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Title	Ultrastructural Characteristics of the Proteid Yolk Formation in the Ovary of the Squid, Todarodes pacificus
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Citation	北海道大學水産學部研究彙報, 29(2), 89-99
Issue Date	1978-06
Doc URL	https://hdl.handle.net/2115/23634
Type	departmental bulletin paper
File Information	29(2)_P89-99.pdf

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Bull. Fac. Fish. Hokkaido Univ.
29(2), 89–99. 1978.

Ultrastructural Characteristics of the Proteid Yolk Formation in the Ovary of the Squid, *Todarodes pacificus**

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Abstract

The origin of proteid yolk in oocytes was investigated electron-microscopically in the squid, *Todarodes pacificus*. In this species, certain precursor materials of yolk granules occur first in the region bordering the basal cytoplasm of the follicular syncytium of ovarian oocytes. In the follicular syncytium, the materials may be transported to Golgi complexes in which construction and maturation of the materials may advance further, then become to be released through Golgi vesicles into the intercellular space between the follicular syncytium and the associated oocyte. The oocyte also develops channel-like infoldings of the ooplasmic membrane facing the follicular syncytium. Yolk materials accumulated in the intercellular space gradually invade the ooplasmic channels, and make the channels expand to cause huge ovoidal invaginations filled with electron-dense yolk materials. The invaginations are separated eventually from the ooplasmic membrane, and are thus transformed into huge electron-dense globules bounded each by a limiting membrane, thus appearing in the peripheral ooplasm as definite yolk granules.

Whereas rather many studies have been concerned so far with proteid yolk appearing in the oocyte of invertebrate and vertebrate animals, there still remains some obscurity especially about the origin of proteid yolk which is considered to vary with the taxonomic groups of animals studied. Two different ways of proteid yolk formation have been suggested to occur[1)2)3)4)]: (1) simple, elementary compounds are taken up by the oocyte to be fully synthesized into yolk within the ooplasm; (2) precursor proteins of yolk synthesized previously by some tissues or organs other than ovaries are taken up by the oocyte. It is interesting to study from the phylogenetic point of view if there is any possible relationship between the different patterns of vitellogenesis and the state of organization or specialization of tissues and organs. In this respect, vitellogenesis in a variety of species belonging to the phylum Mollusca is worthy to be thoroughly investigated because of extensive variations in their anatomical and ecological characteristics.

The present report deals with ultrastructural characteristics of the vitellogenetic

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* This report was presented to the faculty of Hokkaido University as a part of a thesis for the degree of Doctor of Fisheries Science.

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process in a cephalopod, the squid *Todarodes pacificus*, and discusses the results from a comparative standpoint to signify the phylogenetic position of this species.

The writer wishes to express his heartfelt thanks to Professor Emeritus K. Yamamoto, Professor J. Yamada, and Associate Professors H. Takahashi and K. Takano, Faculty of Fisheries, Hokkaido University, for their invaluable criticism and encouragement given to the present study.

Material and Methods

The squid, *Todarodes pacificus*, is a gonochoristic species, and executes reproduction once in its lifetime[5]. The ovary is localized in the terminal part of the mantle cavity. In immature animals the ovary is transparent in its external aspect, whereas in mature ones it shows a brownish colour. Immature and mature females in the course of their spawning migration were captured in the Tsugaru Strait, between Hokkaido and Honshu, and in the Toyama Bay, Toyama Prefecture, respectively.

For electron-microscopic observations, finely cut pieces of the ovary were fixed first with 2.5% glutaraldehyde in 0.5 M phosphate buffer for 2 hours followed by a rinse in the buffer solution overnight, and then post-fixed in Millonig's OsO_4 solution for 2 hours at 4°C. After dehydration in a graded ethanol series and by two changes in *n*-butyl glycidyl ether, the ovarian pieces were embedded in Epon-epoxy resin mixture. Ultrathin sections were cut with glass knives at a thickness of about 500 to 800 Å on a Porter-Blum microtome, and stained by uranyl acetate in 50% ethanol and Reynolds' lead citrate. An observation was done with a Hitachi HS-11 electron microscope.

Observations

In the present study, oocytes at the yolkless stage and the early yolk formation stage were selected, among those of various maturational stages[6], for the observations on fine structural changes of oocytes and their associated follicle cells during vitellogenesis.

I. *Yolkless stage*

In oocytes at the later phase of the early yolkless stage, the nucleus is round in shape and is located in the center. Many small nucleoli dispersed throughout the nucleus are constructed from fibrillar and granular components. In the nucleoplasm, fine fibrils and small granules are present commonly (Fig. 1). A dense zone consisting of fibrillar materials also exists attached closely to the inner nuclear membrane. The dense zone measures approximately 40 mμ in width. Nuclear pores of about 75 mμ in diameter are observable to possess a fibrous structure within those pores.

In the juxtanuclear cytoplasm thread-like structures are usually encountered besides free ribosomes (Fig. 2). The structures measure about 54 mμ in width, and comprise highly electron-dense, amorphous materials. Sometimes, vesicles of varying sizes are also found in the juxtanuclear region. In general, the deep

cytoplasm is provided with mitochondria, Golgi complexes and annulate lamellae. Mitochondria, which are round in shape and measure 1.1 μ in size, consist usually of well-developed tubular cristae and a clear matrix. Annulate lamellae appear to have no connection with other cytoplasmic organelles. They are composed of numerous flattened cisternae with many, regularly arranged pores of about 80 mμ in size. Within the annulate lamellae, there are small, roundish regions which lack lamellar structures (Fig. 3). In these regions, free ribosomes, highly electron-dense, amorphous materials and small vesicles are seen instead of lamellae. Poorly developed Golgi complexes are constructed from 3 to 5 flattened sacs, vacuoles with electron-lucent materials, and small vesicles (Fig. 4). The Golgi vacuoles sometimes contain a minute vesicle. In the peripheral cytoplasm, mitochondria and vesicles of varying sizes are seen to exist dispersedly.

Follicle cells surrounding the oocytes of the early yolkless stage are flat in shape and reach about 1.5 μ in height (Fig. 5), with a nucleus of an elliptical shape. In the nucleus, fibrillar and granular components are distributed uniformly. The cytoplasm possesses ill-developed organelles such as free ribosomes, mitochondria and endoplasmic reticulum. The plasma membrane of follicle cells facing the oocyte runs smoothly, being furnished with desmosomes.

Follicle cells encircling oocytes of the late yolkless stage are round to columnar in shape. The round follicle cells measure about 6 μ in diameter. They have a nucleus occupying the greater part of the cytoplasm (Fig. 6). The nucleus is provided along its periphery with a few nucleoli comprising small aggregations of fibrillar and granular components. Cytoplasmic organelles still remain in an ill-developed state.

In oocytes accompanied by the round follicle cells, some parts of the ooplasmic membrane begin to be caved in. The surface of the oocytes becomes occupied by "ditches" composed of the ooplasmic membrane itself.

As follicle cells develop from round into columnar type, they begin to intrude into the occyte. The columnar follicle cells measure approximately 10 μ in height, and have a nucleus of an elliptical shape in the center of the cell (Fig. 7). The nuclear envelope is wavy in contour, and the interspace between the outer and the inner membrane is expanded to varying extents at some places. In the juxtanuclear cytoplasm, many flattened cisternae and vesicles are noticed to exist extensively. Mitochondria are elliptical in form, and have ill-developed tubular cristae and a fibrillar matrix. Many flattened cisternae of the rough endoplasmic reticulum occupy the whole cytoplasm. Golgi complexes display a gradual development in the cytoplasm facing the oocyte. They are composed of flattened sacs and numerous vesicles. Dense bodies with minute vesicles are present around the Golgi complexes (Fig. 8).

In oocytes with columnar follicle cells, a huge number of ditch-like structures are found to lie on their periphery. The ooplasm among the ditch-like structure is constituted only from fibrillar materials (Fig. 9). In a close proximity of the structures, many vesicles of varying sizes are also detectable.

II. *Early yolk formation stage*

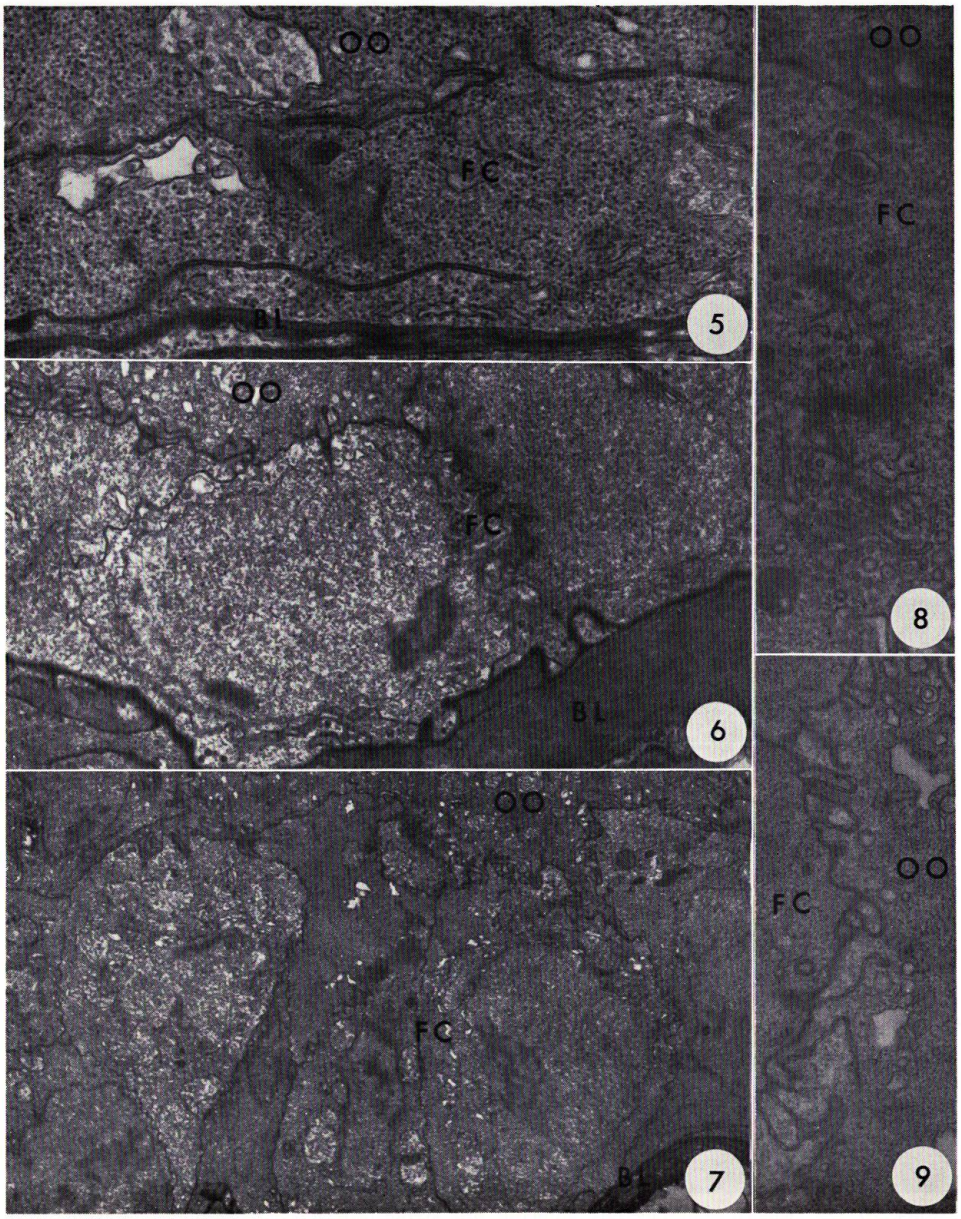
During this stage, follicle cells show interesting ultrastructural changes in

Fig. 1. Ooplasm of an oocyte of the yolkless stage, showing the occurrence of numerous free ribosomes and large mitochondria (*M*). The nuclear envelope runs smoothly. *ON*, nucleus. × 14,000.

Fig. 2. Juxtanuclear cytoplasm of an oocyte of the yolkless stage, with thread-like structures (arrow) composed of highly electron-dense, amorphous materials. × 23,000.

Fig. 3. Annulate lamellae having a small region containing free ribosomes, vesicles and amorphous materials. × 24,000.

Fig. 4. Golgi complex consisting of flattened sacs and vesicles. Some of the vesicles possess a minute vesicle in the center. × 24,000.



Figs. 5–9. Electronmicrographs demonstrating changes of follicle cells (*FC*) surrounding ovarian oocytes (*OO*) of the yolkless stage. *BL*, basement lamina.

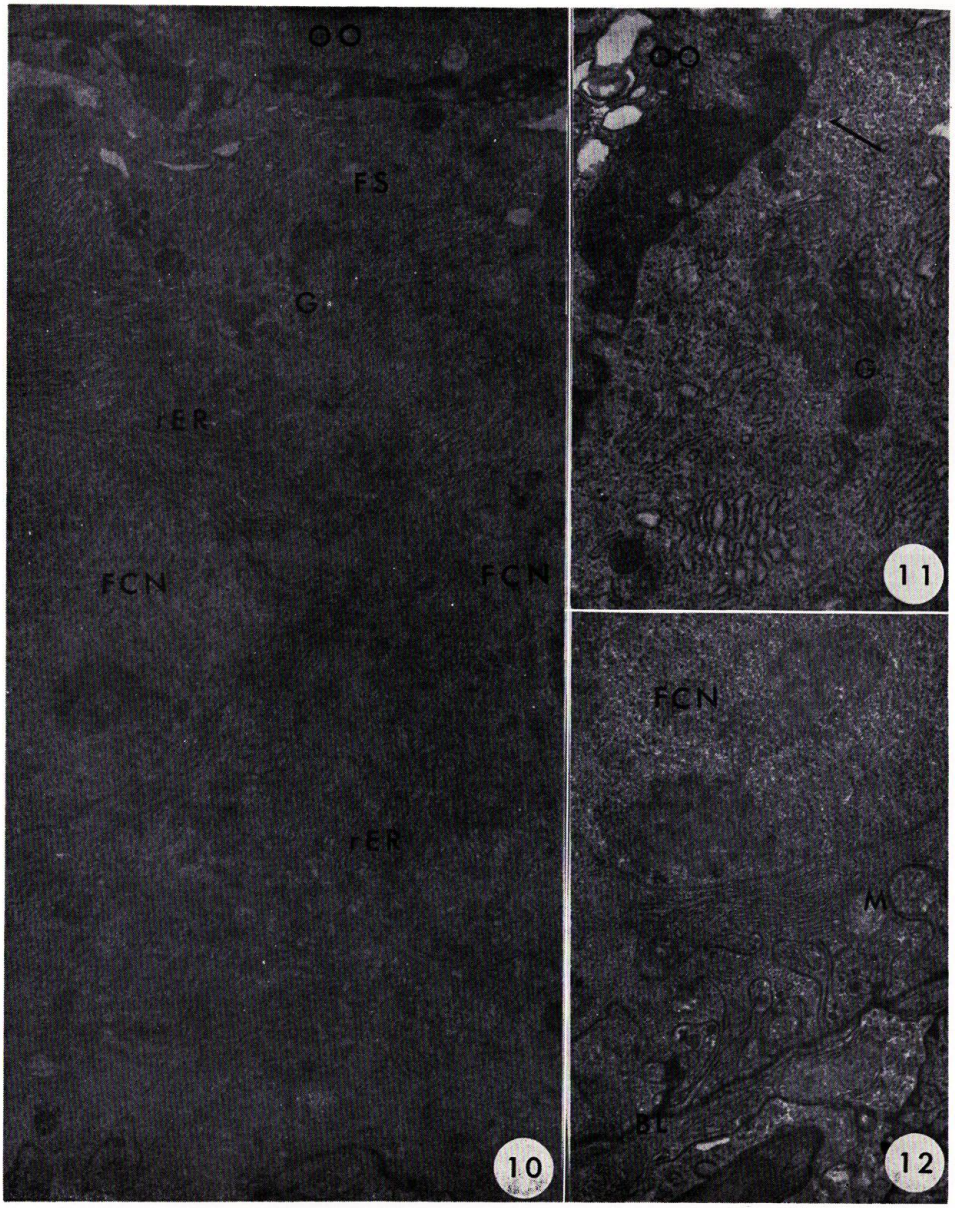
Fig. 5. Follicle cells of a flat shape. × 25,000.

Fig. 6. Follicle cells of a roundish shape. The nucleus takes the greater part of the cytoplasm. × 7,700.

Fig. 7. Follicle cells of a columnar shape. The cytoplasm has a vast number of free ribosomes. The oocyte is furnished with many ditch-like structures of the ooplasmic membrane. × 4,800.

Fig. 8. Golgi complexes of a follicle cell. Small dense bodies lie in the vicinity of the Golgi complexes. × 16,000.

Fig. 9. Growing ditch-like structures of an oocyte. × 16,000.

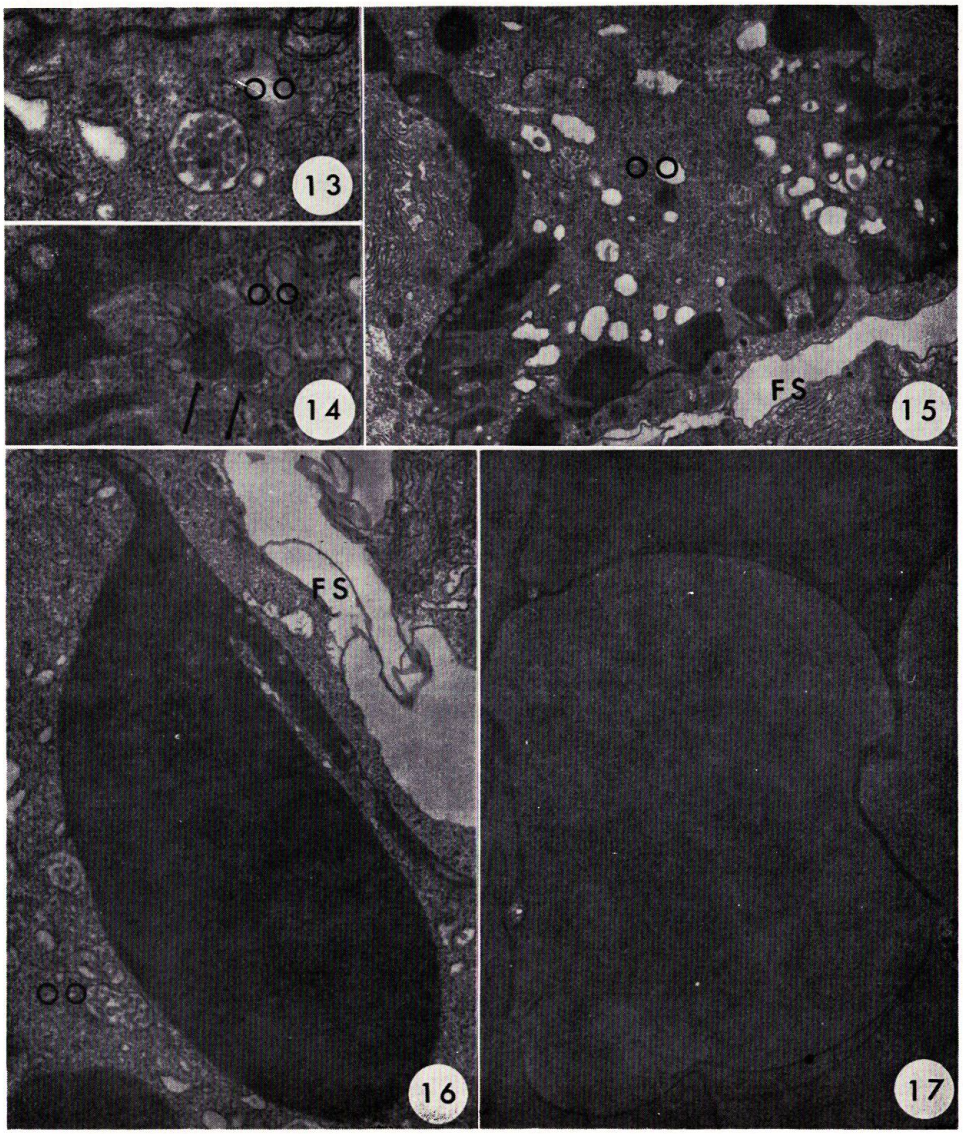


Figs. 10–12. Electronmicrographs showing fine structural characteristics of the follicular syncytium (*FS*) of ovarian oocytes (*OO*) in the early yolk formation stage.

Fig. 10. Follicular syncytium with a large amount of free ribosomes and a well-developed rough endoplasmic reticulum (*rER*) throughout the whole cytoplasm. The lateral plasma membranes separating adjacent follicle cells have disappeared to make a single syncytium. *G*, Golgi complex; *FCN*, nucleus of follicular syncytium. × 8,000.

Fig. 11. Apical cytoplasm of a follicular syncytium, revealing the occurrence of many expanded sacs in the Golgi area (*G*). The expanded sacs have highly electron-dense materials. A small vesicle releasing its contents into the intercellular space is indicated by an arrow. × 16,000.

Fig. 12. Basal cytoplasm of a follicular syncytium, showing the intrusion of some parts of the basement lamina (*BL*). Peculiar structures are seen in association with mitochondria (*M*), a rough endoplasmic reticulum and small bodies. × 7,700.



Figs. 13–15. Changes of the intercellular space between oocytes (*OO*) and the follicular syncytium (*FS*) in relation to the occurrence of yolk materials.

Fig. 13. Multivesicular body and the border of oocytes. × 24,000.

Figs. 14 and 15. Yolk materials displaying an increase in volume in the intercellular space. Small dense bodies (arrows), possibly minute yolk granules, are recognized in the peripheral ooplasm. Fig. 14, × 24,000; Fig. 15, × 7,700.

Fig. 16. Yolk materials taken into an oocyte (*OO*) by macropinocytosis. *FS*, follicular syncytium. × 9,600.

Fig. 17. Yolk masses in yolk granules. The yolk granules bounded each by a limiting membrane possess electron-dense, homogeneous materials. × 11,200.

relation to the formation of yolk materials. The lateral plasma membranes separating adjacent follicle cells begin to disappear, and as a result a developing oocyte comes to be enveloped completely in a single follicular syncytium (Fig. 10). The follicular syncytium contains many elliptical nuclei, with nucleoli of irregular shapes, which are noticed frequently to lean over the inner nuclear membrane. The follicular syncytium has a large amount of free ribosomes and a well-developed rough endoplasmic reticulum throughout the whole cytoplasm.

A prominent change occurs in the basal cytoplasm of follicle cells. The basal plasma membrane of these cells begins to be infolded complicatedly together with the basement lamina (Fig. 12). The interstice between the two plasma membranes of each infolding becomes packed with highly electron-dense materials. On the other hand, in the apical cytoplasm facing the oocyte, there are well-developed Golgi complexes which comprise expanded sacs and vesicles containing electron-dense materials. As shown in Fig. 11, small vesicles, which may possibly originate from the Golgi complexes, are often seen to release their contents into the intercellular space between the follicular syncytium and the oocyte.

During the initial phase of this stage, the intercellular space between the two kinds of cells is usually electron-lucent (Fig. 13). Later, the space begins to accumulate highly electron-dense, homogeneous materials produced by the follicular syncytium, and comes to be expanded to various extents (Figs. 14 and 15). By that period, pit-like invaginations of the ooplasmic membrane are often encountered in the region of the "ditches" of the ooplasmic membrane which have been formed in the peripheral ooplasm during the preceding stage. The invaginated pit seems to be of a nature of the pinocytotic vesicle which is involved in the uptake of precursor materials of the yolk from the intercellular space. As seen in Fig. 14, small bodies bounded each by a limiting membrane have electron-dense materials which are similar in ultrastructural aspects to those existing in the intercellular space. Multivesicular bodies originating possibly from pinocytotic vesicles or from Golgi vacuoles are also present in the peripheral cytoplasm (Fig. 13). They may develop into small dense bodies.

As the accumulation of electron-dense materials, or yolk materials, proceeds in the intercellular space, the ditches in the peripheral ooplasm become obscured as a result of their expansion forced by the accumulation of the materials. In this way, huge pear-shaped invaginations of the ooplasmic membrane containing yolk materials appear along the periphery of oocytes. One of the invaginations indicated in Fig. 16 measures 8.3 μ in its long axis and 3.8 μ in its short axis. After the stalk of the pear-shaped invagination has been torn off, an elongated body which is packed with dense yolk materials in a limiting membrane occurs in the peripheral ooplasm. Subsequently the body becomes roundish in shape, reaching about 4 μ in diameter and is thus termed the yolk granule. Many yolk granules occurring in the whole ooplasm come to be fused with each other resulting in the formation of huge masses of yolk materials (Fig. 17).

Discussion

The present study showed ultrastructurally that follicle cells of the squid,

Todarodes pacificus, during oogenesis become highly differentiated secretory cells as exactly as they are in the squid *Loligo pealei*[7]. Furthermore, the present observation made it clear that the oocyte in this species takes up the secretory products of the follicular syncytium by a macropinocytotic manner. The facts may disclose that the follicular syncytium plays a leading role in the yolk formation.

The mode of yolk formation in the squid is similar to that demonstrated in most vertebrate animals in the sense that precursor proteins of the yolk synthesized by some tissues or organs are taken up by the oocyte. The site of synthesis of the yolk precursors in vertebrates is known to be the liver[4]. In invertebrates, a similar mode of yolk formation appears also mainly in arthropods. Insects have vitellogenin (yolk precursor) usually in their blood serum, and they synthesize it in the hepatopancreas[2]. In many other invertebrates such as a hydrozoan medusa[7], the flatworm (*Prostheceraeos floridanus*)[9], the crayfish (*Cambarus clarkii*)[10][11], the sea-cucumber (*Thyone briareus*)[12], sea-urchins[13][14] and the ascidian (*Ciona intestinalis*)[15], it has been shown repeatedly that simple, elementary compounds of yolk precursors are taken up by the oocyte to be fully synthesized into yolk within the ooplasm by the cooperation of some cytoplasmic organelles such as the endoplasmic reticulum and the Golgi complex. However, in the rotifer (*Priapulus caudatus*)[16] the proteid yolk was reported to be formed in the ooplasm without the contribution of cytoplasmic organelles.

Similarly, many molluscan animals are known to take the intraooplasmic mode of proteid yolk formation. Anderson[17] observed that, in *Mopalia mucosa* and *Chaetopleura apiculata*, yolk granules occur as a result of collaboration of the rough endoplasmic reticulum and the Golgi complex. In some protobranch species such as *Patella coerulea*[18][19], *Bembicium nanum*[20] and *Ilyanassa obsoleta*[21], the proteid yolk is found in association with the Golgi complex. Bolognari[18][19] reported that the vitellogenesis in oocytes of *Aplysia depilans* starts with the transformation of Golgi bodies into small globules. Recently, Coggeshall[22] described that oocytes of *Aplysia californica* synthesize the yolk in the rough endoplasmic reticulum. In plumonates such as *Planorbis*[23][24], *Chipangopaludina malleata*[25] and *Helisoma trivolvis*[26], it was concluded that the vitellogenesis of oocytes comprises very complicated processes of the intraooplasmic yolk synthesis. Beams and Sekhon[27] reported in a bivalve (*Anodonta*) that yolk precursors appear first in the region of the Golgi complex.

On the other hand, the synthesis of proteid yolk in cephalopod molluscs, as shown in *Todarodes pacificus* by the present study and in *Loligo pealei* by Selman and Arnold[7], is definitely an extraooplasmic event. In oocytes of *T. pacificus*, certain precursor materials of the yolk are first accumulated in the extracellular region bordering the basal cytoplasm of the follicular syncytium. The plasma membrane of the follicular syncytium displays complicated infoldings, and the precursor materials are taken up by the cell through a pinocytotic action. In the follicular syncytium, the materials may possibly be transported to Golgi complexes in which the construction and maturation of the materials may advance further, and are then released through Golgi vesicles into the intercellular space between the follicular syncytium and the associated oocyte. The oocyte also develops channel-

like infoldings of the ooplasmic membrane facing the follicular syncytium. The yolk materials discharged from the follicular syncytium gradually invade the ooplasmic channels, and make the channels expand to cause huge ovoidal invaginations filled with electron-dense yolk materials. The invaginations are eventually separated from the ooplasmic membrane and transformed into huge electron-dense globules bounded each by a limiting membrane, and thus yolk granules appear in the peripheral ooplasm. Accordingly, it is certain that, in *T. pacificus*, the site of the extraooplasmic yolk synthesis is the follicular syncytium.

It is interesting for the present writer to consider that the situation of cephalopods as to the mode of yolk formation may have a phylogenetic significance. The site of proteid yolk formation is decisively the follicular syncytium of ovarian oocytes in cephalopods whereas it is the oocytes themselves in other molluscan animals, notwithstanding that all of them are furnished with the hepatopancreas which is known to be the site of proteid yolk formation in insects. These facts seem to be suggestive of the fact that the site of yolk synthesis evolves in relation to a specialization of the organization of organisms or to adaptive changes of a reproductive mechanism for the surroundings. In particular, the specialization of follicle cells of ovarian oocytes in cephalopods may have a concern with an excellent locomotive ability of the animals for feeding and/or with the nature of their reproduction in which vitellogenesis occurs only once in their lifetime. Anyhow, as somatic cells composing the ovary of molluscan animals change also their fine structural characteristics in harmony with the reproductive cycle, viz., follicle cells and "nurse cells", further comparative studies on ultrastructural, cytochemical and biochemical natures of ovarian somatic elements in relation to vitellogenesis are needed to get more exact information about vitellogenetic processes which may probably involve several factors still to be determined.

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