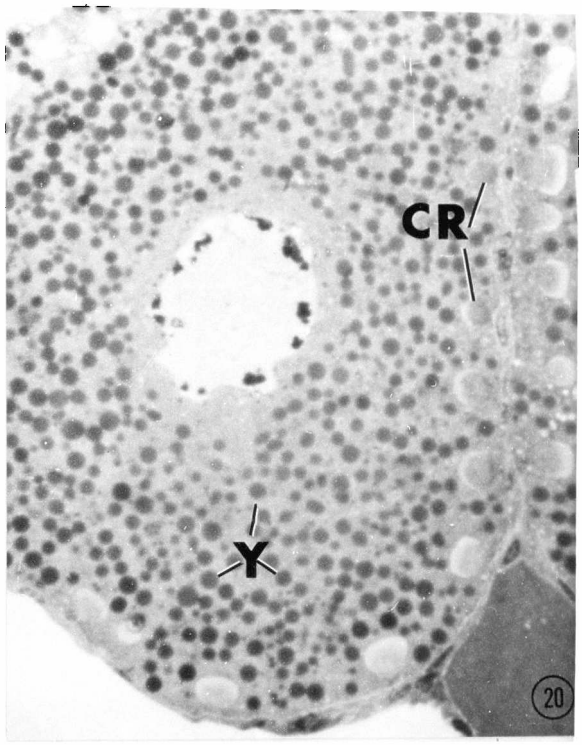
Figure 19 An electron micrograph showing a follicle cell (yolk sphere phase oocyte). FC, follicle cell; BL, basement lamina; arrows, oocyte microvilli contacting the follicle cell membrane. X 26,400.

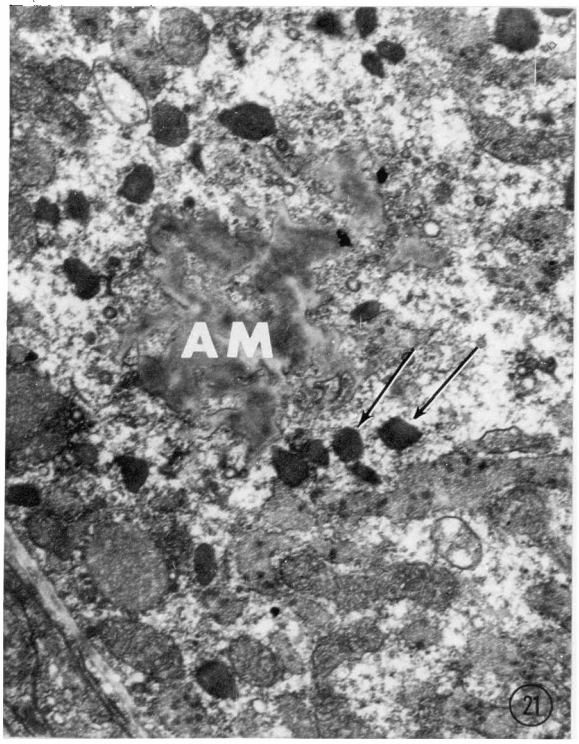
Figure 20 A photomicrograph of a cortical rod phase oocyte. Y, yolk; CR, cortical rods. X 500.

Figure 21 An electron micrograph of the cortical region of a cortical rod phase oocyte. AM, amorphous material; arrows, electron dense inclusions associated with amorphous material. X 20,400.

Figure 22 An electron micrograph of the matrix of a cortical rod. Arrow, "bottle brush" structure. X 35,300.


M
FC
BL
19


CR
Y
20


AM
21

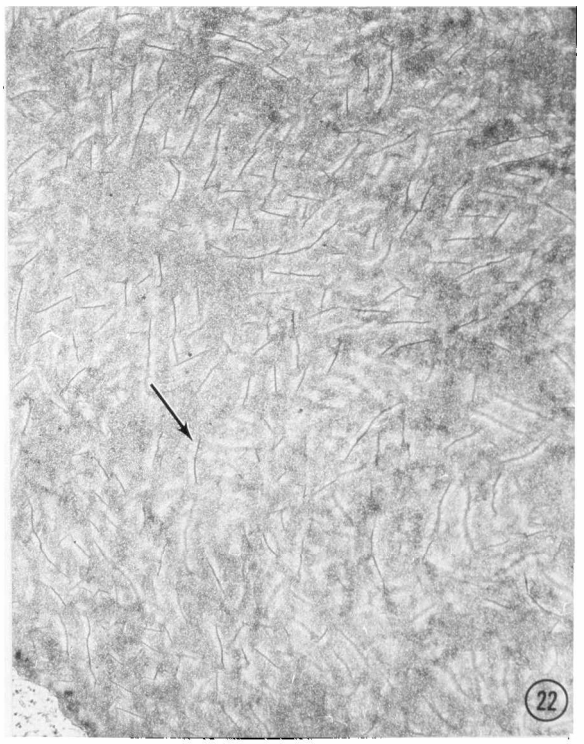
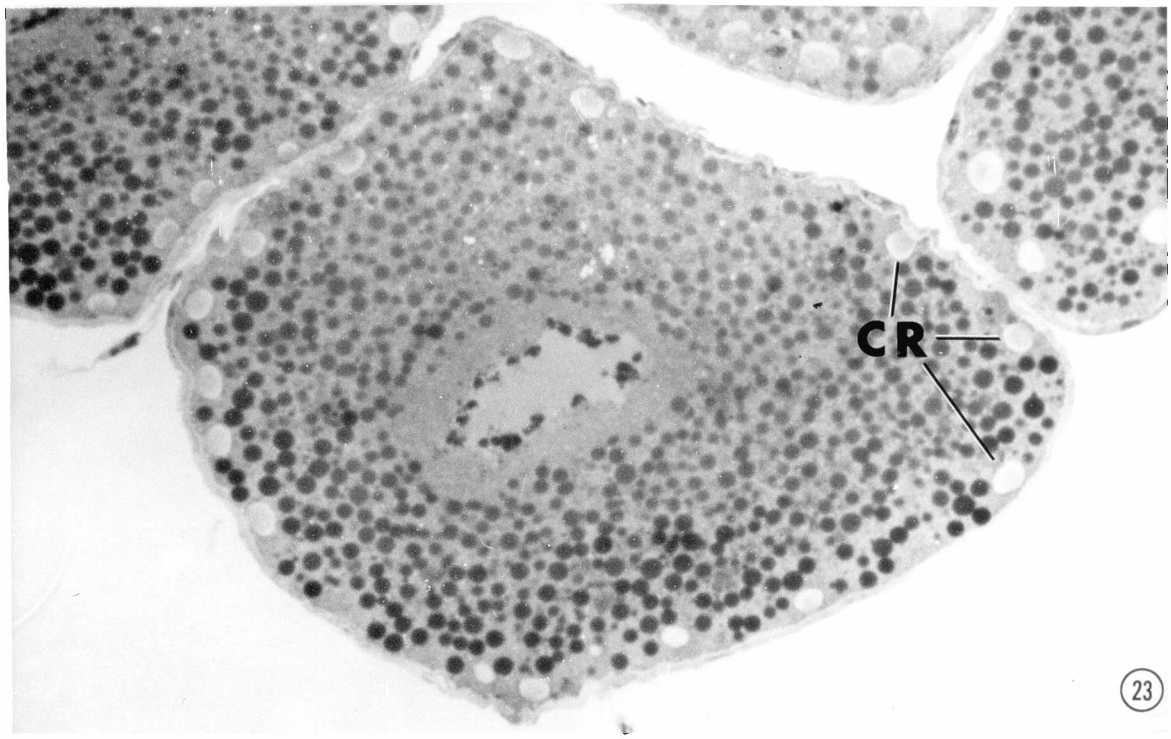

22

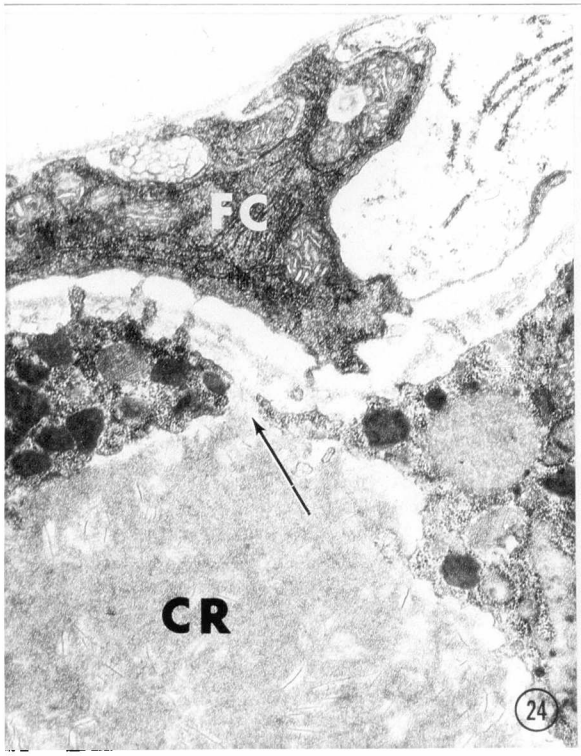
Figure 23 A photomicrograph of a mature oocyte. CR, cortical rods. X 500.

Figure 24 An electron micrograph of a mature oocyte showing an open cortical rod. CR, cortical rod; FC, follicle cell; arrow, point of continuity between the cortical rod and the oocyte - follicle cell intercellular space. X 24,500.

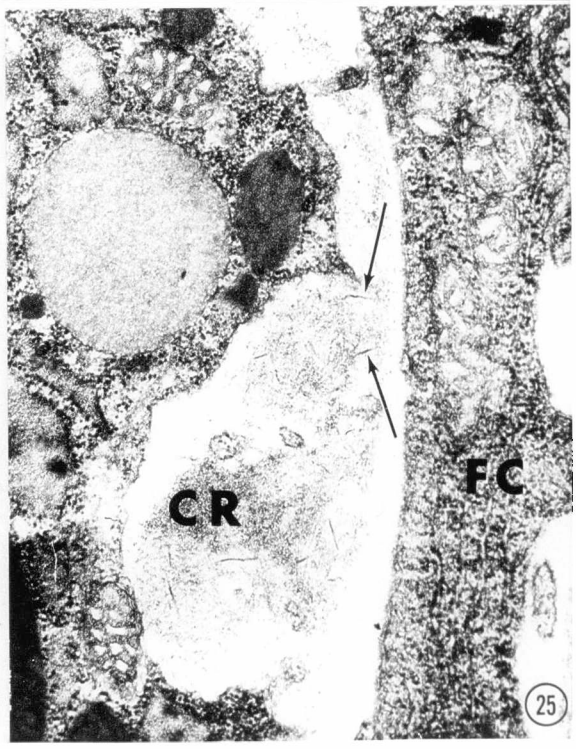
Figure 25 An electron micrograph of the cortical region of a mature oocyte. CR, cortical rod; arrows, "bottle brush" structures in the intercellular space; FC, follicle cell. X 44,800.



23



24



25

BIBLIOGRAPHY

Anderson, E. 1964 Oocyte differentiation and vitellogenesis in the roach, Periplaneta americana. J. Cell Biol., 20: 131.

Anderson, E. 1968 Oocyte differentiation in the sea urchin, Arbacia punctulata, with particular reference to the cortical granules and their participation in the cortical reaction. J. Cell Biol., 37: 514.

Beams, H. W., and R. G. Kessel 1963 Electron microscope studies on developing crayfish oocytes with special reference to the origin of yolk. J. Cell Biol., 18: 621.

Burukovskii, R. N. 1970 Certain aspects of oogenesis in the pink prawn (Penaeus duorarum). Arkhiv Anatomii, Gistologii i Embriologii, 58: 56.

Caillouet, C. W. Jr. 1972 Ovarian maturation induced by eyestalk ablation in pink shrimp. Proceedings of the third annual meeting of the World Mariculture Society.

Clark, W. H. Jr., P. Talbot, R. A. Neal, C. R. Mock, and B. R. Salser, 1973 In vitro fertilization with non-motile spermatozoa of the brown shrimp Penaeus aztecus. Marine Biology, 22: 353.

Cone, M. V., and H. A. Scalzi 1967 An ultrastructural study of oogenesis in the silverfish Lepisma sp. (Thysamura) J. Cell Biol., 35: 163A.

Cummings, W. C. 1961 Maturation and spawning of the pink shrimp Penaeus duorarum (Burkenroad). Transactions of the American Fisheries Society, 90: 462.

Dewel, W. C., and W. H. Clark, Jr. 1974 A fine structural investigation of surface specializations and the cortical reaction in eggs of the Cnidarian Bunodosoma cavernata. J. Cell Biol., 60: 78.

Dumont, J. N., and E. Anderson 1967 Vitellogenesis in the horseshoe crab Limulus polyphemus. J. Microsp., 6: 791.

Hinsch, G. W., and M. V. Cone 1969 Ultrastructural observations of vitellogenesis in the spider crab Libinia emarginata (L.). J. Cell Biol., 40: 336.

Hinsch, G. W. 1970 Possible role of intranuclear membranes in nuclear-cytoplasmic exchange in spider crab oocytes. J. Cell Biol., 47: 531.

Hudinaga, M. 1942 Reproduction, development, and rearing of Penaeus japonicus (Bate). Japanese Journal of Zoology, 10: 305.

Karnovsky, M. J. 1965 A formaldehyde-glutaraldehyde fixative of high osmolarity for use in electron microscopy. J. Cell Biol., 27: 137A.

Kessel, R. G., and H. W. Beams 1963 Micropinocytosis and yolk formation in oocytes of the small milkweed bug. Exp. Cell Res., 30: 440.

Kessel, R. G. 1968 Mechanisms of protein yolk synthesis in crustacean oocytes. Z. Zellforsch., 89: 17.

King, J. E. 1948 A study of the reproductive organs of the common marine shrimp Penaeus setiferus (L.). Biol. Bull., 94: 244.

King, P. E., and J. H. Jarvis 1970 Egg development in a littoral pycnogonid Nymphon gracile. Marine Biology, 7: 294.

Millonig, G. 1961 Advantages of a phosphate buffer for OsO_4 solutions in fixation. J. Applied Physics, 32: 1637.

Oka, M., and S. Shirahata 1965 Studies on P. orientalis (Kishinouye) II. Morphological classification of the ovarian eggs and the maturity of the ovary. Bull. Fac. Fish. Nagasaki Univ., 18: 30.

Raven, C. P. 1961 Oogenesis: The Storage of Developmental Information. Pergammon Press, Ltd., Oxford.

Roth, T. F., and K. R. Porter 1964 Yolk protein uptake in the oocytes of the mosquito Aedes aegypti (L.). J. Cell Biol., 20: 313.

Spurr, A. R. 1969 A low viscosity epoxy resin embedding medium for electron microscopy. J. Ultrastr. Res., 26: 31.

Stay, B. 1965 Protein uptake in the oocytes of the cecropia moth. J. Cell Biol., 26: 49.

Telfer, W. H. 1961 The route of entry and localization of blood proteins in the oocytes of saturniid moths. J. Biophys. Biochem. Cytol., 9: 747.

Telfer, W. H., and M. E. Melius 1963 The mechanisms of blood protein uptake by insect oocytes. Am. Zoologist, 3: 185.

Telfer, W. H. 1965 The mechanism and control of (Insecta) yolk formation. Ann. Rev. Entomol., 10: 161.

Venable, J. H., and R. Coggeshall 1965 A simplified lead citrate stain for use in electron microscopy. J. Cell Biol., 25: 407.