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Oogenesis in a Tubiculous Polychaete, *Schizobranchia insignis* Bush. I Microscopic and Biometric Studies

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棲管 갯지렁이 *Schizobranchia insignis* Bush의 卵子形成 I. 현미경적 및 생물측정학적 연구

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摘 要

棲管 갯지렁이 *Schizobranchia insignis* Bush의 卵子에서 일어나는 變化의 形態學的 및 生化學的 관계를 규명코자 卵子形成中 細胞學的인 變化와 卵子의 年中 分布에 대하여 연구하였다. 卵原細胞分裂 직후 난자는 卵巢에서 離脫하여 體腔囊에서 最大의 크기인 직경 6180μ 까지 성장한다. 난자형성은 年中 계속 일어나고 있으며, 産卵期間은 1월에서 3월간이다. 난자가 最大의 크기에 달하면 그 후로는 크기에 변화가 없으며 體腔囊에 축적된다.

核仁은 직경 5—10μ의 난자에서 처음으로 나타나며 난자형성 초기단계에서 크기의 증가를 보여 직경 100—120μ의 난자에서 가장 크고, 직경 120—180μ의 기간동안 크기의 변화가 거이 없다. 仁은 최초에는 단일구획 이지만 난황물질형성 직전에 이중구획으로 나뉘어진다.

세 종류의 난황물질이 난자형성 기간중 서로 다른 시기에 형성된다. 脂質顆粒과 성분이 불확실한 타원형의 顆粒은 초기에 나타나지만, 대부분이 직경 80μ이상의 난자에서 형성된다. 단백질 난황과 皮質顆粒은 반드시 직경 80μ 이상의 난자에서만 형성된다. 미세융털이 직경 80μ의 난자에서는 卵黃膜에 다량 존재하지만 직경 180μ의 난자에서는 거의 찾아 볼 수 없다.

INTRODUCTION

The understanding of the relationship between oogenesis and embryogenesis is one of the most important goals for the developmental biologists (Raven, 1961). Products made during oogenesis are utilized during embryogenesis. Yolk and other nutritive materials are elaborated in oocytes and used for nourishing the embryos. Machinery for the synthesis of specific proteins is prepared during the prefertilization stages (Brachet *et al.*, 1963; Tyler, 1963; Gross and Cousineau 1964). Factors which will later be important for directing events of differentiation of the embryos often are largely laid down during oogenesis. A remarkable example of this type is the formation of polar granules in *Miaster*, which are elaborated in oocytes, transmitted to the embryos, and destined to become germ cells (Hegner, 1914).

In the last two decades morphological aspects of oogenesis have been extensively studied (Gonse, 1956; Raven, 1961; Norrevang, 1968), but relatively few biochemical studies have been made and most of them have been confined to the later vitellogenic stages. Furthermore, biochemical and morphological aspects have not been correlated in a single species of animal. For the beginning of a series of studies pertaining to the regulatory mechanism of oogenesis and the relationship of oogenesis to embryogenesis, reproductive organs and structural changes in oocytes in *Schizobranchia insignis* are described.

MATERIALS AND METHODS

The tubiculous polychaete, *Schizobranchia insignis*, was collected from Friday Harbor or Roche Harbor, San June Island, Washington, U.S.A. The animals, whose tube size ranges from 15 to 20 cm, are attached to the floats and clustered together. Clusters of worms were collected and kept in the sea water tank. Celloidin Preparation: To study location and the morphology of the gonads, and the relation of the oocytes to the gonads and coelom, cross-sections of whole animal were prepared by the celloidin method (Galigher and Kozloff, 1971). Slices of the whole worm fixed in Bouin's fixative, dehydrated in a series of alcohol, and embedded in 2—8% celloiding dissolved in ether-ethanol (1 : 1). The celloidin-infiltrated piece of the animal was hardened on a wooden block with chloroform vapor, and sectioned into 40μ thick sections with sliding knife. These sections were stained with Schwarz's methyl alcohol Borax carmine (Schwarz, 1934) and destained in 50% ethanol by adding diluted HCl until proper staining quality appeared.

Bright Field Microscopy: Free coelomic oocytes were fixed in 2.5% sodium glutaraldehyde, 0.2M Millonig's phosphate buffer, 0.14M NaCl, pH 7.4 for 1 hour and post-fixed in 1% OsO_4, 0.1M phosphate buffer, 0.38M NaCl for 1 hour. The oocytes were then rinsed with water, dehydrated in ethanol, transferred through three changes of propylene oxide and embedded in Epon according to the method of Luft (1961). One micron thick sections were stained with Azure II and methylene blue (Richardson *et al.*, 1960).

Electron Microscopy: The same Epon blocks were sectioned at approximately 600Å thickness, stained with uranyl acetate and lead citrate (Reynolds, 1963), and examined with a Phillips EM-300 electron microscope.

Enumeration of size determination of size distribution of oocytes present in the coelom of representative females was carried out using a Coulter Counter Model B attached with automatic particle size plotter (Coulter Electronics, Inc.). Once each month for a full year animals were collected and used immediately for this purpose. The worm was taken out of its tube, rinsed with Millipore-filtered sea water (MFS) and weighed on a Torsion balance. Oocytes were collected by dissecting the worm lengthwise along the side with a razor blade. Septa and other coelomic membranes were ruptured with a blunt glass rod to release the oocytes. The oocytes were collected from the coelom directly into a beaker by rinsing with MFS until no oocytes were found in the last rinse. The oocyte suspension was strained through at Nitex cloth of 230 μ pore size and diluted to 100 ml of wet weight of the animal.

Aliquots of oocyte suspension were sucked through a special sampling tube with an orifice 400 μ in diameter and the size distribution of oocytes was measured with a Coulter counter multiplier setting of amplification 64 and aperture 0.707. The size distribution of oocytes obtained from one animal was plotted in a differential form using an automatic plotter, which segregates particles into 24 size classes in terms of volume. The plotter was calibrated in terms of volume with plastic beads 30, 80 and 150 μ in diameter(Scndell Scientific Instruments, Inc. styrene-DVB).

RESULTS

Reproductive structures and annual cycle of oocyte size distribution:

A pair of *Schizobranchia insignis* is located in each segment at the ventral side of the worm and indicated by arrow in Fig. 3a. High magnification of the structure shows that the ovary consists of many oocytes of 5 μ in diameter and is surrounded by fat body cells (Fig.3c). Oogonial divisions are completed in the ovary and these small oocytes proceed the early stages of the first meiotic prophase

(Fig. 3d). At the diplotene stage, the oocytes are released into the cavity of the coelomic sac (Figs. 3a, b, c,), in which the rest of oogenesis, all at the diplotene stage, is proceeded for long period of time. The coelomic sacs are present as a pair in every abdominal segment. Oocytes remain in the coelomic sac throughout the whole growth period,

Growth of oocytes was examined by measuring the size distribution using a Coulter counter. To compare the size distribution at different times of the year, the raw data were normalized by taking the total number of oocytes existing in one animal as 100% and plotting the proportion of each size class. An analysis of this kind was made each month throughout the year. The results of such analyses are given in Fig. 2. During the breeding season no large oocytes are present in the spawned animal and most of the oocytes present in this animal are less than 100μ in diameter (Fig. 2/9/69), but in the non-spawned animal at the same time more than half of the oocytes are larger than 160μ. Even though the proportion of the small oocytes of the latter animal is lower than that of the former, the absolute number of the small oocytes would be equal between spawned and non-spawned animals, because the difference in proportion of small oocytes is due to the high proportion of large oocytes in the non-spawned. The proportion of the small oocytes decreases as that of the large oocytes increases. The largest size class begins to show already in April and accumulates throughout the rest of the year until the breeding season. The low frequency of these oocytes indicates that when an oocyte begins to grow it passes without delay throughout the intermediate sizes and rapidly grows to the largest size.

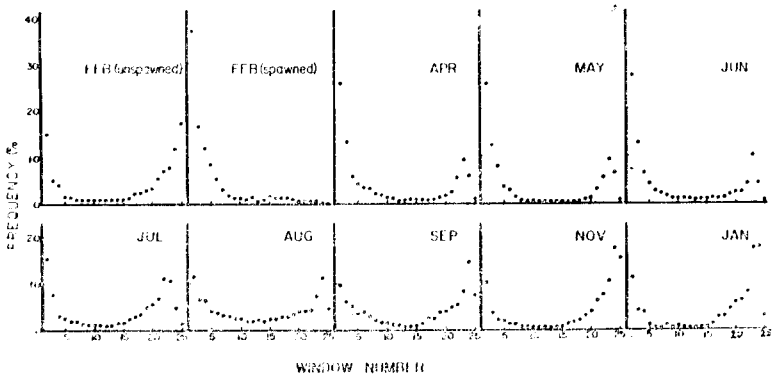

Fig. 1. Annual cycle of size distribution of oocytes using Coulter counter.

Seasonal variations of three main size classes were examined. Oocytes which had been counted in Window number 2 through 6, corresponding to the size of

20μ through 100μ in diameter, were catagorized into small size class. The intermedaite size class ranges from window number 7 to 19 (100-160μ) and the lage size classes from window number 20 to 25 (160-180μ). Fig. 2 is the results of such analyses. Immediately after spawning the small size class is the highest in frequency, which gradually decreases during the rest of the year. The frequency of the large oocytes shows a mirror image of that of the small oocytes, gradually increasing and becoming the maximum just prior to the spawning.

The intermediate size class is relatively constant throughout the year except the period between July and October. These biometrical studies carried over three years on animals freshly collected from Roche Harbor indicate that the breeding-season is the period between January and March, when the number of the largest oocytes suddenly drops to zero in individual animals. Natural spawning of animals maintained in the laboratory aquaria was observed during the period between mid-December and mid-March, Predominantly in February and March. Spawning was observed in nature in Parks Bay, Shaw Island, in March, 1968. Often a few worms were found with fully grown oocytes in the coelomic sac until the end of April, and a single observation was made in the laboratory in April, 1976.

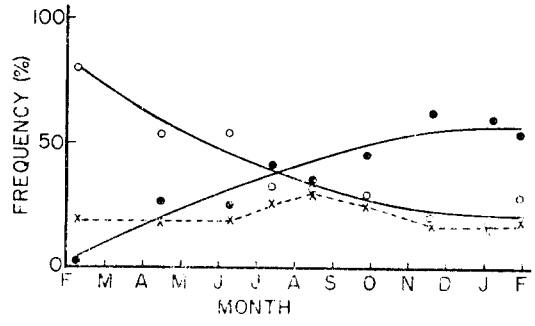

Fig. 2. Annual distribution of small (20—100μ), intermediate (100—160μ) and large size classes (160—180μ).

Spawning behavior has been observed in this animal several times. Females were taken out of their own tubes and transferred into glass tubes of 0.8cm I.D., which were left open at both ends but made to be tapered gently at the posterior end. The worms secrete mucus, which probably normally becomes part of the tube, and adapt completely to the glass tube. The effective diameter of the tube is actually adjusted by the worm by laying down more or less of the mucoid substance. In this way worms were kept alive as long as six months. The process of spawning can be readily observed in these tubes. When the worms spawn, the unfertilized eggs released from the most or all of the abdominal segments. The ova are extruded from openings near the ventral groove, and carried anteriorly along the groove by ciliary movement. This process was observed to continue over a period of three to four hours. In a large population of worms, in their normal tubes in the sea water tables, spawning began in the late evening, about 10 PM, continued through the night, and sometimes on through the morning until about 10 AM. The unfertilized eggs become spherical while spawning in contrast

to irregular shape of the large oocytes, suggesting that the oocytes have become matured during this process. Several mls of unfertilized eggs were usually collected from a female of 3-6 gm wet weight in one spawning.

Cytology of Oogenesis:

Oogenesis was observed mainly with the light microscope and to some extent with the transmission electron microscope with an emphasis on yolk formation and changes in the nucleolus and surface membrane.

The nucleolus: The nucleolus is only a single compartment in oocytes 5-10μ in diameter, which are still attached to the ovarian tissues. The nucleolus is uniformly basophilic, small in size and attached to the nuclear membrane. In oocytes 20μ in diameter, released into the coelomic sac, only a single, strongly basophilic nucleolar compartment is present (Fig. 4a). With the growth of the oocyte to approximately 20-40μ, the nucleolus becomes detached from the nuclear membrane and increases in size(Fig. 4b). When the oocytes reach a diameter of 80μ, the nucleolus becomes bipartite, containing an outer very basophilic zone surrounding a less basophilic inner region (Fig. 4c). Inasmuch as the former stains as darkly as does the immature nucleolus of the earlier stages, the two are probably equivalent and functionally correspond to the nucleolar core as typically described. The more lightly stained zone is probably the newly added compartment and corresponds to the cortex (Raven, 1961). The nucleolus becomes maximum in size when the oocyte becomes 100-120μ in diameter (Fig. 4d, e). The highly basophilic core region is not homogeneous in electron micrographs (Fig. 5a), but contains regions that are relatively less electron dense. The less basophilic cortex contains granules which are highly basophilic in stained sections, and in electron micrographs have an electron density similar to that of the core (Fig. 5b,). The significance of these structures is not clear.

Yolk Formation: Yolk granules are visible final products of oogenesis. The kinds of yolk granules and the time of their appearance during the process were observed. Three different types of yolk granules were found in this worm. Types of yolk granules were classified according to their shape, basophilia, electron-density, and size. Type *a* yolk granules, which are non-basophilic and round, have low electron-density, and probably are lipid granules, first appear in oocytes 20μ in diameter (Fig. 4a). These granules increase in number, and form perinuclear clusters as oocytes grow to 80μ in diameter(Fig. 4c). Subsequently there is a massive increase in their number as the oocyte grows to its final size, and they become uniformly distributed throughout the cytoplasm (Fig. 4f). In oocytes 20μ through 80μ in diameter these granules are nearly constant in size (Fig. 4c), but have grown to nearly twice their original diameter in the largest oocytes (Fig. 4f). Type *b* yolk granules, which are variable in size, round, baso-

philic and highly electron-dense, are first seen in oocytes greater than 80μ in diameter (Fig. 4c), but become conspicuous only in oocytes larger than 100μ diameter (Fig. 4d). These granules are considered to be protein yolk granules. When these granules are first seen, they are located in the periphery of the oocyte. Later they become randomly distributed throughout the cytoplasm. Along with the lipid yolk, proteid yolk granules constitute the most abundant elements visible in the cytoplasm of the fully grown oocytes. Type *c* yolk granules are basophilic, moderately electron-dense, oval in shape, and constant in size. The first appearance of these granules is in 20μ oocytes, in which the granules are mainly located near the plasma membrane (Fig. 4a). A substantial increase in their number takes place when oocytes are about 80μ in diameter (Fig. 4c). In the late stages the number of these granules increases relatively little, but their distribution becomes random as they migrate toward the center. At this time they often become arranged in rosette clusters (Fig. 6a). The rosettes are apparently sections through packets of such granules clustered into the form of balls.

In the electronmicrograph of 80μ oocyte the oval granules are shown to be located at the periphery near the cortical granules (Fig. 5a). There is a variation in the electron-density of these granules; the relatively high electron-density of the granules is very similar to that of cortical granules. In the largest oocyte the oval granules have the same electrondensity as that of the cortical granules (Fig. 5b).

The yolk granules can be classified according to their density. These granules and other inclusions were displaced by centrifuging oocytes on a step gradient made by layering 15 ml of 7 parts of 1M sucrose to 3 parts of sea water over 15 ml of 1M sucrose. After centrifuging at 5,000x g for 10 minutes, three layers of cytoplasmic inclusions were observed in the largest oocytes (Fig. 6b). Most of the round, basophilic granules, type *b*, were displaced to the centrifugal pole and the large, non-basophilic granules, type *a*, were displaced to the centripetal pole. The oval, basophilic granules, type *c* were scattered throughout the intermediate layer. From the stainability and the relative densities it is inferred that the non-basophilic granules, type *a*, are lipid, and the highly basophilic, round granules, type *b*, are proteid in composition. Relative abundance of these three kinds of granules is shown in Fig. 6b. More than half of the space in the oocyte is filled with lipid and proteid yolk granules.

In the stratified oocytes granules which are not displaced by centrifugation and which are located underneath the plasma membrane are apparently enmeshed in stiffer cortical cytoplasm or attached to the membrane. These granules are less basophilic than types *b* and *c* granules, and are round. They are considered to be cortical granules (Figs. 5a, b, Fig. 6b). Such granules are first seen in oocytes 80μ in diameter. In the fully-grown oocytes the cortical granules from a nearly com-

plete layer just beneath the cell membrane. The cortical granules, after osmium-glutaraldehyde fixation, often have a clear region from which material apparently has been dissolved.

Change in Surface Membrane: A delicate vitelline membrane is present in oocytes 20μ in diameter, and becomes thickened and conspicuous in 80μ oocytes. Microvilli were observed to be abundant in 80μ oocytes and to be embedded in the vitelline membrane (Fig. 5a). In the fullygrown oocyte the microvilli have retracted from the vitelline membrane (Fig. 5b).

DISCUSSION

Schizobranchia insignis, a tubiculous polychaete, is an excellent experimental material to study oogenesis. Oocytes are not bound or buried but freely floating in the coelomic fluid. In addition, these worms do not contain any other accessory cells, which are identical to oocytes in terms of size and density, while most of other animals including polychaetes carry accessory cells. Technically it is now possible to separate oocytes into several size classes. The eggs can be collected during breeding season and cultured up to the adult, and thus, all stages of life cycle in this animal are available.

The ovary and oocytes are locates are located within the coelomic sac of every abdominal segment of *Schizobranchia*, and the long process of oogenesis is undertaken in this sac. This sac is a structure which has not been previously described. In other species of polychaete, however, oocytes are released into the coelomic cavity from the ovary and membranous structures such as coelomic sac are not involved in keeping the oocytes during their growth period (Howie, 196h; Schroeder, and Herman 1975). Nephromixium is a structure which is closely related to spawning behaviour in these polychaetes. The coelomic sac of *Schizobranchia* might derived from the peritoneal membrane. When the *Schizobranchia* spawn, only the large mature oocytes are selectively released into the sea water, while the small immature oocytes are remaining in the coelomic sac. Such selective release of the mature oocytes seem to be due to the ciliary action of the peripheral end of the coelomic sac.

Schizobranchia do not have nurse cells in the coelomic sac, and thus, nutrients for the yolk granules should be transported from the coelomic fluid into the oocytes. Since the lipid droplets were found in the oocytes 20 μ in diameter, lipid or lipid precursor must be transported into 20 μ or even smaller size classes. This lipid precursor has to be continuously transported into the oocytes, because the lipid droplet grows in the large oocytes in size and number, reaching about one-third of the oocyte volume. However, the most conspicuous transport takes place

in oocytes 80 μ in diameter; much of the lipid precursor is transported at this stage.

Transport of proteinaceous precursor is stage-specific, since proteid yolk granules do not appear until the oocytes reach 80 μ in diameter. The mechanism of the stage-specific transport seems to be built up within the oocyte itself, because oocytes of all size classes are exposed to the same coelmic fluid at the same time, but the oocytes which elaborate proteid yolk granules are only 80 μ size class or larger. The mechanism of the specific transport seems to be closely related to the activation of the oocyte surface. Abundance of microvilli in the vitelline membrane of 80 μ oocytes appears to indicate the surface activation.

Although the lipid droplets and oval-shaped granules appear in early stages of oogenesis, the process of vitellogenesis is considered to begin with the appearance of the proteid type *b* yolk granules at the 80 μ stage. The subsequent vitellogenic period is marked by a massive production of thes latter granules.

The function of the oval-shaped granules is not clear. Although it was suggested from the electron-micrograph that these granules are transformed into the cortical granules, transformation appears to be most unlikely, since the oval-shaped granules migrate into the center at the later stage and the cortical granules have stainability different from that of the oval-shaped granules.
The peculiar clustering of these granules into rosettes is suggestive of some function at this time in the endoplasm.

The nucleolus is known to be involved in the synthesis of ribosomal RNA. The differentiation of the nucleolus with respect to changes of its size and shape can be correlated with the RNA synthetic activity during oogenesis. Nucleolar growth during oogenesis of *Haliotis* (Bolognari, 1956) and *Priapulus* (Nørrevang, 1965), and increase in number of nucleoli in frog oocytes (Brown and Littna, 1966) are indications of increased activity in RNA synthesis. The requirement of the nucleolus for this rRNA synthesis is indicated by studies on the anucleolate embryos of *Xenopus*, which do not synthesize any rRNA during their limited life span (Brown and Gurdon, 1964). Furthermore DNA of anucleolate embryos does not hybridize with rRNA (Brown and Weber, 1968), indicating the absence of the ribosomal cistrons.

The typical bipartite structure of the nucleolus may be related to processing of rRNA, even though the mechanism of formation of the cortex of the nucleolus is not yet known. According to MacGregor (1967) RNA molecules found in the core were observed in the cortex after a prolonged incubation. If the cortex is needed for processing and transport of the newly synthesized ribosomal RNA into the cytoplasm, these molecules should not appear in the cytoplasm of *Schizobranchia* oocytes until the bipartite nature of the nucleolus is established. These

relationships will be considered in a subsequent paper.

SUMMARY

A study has been made to correlate morphological and biochemical differentiation in the oocytes of a tubiculous polychaete, *Schizobranchia insignis*. The pressent paper is concerned with an examination of the cytological changes during oogenesis and annual size distribution of oocytes, The oocytes are released from the ovary into the coelomic sac at the end of the oogonial division and grow to a maximum size (180 μ diameter). Oogenesis takes place continuously throughout a year, although the breeding season is the period between January and March. When the oocytes reach the largest size class, they remain constant in size thereafter and accumulate in the coelomic sac.

The nucleolus, which first appears in the oocytes 5-10μ diameter, grows in the early stages of oogenesis, becomes maximum in the oocytes 100-120 μ diameter, and is constant throughout the rest of the period. The nuclelus initially has a single compartment but becomes bipartite prior to vitello genesis.

Three types of yolk including lipid droplets, proteid granules and oval granules of unknown composition form at different times of oogenesis. The lipid droplets and oval granules appear in the early stage, but mainly in the oocytes larger than 80 μ diameter. Proteid yolk and cortical granules appear only in the oocytes larger than 80 μ diameter. Microvilli are abundant in the oocyte 80 μ diameter and embedded in the vitelline membranc. In the oocytes 180 μ diameter they have retracted from the vitelline membrane.

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REFERENCES

Bolognari, A., 1956. Particolari manifestazioni del nucleolo degli ovociti in accrescimento di *Haliotis Lamellosa Lam.* (*Moll. Gast. Pros.*) e di *Aplysia depilans L.* (*Moll. Gast. Opis.*) *Acta Histochem.* **2** : 229.

Brachet, J., M. Decroly, A. Ficq, and J. Quertier,1963. Ribonucleic acid metabolism in unfertilized and fertilized sea urchin eggs. *Biochim. Biophys. Acta* **72** : 660—662.

Brown, D.D. and J.B. Gurdon, 1964. Absence of ribosomal RNA synthesis in the anucle-

olate mutant of *Xenopus lavis. Proc. Nat. Acad. Sci.* U.S. **51** : 139—146

Brown, D.D. and E. Littna. 1966. Syntesis and accumulation of low molecular weight RNA during embryogenesis of *Xenopus laevis. J. Mol. Biol.* **20** : 95—112.

Brown, D. D. and C.S. Weber, 1968. Gene linkage by RNA-DNA hybridization. I. Unique DNA sequences homologous to 4SRNA. 5S ribosomal RNA. *J. Mol. Biol.* **34** : 661.

Galigher, A.E. and E.N. Kozloff, 1971. Essentials of practical microtechnique. Lea and Febiger. pp. 255—285.

Gonse, P.H., 1956. L'voogenese chez *Phascolosoma vulgare.* I. Definition cytologique des stades de croissance des ovocytes. *Acta Zool.* **37**··193.

Gross, P.R. and G.H. Cousineau, 1964. Macromolecule synthesis and the influence of Actinomycin on early development. *Exptl. Cell Res.* **33** : 368—395.

Hegner, R.W., 1914. The germ cell cycles in animal, New York. Macmillan. pp. 114.

Howie, D.I.D., 1961. The spawning of *Arenicola marina* (*L*).III. Maturation and shedding of the ova. *J. Mar. Biol. Assoc.*, **41** : 771—783.

Luft, J., 1961. Improvements in epoxy resin embedding methods. *J. biophys. biochem. cytol* **9** : 409—414.

MacGregor, H.C., 1967. Pattern of incorporation of H^3-uridine into RNA of Amphibian oocyte nucleoli. J. Cell. Sci. 2 : 145.

Nørrevang, A., 1965. Oogenesis in *Priapulus caudatus Lamarck. Vidensk.Medd, Fra Dansk naturh. Foren.*, **128** : 1—83.

Nørrevang, A., 1968. Electronmicroscopic morphology of oogenesis. Intern. *Rev. Cytol.* **23** : 113—186.

Raven, C.P., 1961. Oogenesis: The storagge of developmental information. Pergamon Press.

Reynolds, E.S., 1963. The use of lead citrate at high pH as an electron-opaque stain in electron microscopy. *J. Cell Biol.* **17** : 208—212.

Richardson, K.C., L. Jarrett, and E.H. Finke, 1960. Embedding in epoxy resin for ultrathin sectioning in electron microscopy. Stain Technol. **35** : 313—323.

Schroeder, P.C. and C.O. Hermans, 1975. Annelida: Polychaeta In. Reproduction of marine invertebratesVol. III. Ed. A.C. Giese and J.S. Pearse. Acad. Press.

Schwartz, F., 1934. Untersuchungen ueber vorteilihafte Stueckfaerbung mit Karminen. Zeitschr. f. wiss. *Mikroskopie* **50** : (3) : 305—322.

Tweedle, K.S., 1967. Oocyte development and incorporation of H^3-thymidine and H^3-uridine in *Pectinaria* (*Cistenides*) *Gouldi. Biol. Bull.*, **131** : 516—538.

Tyler, A., 1963. The manipulations of macromolecular substances during fertilization and early development of animal eggs. *Am. Zoologists* **3** : 109—126.

ILLUSTRATIONS OF FIGURES

Fig. 3. Reproductive structures and beginning of oogenesis in *Schizobranchia insignis.* (a) Cross-section of the whole worm prepared by celloidin embedding. The ovary is indicated by the arrow.(b) The coelomic sac of the live animal. (c) The structure

of the ovary (indicated by the arrow in a) in high magnification. (b) The oocytes which just emerge from the oogonial division are clustered in the ovary. g: intestine, c: coelomic sac, s: septum, o: ovary, f: fat body.

Fig. 4. Different size classes of oocytes. All these photos were taken in the oil immersion. (a) oocyte 25 μ diameter, (b) oocyte 40 μ diametecr, (c) oocyte 80 μ diameter, (d) oocyte 100 μ diameter, (e) oocyte 120 μ diameter, (f) oocyte 180 μ diameter.

Fig. 5. Electron micrographs of oocyte 80 μ (a) and 180 μ diameter (b). v: vitellinemembrane, o: oval granules, d: lipid droplet, p: proteid yolk granules, c: cortical granules.

Fig. 6. (a) Rosette array of the oval granules. (b) Stratified oocyte. The arrow indicates the contrifugal pole. d: lipid droplet, c: cortical granules, p: proteid yolk granules.

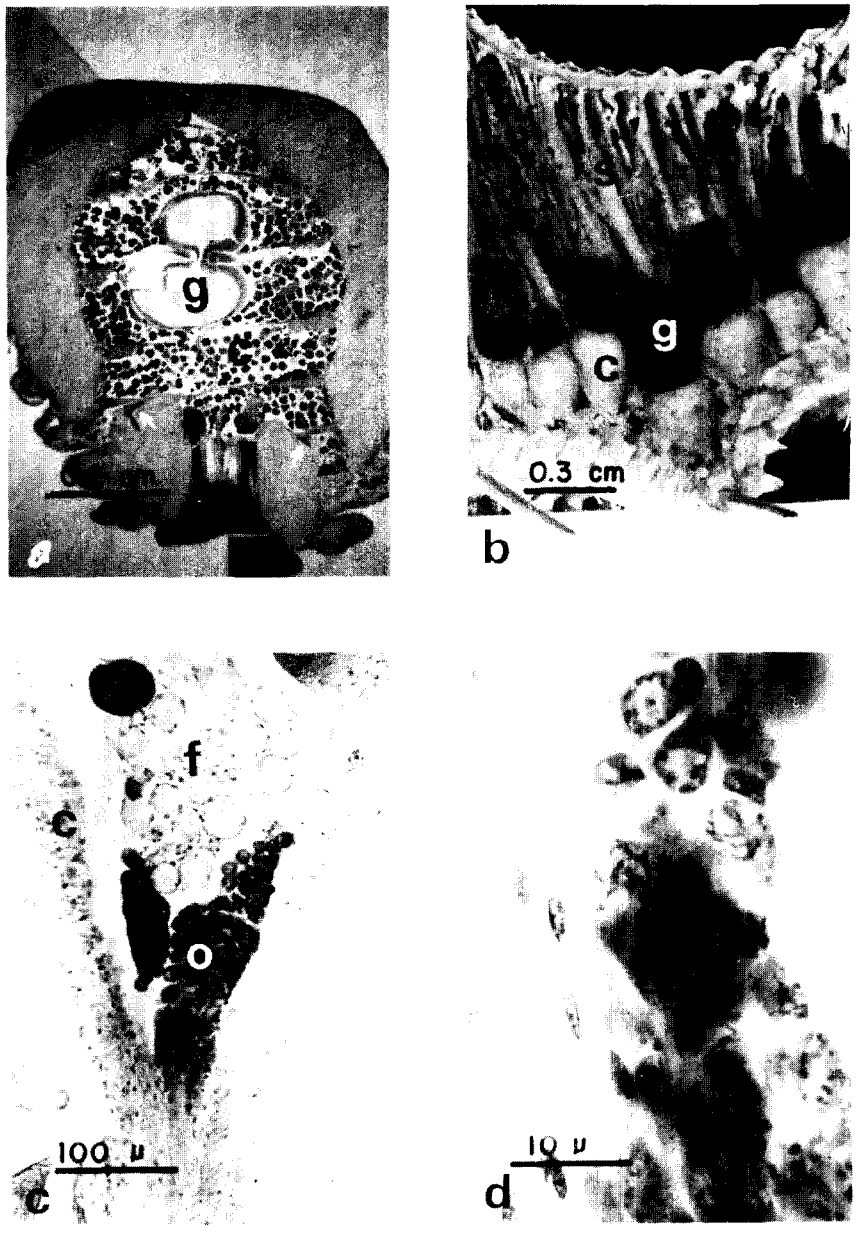
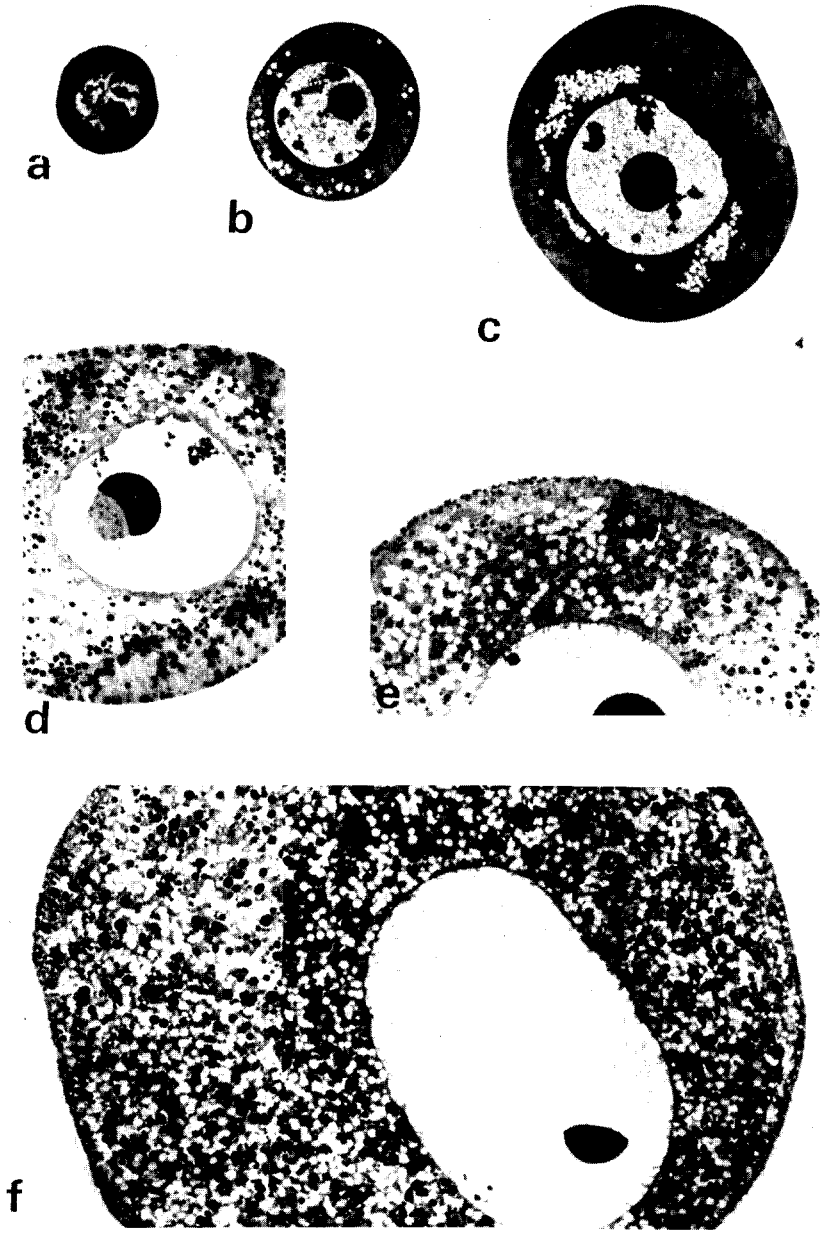


Fig. 3.



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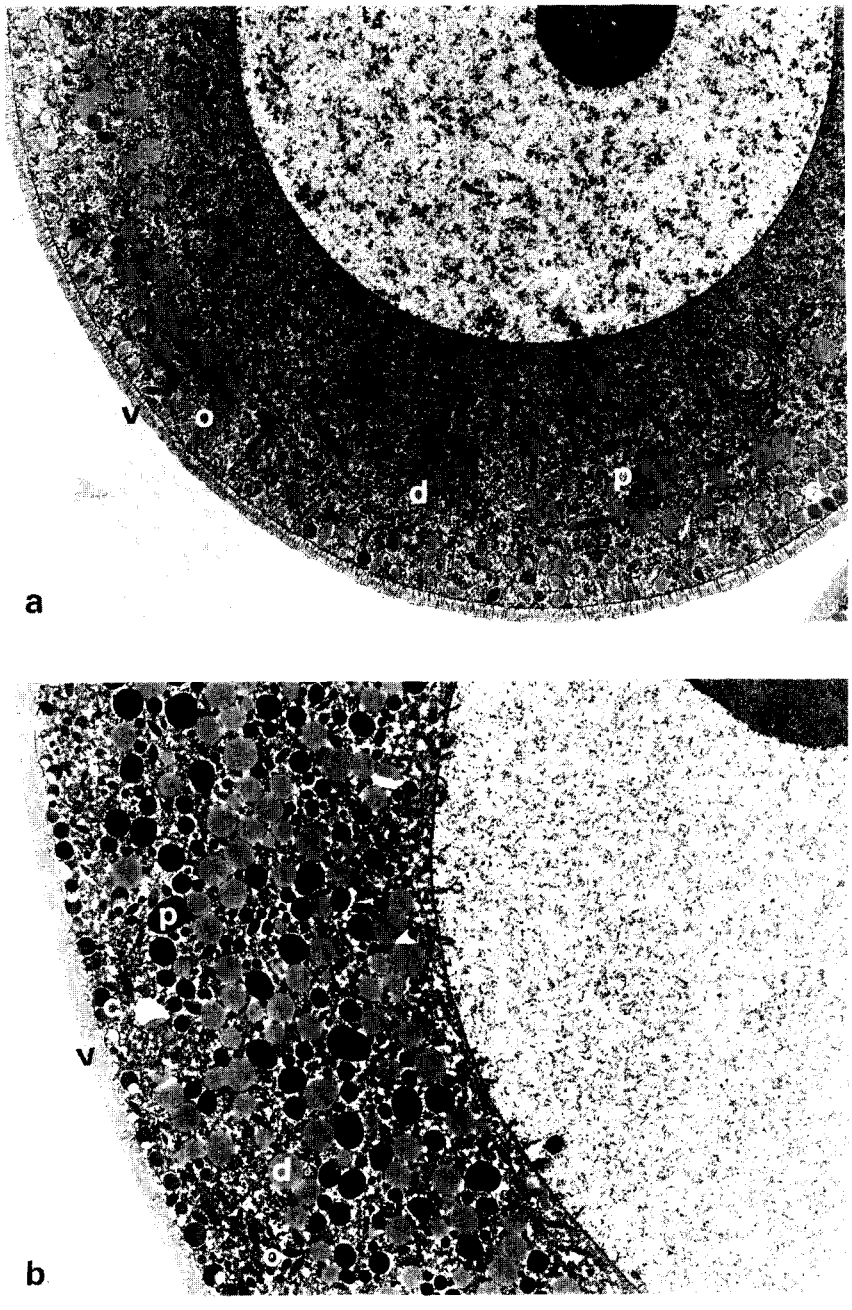

Fig. 5.

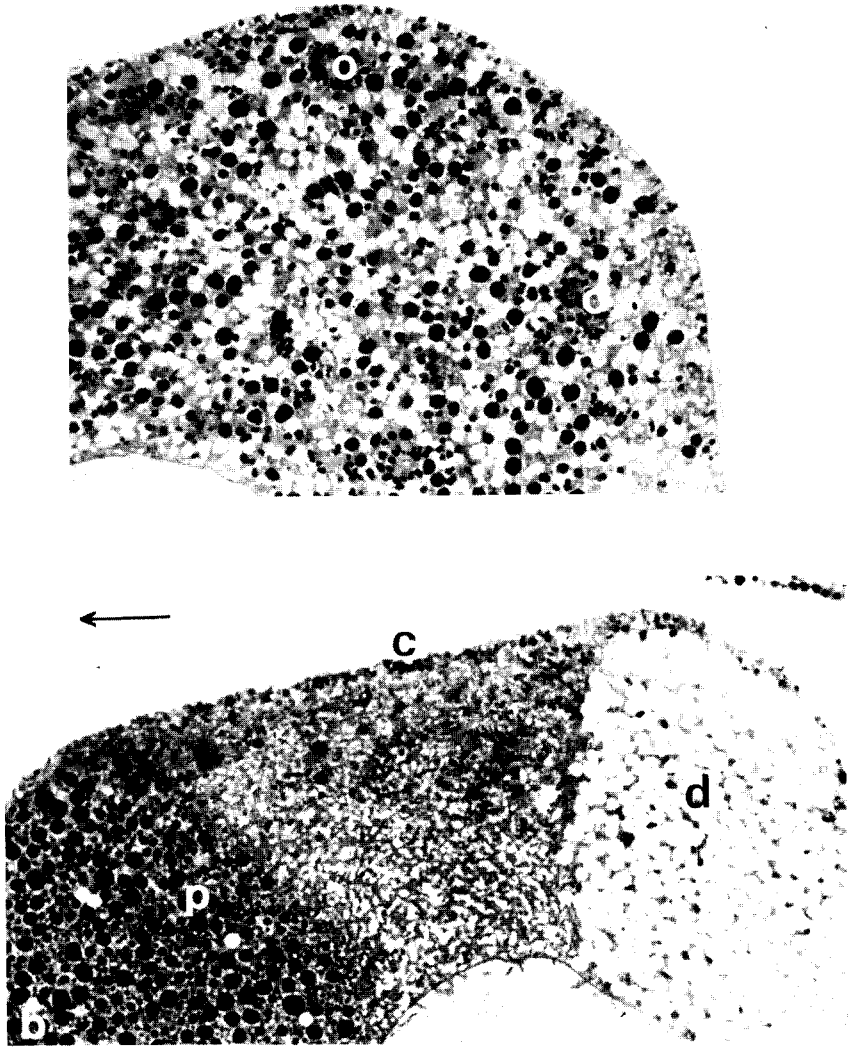

Fig. 6.