ELECTRON MICROSCOPIC STUDIES ON THE GONAD OF THE BORING CLAM, *Tridacna crocea*

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Summary

The gonad of the boring clam, *Tridacna crocea* has been studied under light and electron microscopes. The egg is found covered with a thick coat and attached to the follicle wall by a peduncle. The coat is seen in three layers and is composed of microvilli and intercrossing amorphous substances. The peduncle is the passage for nutritive substances to the egg.

Introduction

The boring clam, *Tridacna crocea* can be successfully farmed with gametes produced by cutting ripe gonads into pieces.[1] The ovum obtained by this method is pear-shaped and almost completely enveloped in a thick coat. It becomes spherical after treatment with ammonium solution. Then, it is fertilized normally. The ammonium method employed was essentially the same as that described by Wada[2] for a pearl oyster, *Pinctada maxima.*

We want to clarify the method employed here for further improvements by means of an electron microscopic observation on the gametes in the gonad. Electron microscopic pictures reveal structures of the coat, modes of ovum attachment to the ovarian follicle and wall structures of the follicle. They provide suggestions on the process of maturation of egg cells and also for the process of transformation into a spherical form.

Material and Method

Materials used were ripe specimens of the boring clam, *Tridacna crocea,* which were collected at Kabira, Ishigaki Island, one of the Ryukyu Islands at the Fisheries

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Station of Okinawa Prefecture. The vales were opened by cutting the adductor and one valve was removed to expose the body. Some blocks of the gonad were cut off and fixed in 2% glutaraldehyde in a buffered solution. After about 1 hour these blocks were cut into small pieces which were further fixed in a renewed solution of the same for 2 hours. These pieces were washed by buffer solution to remove glutaraldehyde and preserved in the buffer solution. They were brought back to the laboratory of Kawasaki Medical School where further treatments were carried out.

They were postfixed for 1.5 hours at 0°C with 1% osmium tetroxide in phosphate buffer, dehydrated and embedded in Epon. Sections were cut on a Porter-Blum ultramicrotome and stained with uranyl acetate and lead citrate. They were observed with a Hitachi HS-9 electron microscope.

Thick sections from the sample for electron microscopy were cut for light microscopic observations. These sections were stained in 1% toluidine blue.

For a comparative study of the development of gonads some histological preparations were made for a study on seasonal variations of the gonad. They were made from gonad specimens preserved in 90% alcohol after fixation in Bouin solution and stained with hematoxylin and eosin (Fig. 1).

Results

1. Light microscopic observation

The gonad is constituted of a great accumulation of both male and female follicles. Distribution patterns of both kinds of follicles vary greatly from specimen to specimen. In younger ones male follicles prevail, but in older ones female follicles are more common. Female follicles are usually about 250−300 μ in diameter, whereas male follicles are irregular in size and shape sometimes attaining great dimensions. Each egg follicle is enveloped in a thin membrane (Fig. 1). In a ripe specimen in summer, it is filled with large egg cells. Each egg measures about 100 μ and is attached to the follicle wall with a peduncle which faces the connective tissue or blood lacunae. Except for this attachment of the peduncle, it is covered with a thick coat[3]. The coat is stained by toluidine blue into three layers: namely, outer deep pink, middle light pink and inner light blue one (Fig. 2).

There are many spherical bodies in the cytoplasm: dark blue ones of various sizes (lipid droplets) and light greenish blue ones of comparatively large and uniform dimensions (yolk protein granules). In some female follicles, there are egg cells at various stages of development. This fact is remarkable in winter specimens or

exhausted ones. Small ova have a small nucleus with spherical nucleolus at the peripheral region but no thick coat. They are found close to the follicle membrane. However, there are not so many of them as to form a line along the follicle wall as are reported in oysters[4].

2. Electron microscopic observation

Fig.3 shows a part of an ovarian follicle wall and its vicinity. The wall appears as a thin layer running obliquely in the middle of the picture. It is covered with a lining of thin muscle fibers and with a connective tissue containing wide blood lacunae with blood cells and zooxanthellae. Zooxanthellae are found encircled in wandering cells in various stages of digestion.[5] They are few in the inner portions of the gonad, especially in narrow connective tissues. The lower portion is a part of an egg cell with a thick coat, facing the follicle wall. The cytoplasm of the egg cell is composed of a dense distribution of two kings of spherules. One consists of dark stained lipid spherules of various dimensions from 0.2 μ to 1.5 μ in their diameter. The others are of light stained yolk spherules of $1-3\mu$ in diameter. The matrices of cytoplasm between these spherules are made up of heavy distributions of endoplasmic reticula, Golgi bodies, small spheroidal mitochondria and dense particles (Fig.4). These pictures show the active formation of yolk and lipid spherules from these organellae in the matrices.

The nucleus is very large but nearly homogeneous, with even distributions of small particles except for a small nucleolus in a peripheral position. The nuclear membrane is rather thick and provided with many nuclear pores (Fig.4).

The coat of the egg or vitelline layer is very thick measuring about $4-5$ μ in thickness. It is composed of arrangements of fibrous substances. It can be divided into three layers, as described above, in toluidine blue stained preparations. However, in electron micrographs the distinction between these layers is not so clear as in optical observations (Fig.5). The inner layer spans about $0.8-1.0$ μ in thickness and is composed of parallel arrangements of microvilli which stand out vertically from the egg surface. Each microvillus measures about 80 nm in diameter and is densely surrounded with amorphous substances which are frequently observed as numerous horizontal lines connecting with neighbouring ones. It extends further into the outer layer of the coat through the middle layer of the coat into the outer one. It turns very thin (20 nm in thickness) and shows a slightly wavy shape in the middle layer, which measures about 3μ in thickness with diffuse amorphus substances. In the outer layer, measuring about 0.5 μ in thickness, it swells again a little and has rather dense amorphous substances around it.

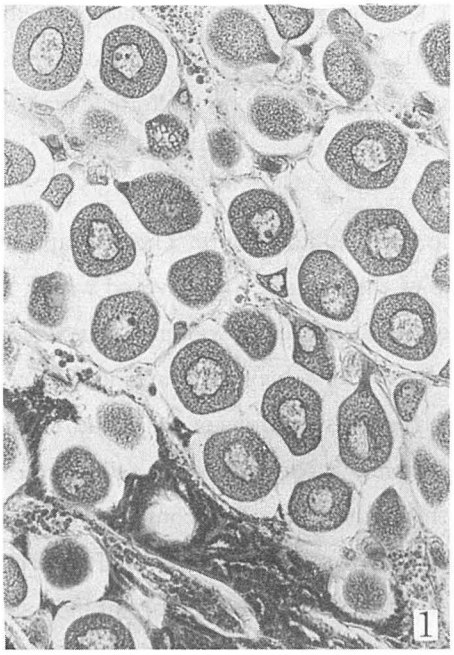

Fig.1. A part of a gonad of *Tridacna crocea,* from a specimen preserved in alcohol after Bouin fixation. The coat is seen as a clear ring of 10 μ in thickness. ×130

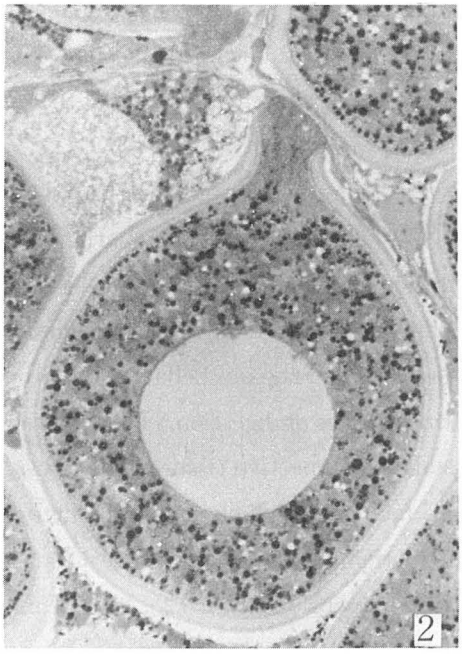

Fig.2. An egg in a toluidine blue stained section showing three layers in the coat and attchment to the follicle wall. ×570

Fig.3. A part of a surface portion of an egg with a thick coat (C) and a thin follicle wall (W) with a lining of thin muscle fibers (F) and connective tissues containing blood cells (B) and zooxanthellae (Z). ×6200

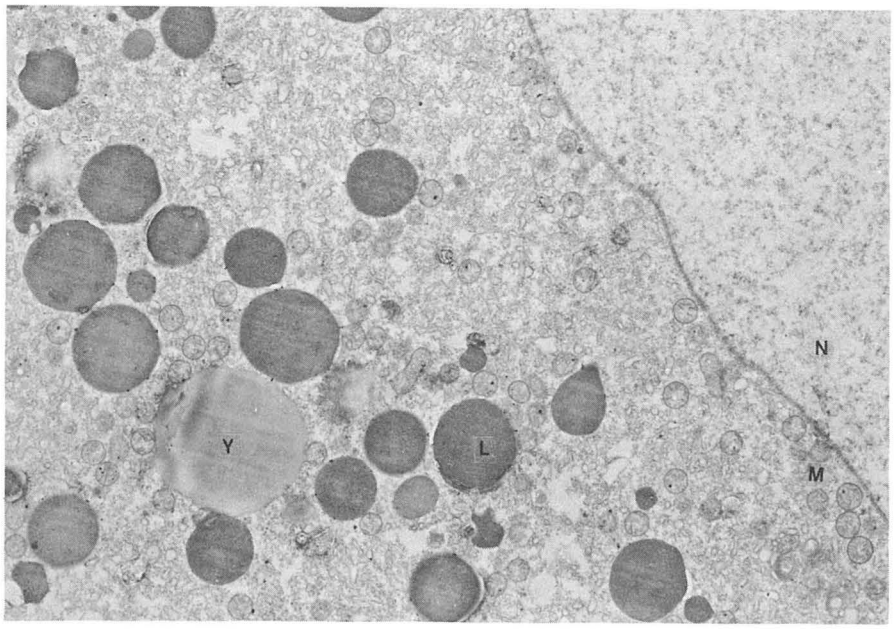

Fig.4. A part of the nucleus (N) and a consecutive area of cytoplasm with dark stained lipid droplets (L), yolk bodies (Y) and matrices including small spheroidal mitochondria (M) and endoplasmic reticula. ×8300

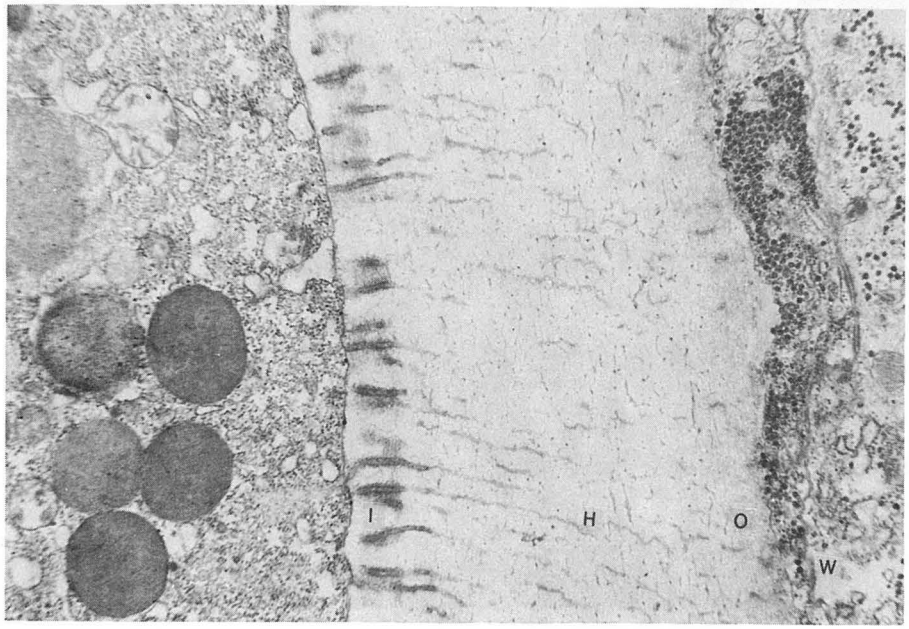

Fig.5. Englarged view of parts of cortical area of the egg and the coat showing details of three layers. Inner layer (I), middle layer (H), outer layer (O). ×12000,

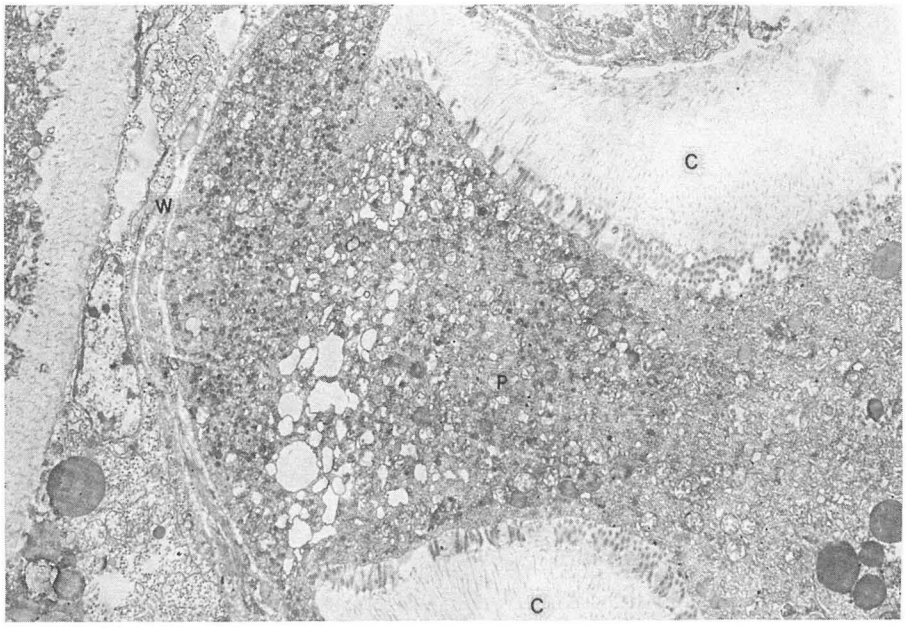

Fig.6. A part of the egg peduncle (P) and its attachment to the follicle wall (W) and connective tissues. ×4700

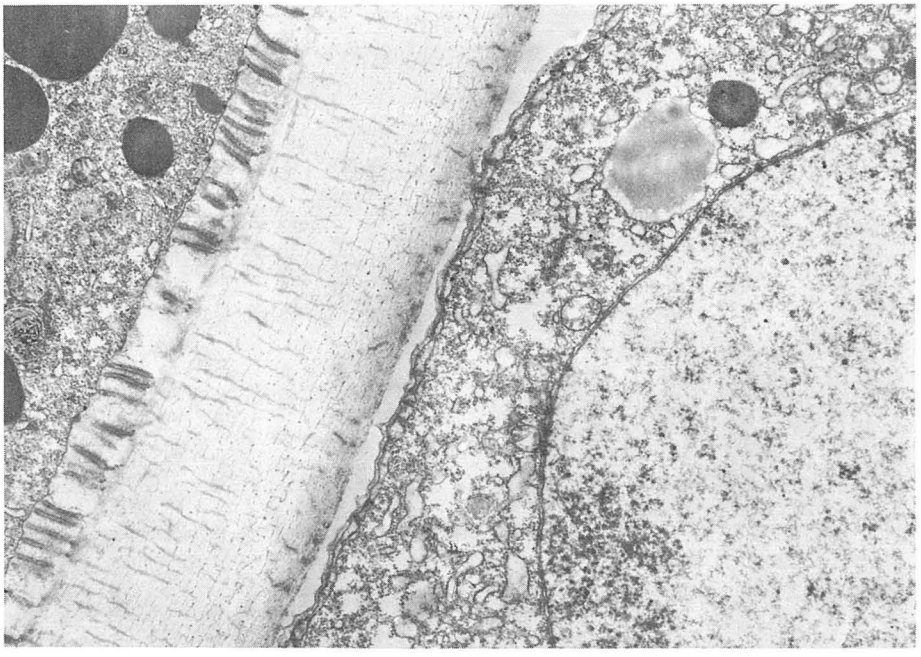

Fig.7. A part of a young egg (right) and a part of a mature egg (left) showing striking differences in structures of coat and also in those of cytoplasmic inclusions. ×8700.

In some cases there is another thin layer between the inner and the middle layers as is seen in Fig.7 at the top of thick microvilli.

Attachment: A part of the peduncle of.an egg is shown in Fig.6. The peduncle attaches to the follicle wall at the left side of the picture. It is still covered with the coat which sharply decreases in thickness and terminates at the edge of the attachment. Its cytoplasm is free from large yolk and lipid granules, but is filled with endoplasmic reticula, mitochondria, small granules and vesicles. These structures indicate the passage of nutritive substances from blood lacunae to the egg through the attachment. Frequently, there is a wandering cell or cells covering the attachment.

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ヒメジャコ性巣の電子顕微鏡的研究

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ヒメジャコ卵巣は小胞状体の集合で，卵は胞壁に短い柄で付着し栄養をここから吸収する。これが切出卵の人工受精に障害となる。柄の付着点以外の卵表面は厚い被膜で覆われる。この膜の電子顕微鏡的構造を明らかにした。

＊ 沖縄県水産試験場　石垣市川平