The Development of Two Species of *Tetilla* (Demosponge)[1,2]

Yoko Watanabe

Department of Biology, Ochanomizu University, Tokyo, Japan.
and
The Tateyama Marine Laboratory of Ochanomizu University, Chiba.

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CONTENTS

1. Dedicated to the memory of Prof. Matazo Kume.
2. This paper was based on a portion of a dissertation submitted to the Tokyo University of Education, in partial fulfilment of requirements for the degree of Doctor of Science.

INTRODUCTION

Studies of development of the Porifera have been made since the 1800's, having their center in Europe. In spite of many researchers for various speicies, there are many problems left unsolved. For example, the following problems are waiting to be studied: sexuality, oviparity and viviparity, gametogenesis, structure of egg, fertilization, formation of blastoderm, metamorphosis and others.

The author has observed the development of the two species of *Tetilla*, *T. japonica* and *T. serica*. Both these species are found to have a form of development that is peculiar among the sponges. Most sponges have been thought to be hermaphroditic, with fertilization taking place inside of the body. The fertilized eggs develop in the maternal tissue until they become free-swimming larvae. The released larvae adhere to the substratum after some free-swimming period. As compared with this, the two species of *Tetilla* follow a peculiar course of development, as follows. 1). Both species are gonochoristic, and individuality is very clear. 2). They are oviparous sponges. They release mature eggs, and fertilization takes place outside the body. 3). They have numerous radiating fiber bundles on the surface of the mature eggs, but they do not have any accessory cells like other oviparous sponges. 4). After fertilization, the fiber bundles on the surface of the egg are drawn into the fertilization membrane surrounding the egg. At this time, the egg shows a peculiar type of movement. 5). The fertilized egg adheres immediately to the substratum, and undergoes direct development without the stage of free-swimming larva.

The development of *Tetilla* is very different from that generally seen in sponges, but as a material for experiment it has the following advantages: 1). With viviparous sponges, the fertilization and early development can be observed only in fixed materials or sectioned preparations. But since *Tetilla* is oviparous and its fertilization takes place outside the body, we can observe these phenomena directly and continuously, and experimental manipulation is possible. 2). If males and females are cultured separately, we are able to obtain unfertilized eggs. 3). After fertilization, since the eggs immediately adhere to the substratum and show direct development, we can easily distinguish the individuals.

On the other hand, they have some disadvantages as experimental materials; that is, 1). Since the eggs and sperm are gradually released as they mature, we cannot obtain enough eggs or sperm at the same time. 2). Spawning conditions are not known, so we cannot induce spawning artificially. 3). Adult sponges, especially *T. serica*, cannot be cultured in a laboratory tank for a long time.

In 1910, Nagai observed the fertilization and early development of *T. serica*. 1952, Kume reexamined this investigation. Watanabe studied the

later development and life history of the same sponge (1957, 1960). In 1967, Endo *et al.* published the results of Endo's research on the process of its fertilization under the electron microscope, including Watanabe's observations of the development. After that Watanabe made a histological study, using electron microscopy. She also found *T. japonica* as a new material and investigated its development. She made it clear that this species also has a peculiar course of development like *T. serica,* and that it has many phenomena in common with *T. serica.*

The author has used *T. japonica* as research material and has studied it from 1975. In this report, she includes the results of the observations of *T. japonica* and unpublished material from *T. serica.*

In this paper, she does not use the customary terms, but she uses a terminology which was proposed by Borojević *et al.* (1967). The customary terms are indicated in parentheses.

ACKNOWLEDGEMENTS

I deeply express my great gratitude to the late Prof. Matazo Kume for giving me such an absorbing subject of study and for leading me to take advantage of studying sponges. I am also grateful to Dr. Yoshiyuki Endo, Professor of Keio University, who took interest in my study and gave me many valuable suggestions.

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Finally, I wish to express my thanks to Mr. Jujiro Deguchi of the Misaki Marine Biological Station, for his kind help in collecting material, and also to the staff of the Station, who kindly facilitated my use of the laboratory.

MATERIALS AND METHODS

Tetilla serica and *Tetilla japonica* were used as the materials. *T. serica* was collected at Aburatsubo Bay and Koajiro Bay near the Misaki Marine Biological Station. *T. japonica* was also collected at Tateyama Bay near the Tateyama Marine Laboratory. The breeding season of these species lasts from July to September, most actively in August. Adult sponges were cultured in the laboratory tanks, where they eventually spawned spontaneously. The eggs are easily fertilized by the sperm discharged from the male sponges. As the fertilized egg has the characteristic of adhering to the substratum, slide-glasses were kept in the tank to make them adhere.

To obtain unfertilized eggs I separated female from male sponges and

cultured them in different tanks. Eggs discharged from the female sponges were collected by a pipette. The difference between male and female is quite difficult to determine from the external morphology. So I waited for the sponges to discharge the eggs to isolate them, or I examined by cutting a slice from the adult body to see if there were any eggs in the tissue. Cutting a slice is not desirable because it shortens the life of the sponges especially in *T. serica,* but *T. japonica* can regenerate easily, so cutting does not shorten its life so much.

During the first five or six days, the eggs on the slide-glasses were cultured under running sea water in the laboratory. After a week, these slides carrying the larvae were put into a rearing box submerged in the sea for further culture.

The larvae were fixed *in toto* according to the developmental stages. The fixative was 1% osmium tetroxide for both light and electron microscopy. The fixed materials were dehydrated with ethanol and embedded in styrene methacrylate. Sections were cut with glass knives on a Porter-Blum MT-1 ultramicrotome and stained with uranyl acetate for thin sections and toluidine blue for thick sections. Observations were performed with a Hitachi HS-7D electron microscope for thin sections and a Nikon phase contrast microscope for thick sections. I also used Olympus water immersion lenses for live materials.

Sect. 1. General features

(1) General morphology

Tetilla serica and *Tetilla japonica* are solitary sponges, and exhibit definite form and radial symmetry. They have one osculum on the top and a root tuft at the base by which they adhere to the substratum. Both species live in sandy shoals.

In *T. serica,* two size groups are found in summer: 100–150 mm in height and 15–20 mm in height. An adult *T. serica* generally has a diameter(r) greater than its height(h); the ratio is $r/h>1$. Sometimes in young or smaller specimens the ratio is $r/h\leqq 1$ (Fig. 1a, 1b). In *T. japonica,* however, the ratio of the diameter to the height is always $r/h<1$ and the animal has an elongated egg-shape (Fig. 1c).

(2) Systematics

When *T. serica* was first used as the material for study, Nagai (1910) referred to it only by the Japanese vernacular name "Tonasu", and did not use the scientific name. "Tonasu" means pumpkin, and the shape and colour of the sponge looks like a Japanese pumpkin. Nagai thought that *T. japonica* belonged to the same species as *T. serica.* But in 1914, Lebwohl described *T. serica* in his investigation of Japanese tetraxonian sponges and named it *Tethya serica.* In 1888, Sollas described *Tetilla japonica* in the "Challenger" report as belonging to the Tetillidae. In Hyman's description (1940), the

the genus *Tetilla* is devoid of microscleres. Some genera of the Tetillidae lack microscleres, but the genus *Tetilla* has sigma microscleres. In 1965, Tanita changed its genus from *Tethya* to *Tetilla* in "New Illustrated Encyclopedia of the Fauna of Japan". The Tethyidae and Tetillidae are solitary massive sponges. *Tetilla serica* and *Tetilla japonica* have sigma microscleres, but lack the steraster, which is a kind of microsclere specific to Tethyidae. Tanita's changing of the family is considered to be appropriate.

The following is the systematic position of the two species: this accords with the system of C. Levi (1973).

Phylum Porifera
Class Demospongeae
Subclass Tetractinomorphes
Order Spirophorida
Family Tetillidae
Genus Tetilla
Species *Tetilla serica*
Tetilla japonica

(3) Discussion

The young *T. serica* is closely similar to the adult *T. japonica* in its external morphology and colour. When I saw *T. japonica* for the first time, I wondered if it was a young *T. serica*. After having found mature eggs in *T. japonica*, I considered that *T. serica* and *T. japonica* belong to the same species, with ecological differences.

T. serica has been belived to be an annual sponge and to die after spawning within one year. *T. serica* has now become extinct in Aburatsubo Bay, but until 1950 the Bay was rich in *T. serica* and there were so many sponges that the sea turned red with them; this was called the "pumpkin farm". In Nagai's report the "pumpkin farms" were observed on both shores of the Bay every other year. He thought that the phenomenon was caused by the waves, but I inferred that it is because *T. serica* is biennial. As described above, there are two kinds of *T. serica* with 100–150 mm length and 15–20 mm length which were collected in August. *T. serica* which I cultured in the field grew to only 4 mm in 77 days after fertilization. The eggs fertilized in the previous year grew to 15–20 mm in a year. These are believed to constitute the smaller size group. After another year they grow to 100–150 mm and die after spawning. These are supposed to belong to the group of larger sponges. *T. secica* has no free-swimming larval stage, so its distribution depends on spreading the eggs over a wide area, and the eggs spawned by many adults paint the sea surface red every two years. Since *T. serica* has become extinct locally, it will be necessary to find another habitat abundant in *T. serica* in order to confirm this supposition.

Sect. 2. Sexuality and breeding habits

(1) Sexuality

As with *T. serica*, it is also very difficult to distinguish males from females of *T. japonica* by the external morphology. During the breeding season, eggs or sperm can be found in the tissue cut from the body wall. Although cutting the body is better avoided to keep the animal alive as long as possible, *T. japonica* soon regenerates. A small cut made with a sharp razor will heal up and recover in a few days and there is no permanent effect that would prevent its use as research material.

In histological preparations, eggs and sperm cannot be found at the same time in the same individual sponge. Both *T. serica* and *T. japonica* are clearly gonochoristic. The life span of *T. japonica* is not certain, but it must be about one year, since there is not a marked difference in size. After spawning, *T. japonica* is seen to disintegrate in the natural environment at the end of September. So it is considered to be a gonochorist which has one period of maturation in its life.

(2) Breeding habits

The spawning period of *T. japonica* extends from the end of July to the middle of September. The peak is August, as in *T. serica*. Spawning of *T. japonica* is seen from 3 a.m. to 7 a.m. In *T, serica*, spawning takes place all day at the best season when the animals are in good condition (Watanabe, 1960), but early or late in the breeding season it is observed only in the early morning (Endo et al., 1967).

Mature eggs of *T. japonica* are discharged through the female osculum into the sea water. Spermatozoa are also discharged through the male osculum in the same manner. A relatively small number of eggs and spermatozoa are discharged at the same time; the eggs are therefore collected by a pipette. When unfertilized eggs are required, the males and females are kept in separate tanks, but in such cases, sometimes spawning does not occur synchronously, suggesting that the animals may be stimulated by the spawning of the opposite sex.

(3) Discussion

For a long time, most of the Porifera had been considered to be hermaphroditic and viviparous, and only a few exceptions to be gonochoristic and oviparous. But recently some sponges which had been considered to belong to the hermaphrodites were proved to be gonochoristic sponges, and the problem of sexuality is being watched with keen interest (Tuzet and Pavans de Ceccatty, 1958; Sarà, 1961; Borojević, 1967; Liaci and Sciscioli, 1967; Fell, 1976; Gilbert and Simpson, 1976; Reiswig, 1976).

Some successive hermaphrodites were found, which release only eggs or sperm in one breeding season and show the alternating male or female sexual activity in the next season (Lévi, 1956; Sarà, 1961; Tuzet and Paris, 1964). Fell (1970) and Gilbert and Simpson (1976) reproted, as the results of continuous examination of the life history and gametogenesis, that the sexuality of sponges is not settled. Sarà (1974) and Fell (1974) described that gono-

choristic and successive hermaphrodite forms are mostly found in the oviparous sponges, and contemporaneous hermaphrodites among the viviparous sponges, so sexuality stands in some relation to the breeding pattern. In the Demosponges, the sex phenotype appears to be linked to the phylogenesis of the sponge through a genetic basis. In this observation, *T. japonica* and *T. serica* have only one breeding season in the life of each, and in that season each individual releases its gametes. So these sponges must be true gonochochorists.

In an experiment of heterosexual combination of *T. serica* by Egami and Ishii (1956), they observed that the gametogenesis was not not affected in either sex by such heterosexual combination. The experiment was undertaken in June; when the two sponges were united, their size had already reached 4 to 8 cm in diameter. As mentioned above, *T. serica* is considered to be a biennial animal, so June is too late for one sex partner to exert an effect on the other. Although I often observed natural fusion of eggs and larvae in an early stage of development (Fig. 10c), I have never seen any gynandromorphs among adult sponges. In the experiment by Egami and and Ishii, sexuality is considered to have been determined in the one or two months before spawning. If the fusion had been performed earlier, the sex might well have been regulated in one direction or the other.

Sect. 3. Eggs and spermatozoa

(1) Oogenesis

Some observations by light and electron microscopy on oogenesis in *T. serica* are reported as follows. In the adult *T. serica,* we find oocytes of various shapes and sizes within the mesohyle (mesenchyme). There are no gonads, as in other sponges, and oocytes are not localized but scattered within the whole mesohyle. The origin of the germ cells of *T. serica* and *T. japonica* is not clear. In many sponges the germ cells are known to develop from choanocytes, but in *Tetilla,* no choanocyte has been observed to be changed to the phase of germ cells. It is difficult to determine whether young oocytes with an irregular amoeboid shape develop directly from archaeocytes, or choanocytes develop into oocytes through an amoeboid phase.

The small oocyte has an irregular shape and lies free in the mesohyle (Figs. 2b, 3a), but growing oocytes are observed to be surrounded with nurse cells (Figs. 2a, 3b), which are engulfed at the surface of the oocyte (Figs. 2a, 4). In the choanocyte chamber (flagellated chamber) around a growing oocyte, several choanocytes are observed to break ranks and change into the amoeboid phase (Fig. 2a). On the other hand, in a choanocyte chamber far from a growing oocyte the choanocytes are arranged in order and do not change their shape in the same specimen (Fig. 2b). These transformed choanocytes may be going to surround the oocyte and develop into nurse cells. Among the calcareous sponges the nurse cells usually seem to be either choanocytes or cells that are derived from choanocytes (Duboscq and

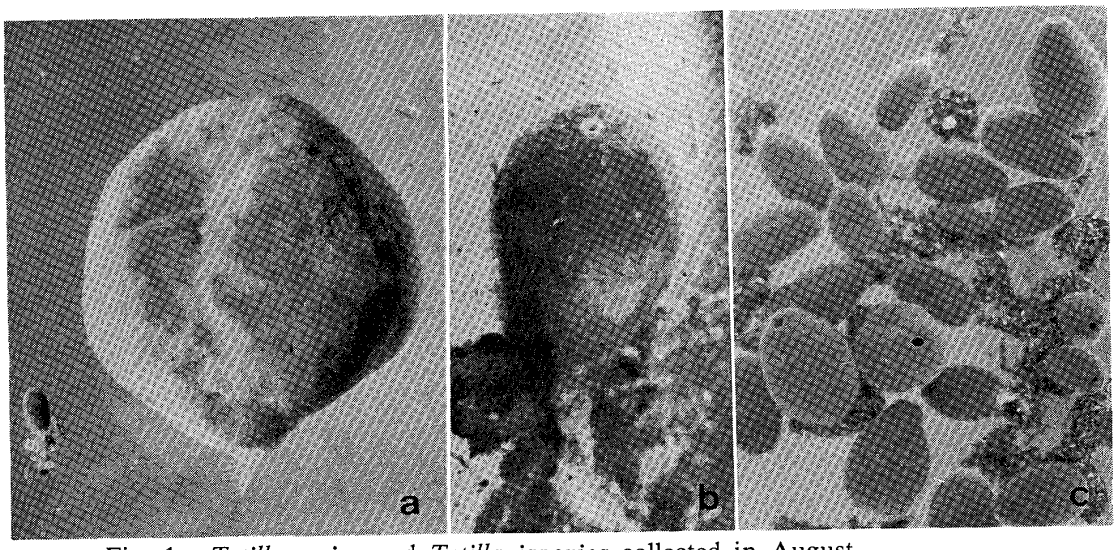


Fig. 1. *Tetilla serica* and *Tetilla japonica* collected in August.
a. There are two kinds of *T. serica*, small (1.2 cm) at left and large (10 cm) at right.
b. Young *T. serica* of elongated egg-shape. Body length is 3 cm.
c. Mature *T. japonica*, body length is 1 cm–3 cm.

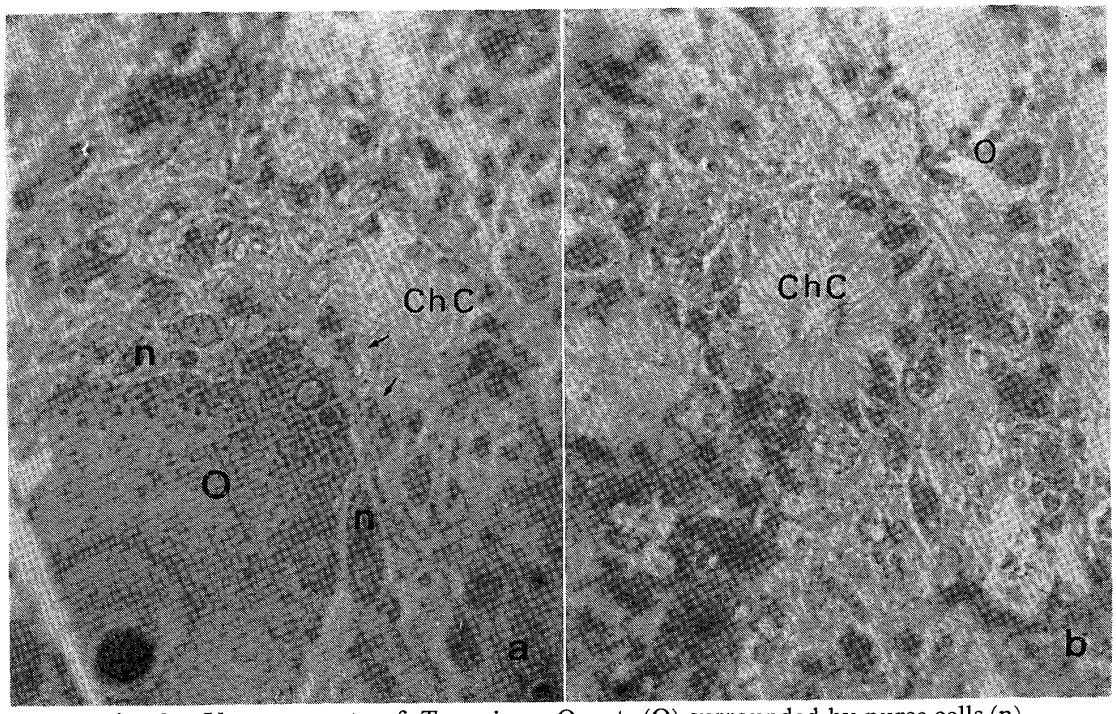


Fig. 2. Young oocyte of *T. serica*. Oocyte (O) surrounded by nurse cells (n) and engulfs them at the surface (light microscope).
a. Choanocytes in a choanocyte chamber (ChC) near an oocyte, developing into the amoeboid phase (arrows).
b. Normal choanocyte chamber in the same specimen. Choanocytes do not change their shape or arrangement.

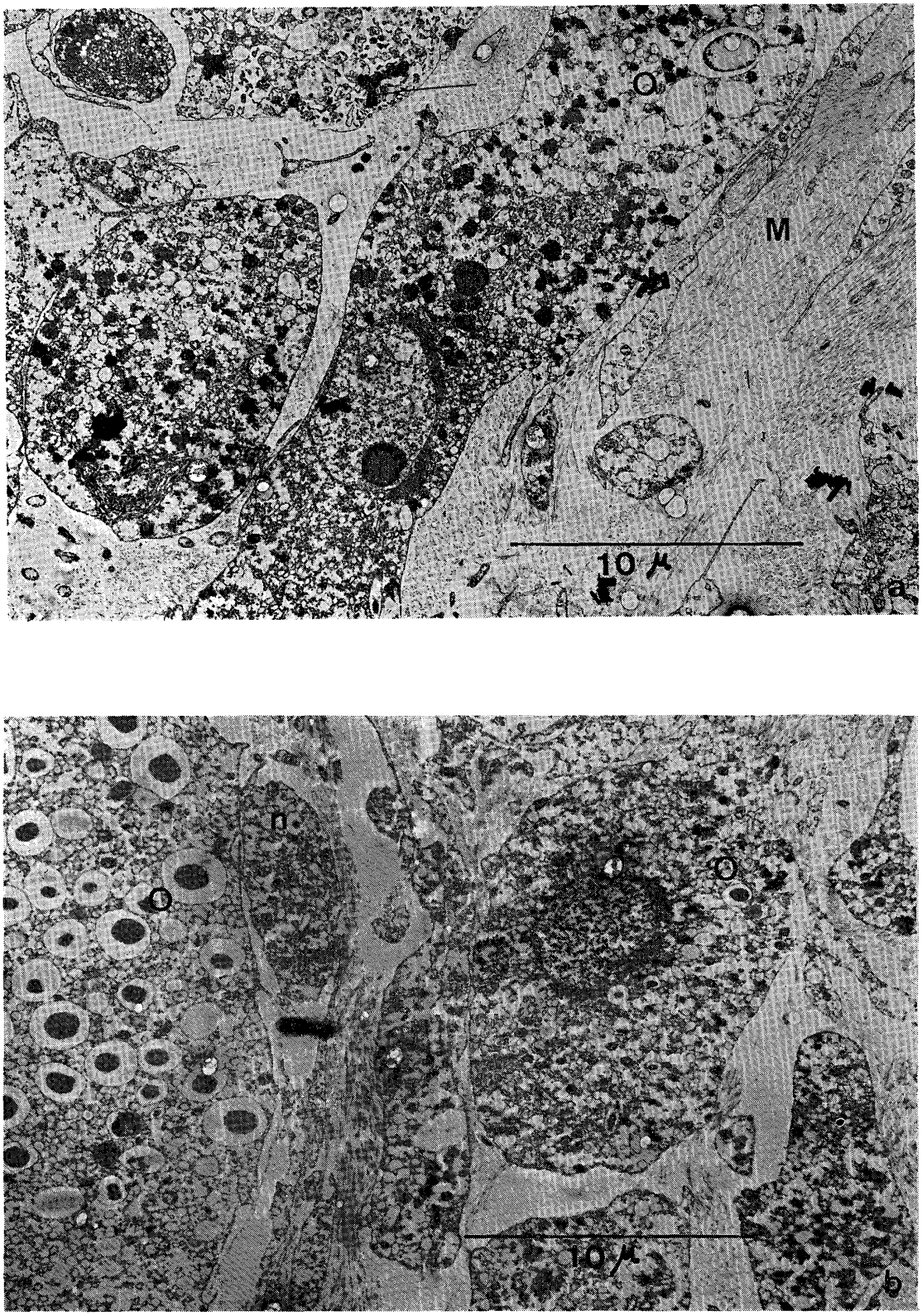
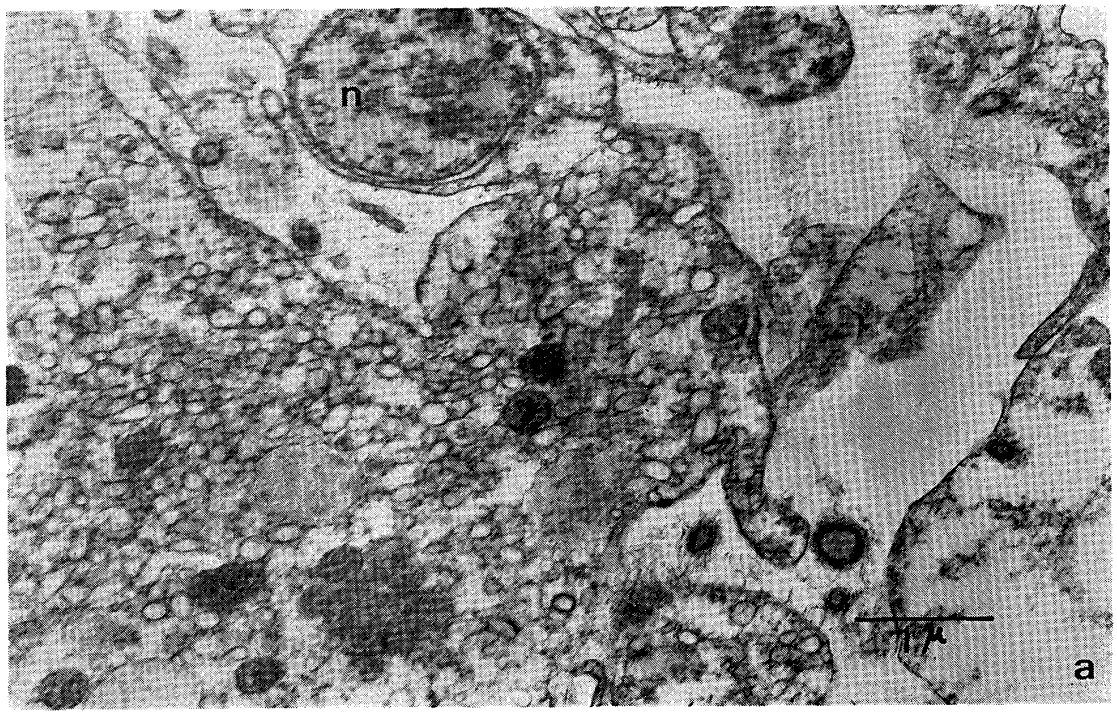


Fig. 3. Electron micrographs of young *T. serica* oocyte before major growth period.
a. Irregular shape, lying free in mesohyle.
b. Growing oocyte at left and young oocyte at right.
O: oocyte; n: nurse cell; M: mesohyle



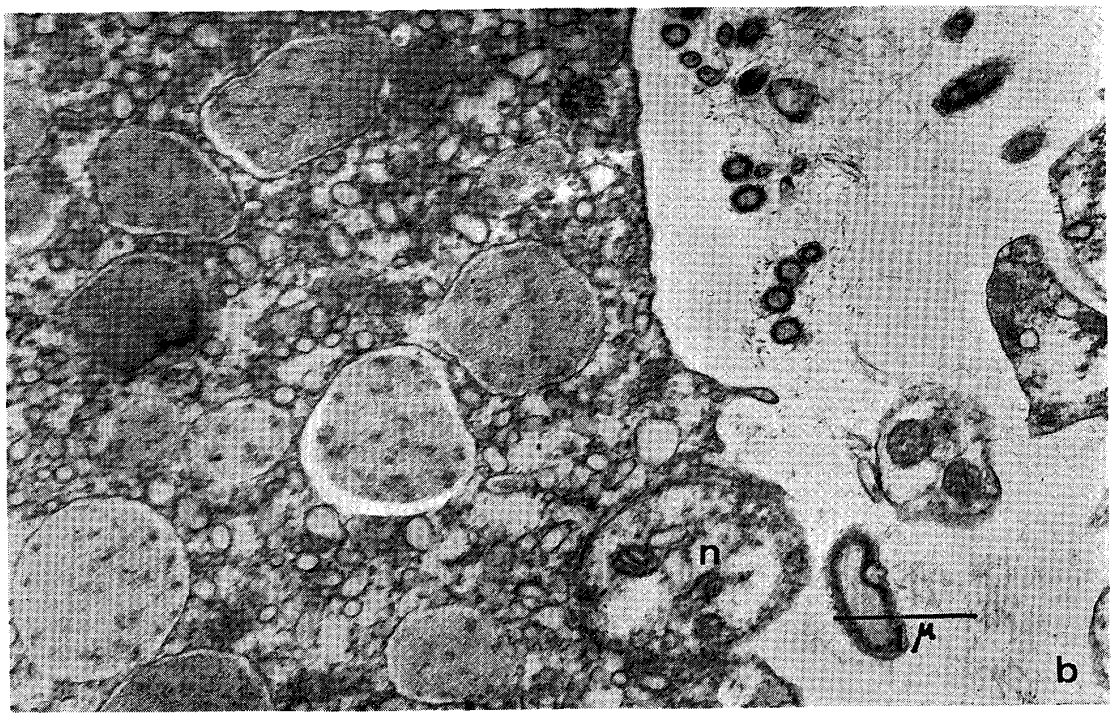


Fig. 4. Growing oocyte of *T. serica*. Oocyte is engulfing nurse cells.
n : nurse cell

Tuzet, 1937, 1942, 1944 ; Vacelet, 1964 ; Tuzet, 1964).

When major growth begins, the oocyte grows rapidly, engulfing nurse cells and storing yolk granules within itself. There are two kinds of conspicuously apparent granules in the ooplasm. One has a large size and high electron density, and with a region of rather low electron density in its center (Figs. 5a, 5d); the other has rather low electron density (Figs. 5a, 6).

The former are considered to be yolk granules, because they increase their number with the growth of the oocyte, and they disappear during the process of larval development. In the ooplasm, many mitochondria are seen to gather in it. Among these mitochondria, some have clearly visible cristae and others are too compact to distinguish the cristae. The aggregations of mitochondria are considered to condense gradually, finally forming compact masses; in the order shown by the numbers 1 to 6 in Figs. 5a to 5d. These figures suggest that the aggregation can be considered to be a yolk nucleus, where yolk granules are to be formed.

The other granules are irregular in shape and smaller than the yolk granules (Fig. 6). Their electron density is the same as that of the center of the yolk granules, and they contain some highly electron-dense particles distributed randomly in them. The nature of these particles has not been clarified. As the granules are noticed in the vicinity of the endoplasmic reticulum around the nucleus and a substance similar to them is seen in the endoplasmic reticulum vesicles, they are considered to be formed by the endoplasmic reticulum (Fig. 6b).

Since no granules similar to the yolk granules of the oocyte are detected in the nurse cells, the yolk granules are considered to be formed inside the egg, rather than to be formed outside of the egg and carried into it.

I have scarecely observed spermatogenesis. Studies of the gametogenesis of *Tetilla* are to be continued in the future.

(2) The structure of the egg

The egg released from the osculum of *T. japonica* is about 130 μm in diameter, richly laden with yolk. It has long, radiating fiber bundles on its surface (Fig. 7a). Endo et al. (1967) reported that *T. serica* had such radiating fiber bundles. The fiber bundles in *T. japonica* are about 60 μm, shorter than those of *T. serica*. However, the ratio of the egg diameter and fiber length is the same as that of *T. serica*. The presence of such structures on the surface of the egg is unknown in Porifera as well as in other Metazoa.

According to Endo's electron microscopic observations, the fiber bundles of *T. serica* consist of very thin fibers, and striations are observed at intervals of 17 nm on each fiber. I did not secure enough material for electron microscopic study in *T. japonica*. I have not yet observed the fiber bundles of *T. japonica* under the electron microscope, but on the basis of their appearance with light microscopy, they are believed to have a similar structure.

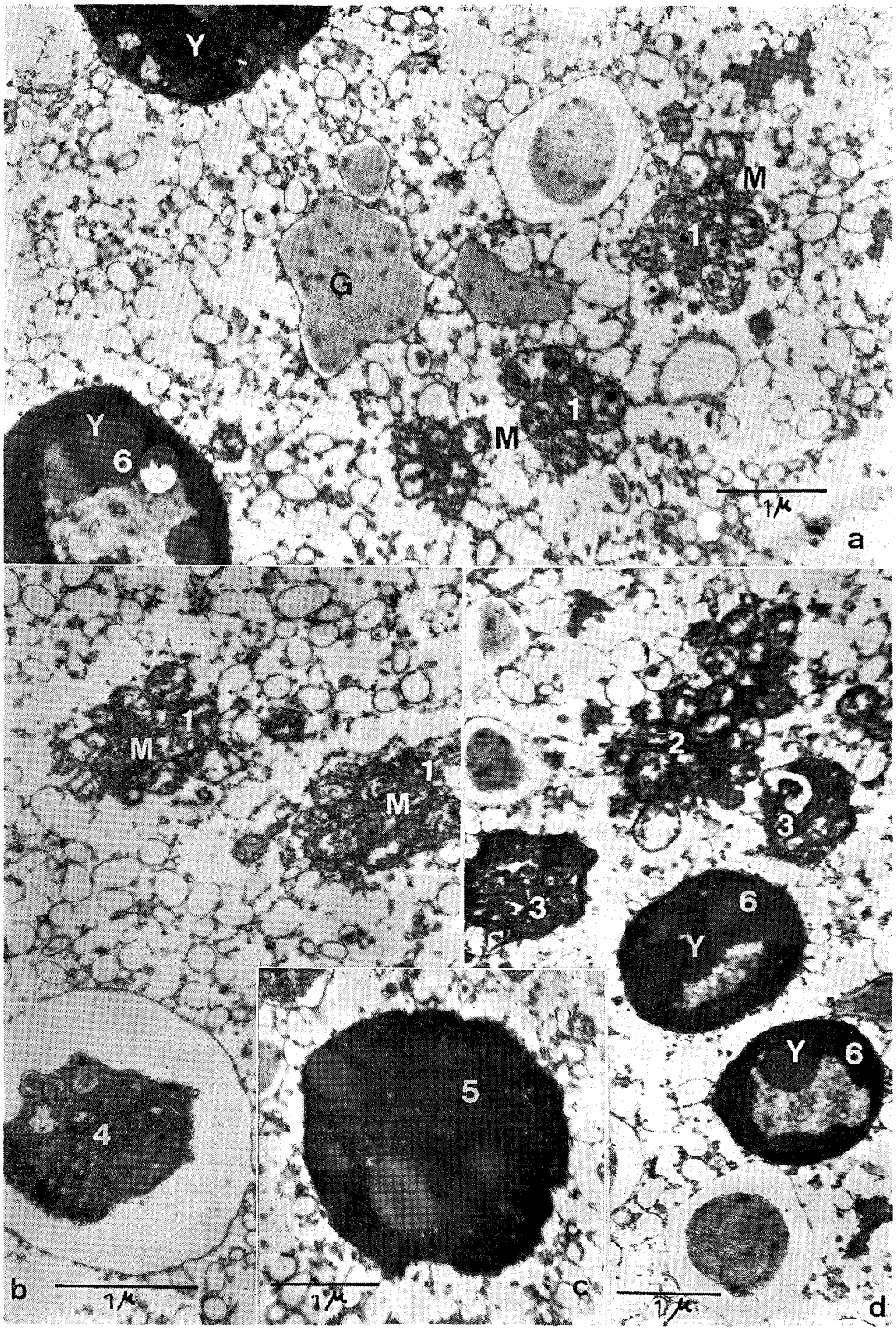
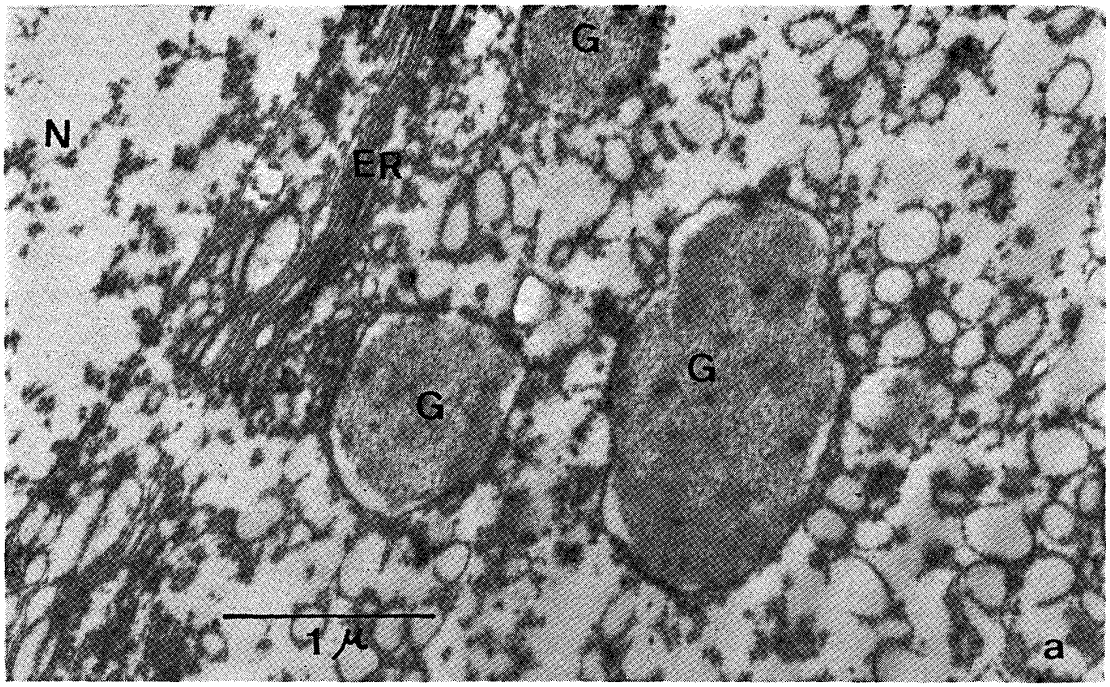


Fig. 5. Yolk granules in oocyte of *T. serica*.

a. Accumulation of mitochondria (M) and completed yolk granules (Y). G : See Fig. 6.

b.—d. The aggregations of mitochondria become compact in order of the numbers 1-6.



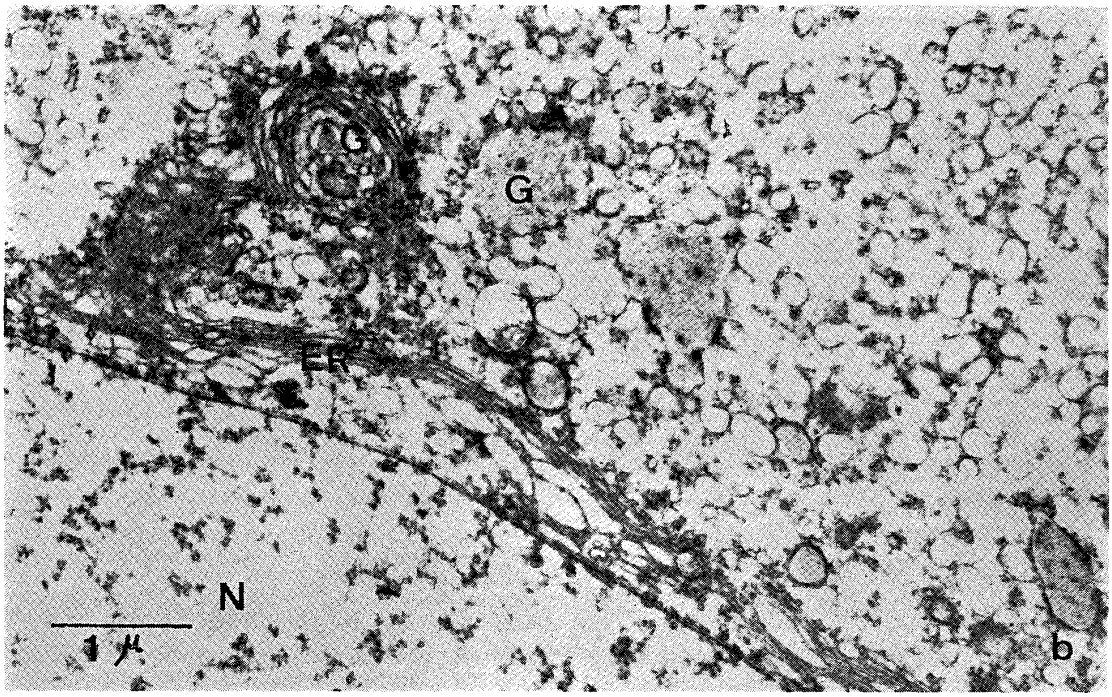


Fig. 6. Endoplasmic reticulum and low-electron-dense granules in *T. serica* oocyte.
a. Vesicles of ER closely surrounded the nucleus, and low-electron-dense granules lie in the vicinity of the ER.
b. Granules of low electron-density in ER vesicles.
ER : endoplasmic reticulum ; G : low-electron-dense granules ; N : nucleus

(3) Spermatozoa

Spermatozoa are released through the osculum of the male sponge into the sea water. Enough of them are released to fertilize the eggs shed at the same time, but after dilution in the sea water it is very difficult to find sufficient spermatozoa to make observations. I could only find them on the surface of the egg under the light microscope, but it was also hard to observe them with the obstacle of the radiating fiber bundles around the egg. The length of the sperm tail is about 20 μm and its head is about 3μm in length, with a conical shape.

(4) Discussion

With respect to the origin of the germ cells in various sponges, two different sources, the choanocytes and the archaeocytes are known. Although there are many descriptions in connection with this problem, it is very difficult to determine the origin of the germ cells solely by the observation of fixed materials. In the recent studies, spermatocytes and oocytes of calcareous sponges were found to be derived directly or indirectly from the choanocytes (Tuzet, 1964 ; Vacelet, 1964). On the other hand, in Demosponges, the oocyte seems to arise from an archaeocyte, but the spermatocytes are derived from choanocytes (Tuzet et al., 1970). But, it is difficult to obtain, from fixed specimens, evidence that choanocytes develop into oocytes through an amoeboid phase; i.e., that indirect transformation takes place. In *Tetilla*, oocytes are not known to originate from archeocytes or from choanocytes, but in this observation a choanocyte has never been observed to transform into an oocyte.

In many calcareous sponges and some Demosponges including *Octovella galangaui* (Tuzet and Paris, 1964) and *Haliclona ecbasis* (Fell, 1969), major growth of oocyte is reported to originate in engulfing nurse cells. In calcareous sponges, in *Sycon* and *Grantia* (Duboscq and Tuzet, 1937, 1942, 1944), *Petrobiona* (Vacelet, 1964),nurse cells are known to originate from choanocytes. But in Demosponges, they are reported to originate generally from amoebocytes. In *Tetilla*, choanocytes in the vicinity of growing oocyte are seen to change their shape into an amoeboid phase. It is considered that the cells are changing their nature from choanocytes to nurse cells. There can not be found any granules simiar to the yolk granules in the nurse cells. The yolk granules are considered to form inside the oocyte.

In the ooplasm, there are many aggregations of mitochondria, which seem to be a step toward condensation (Figs. 5a to 5d). Around the agregation, vesicles accumulate (Figs. 5c, 5d); this complex is considered to be analogous to the yolk nucleus of other animals. The component of yolk nuclei are different according to the animal group and about its function it is considered to be connected with protein synthesis, but not clearly with yolk formation in many animals (Nϕrrevang, 1968). In *Tetilla* it seems certain that yolk formation takes place at centers which consist of numerous

mitochochondria. However, the results in connection with gametogenesis are inadequate; more research must be done on this aspect of *Tetilla* reproduction.

The radiating fiber bundles around the eggs of *T. serica* and *T. japonica* are not observed in other sponges and have a very unique structure. In oviparous sponges, some accessories are often noticed around the eggs. In *Polymastia robusta* (Borojević, 1967) and *Agelas sp.* (Reiswig, 1976), eggs are covered with a thick jelly layer. *Cliona* (Warburton, 1961) and *Hemectyon ferox* (Reiswig, 1976) have cells which are derived from maternal somatic cells around the eggs. Recently Gallissian and Vacelet (1976) reported that mature eggs of the oviparous *Verongia* have spherulous cells and a collagen envelope around them. This is very interesting in view of the fact that the radiating fiber bundles of *T. serica* has striated fibers like collagen (Endo et al., 1967).

Sect. 4. Fertilization and early development

(1) Fertilization

In *Tetilla,* the eggs released from female sponges are fertilized by the spermatozoa released from male sponges. There are several reports that external fertilization takes place in the Porifera, but no actual observations of fertilization in living materials. When the egg of *T. japonica* is fertilized, fertilization membrane elevation seems to start from the place where the sperm entered. It requires about one minute for fertilization membrane elevation. The height of the membrane is 13–18 μm; this is one-third to one-fourth of the length of the radiating fiber bundles, so the fiber bundles still project outside the membrane (Fig. 7b to 7f), but within 10 minutes the fiber bundles are completely included in the perivitelline space (Figs. 7g, 8).

It is seen by watching carefully under the light microscope that a very slow rotation of the egg causes the phenomenon of inclusion of the radiat- fiber bundles. The rotation of the egg is clearly observed by analyzing it with 8 mm timelapse cinematography. The fertilized egg rotates around one axis, and the radiating fiber bundles are drawn into the perivitelline space. It rotates in one direction for a while and then reverses and returns to the begining position. At that time the fiber bundles are folded in the perivitelline space and further drawn into the opposite direction (Figs. 7c to 7f). The rotation and reverse rotation are repeated several times, and the fiber bundles show a zigzag folding from these reversals (Fig. 8).

In the perivitelline space, the fiber bundles swell and gradually lose their morphological integrity. Since the rotation occurs around one axis, the fiber bundles on the two poles remain outside the membrane, but finally they also swell and become obscure. It is considered that some substance, which changes the nature of the radiating fiber bundles, is secreted into the perivitelline space (Figs. 9d, 10a). This substance is believed to be secreted

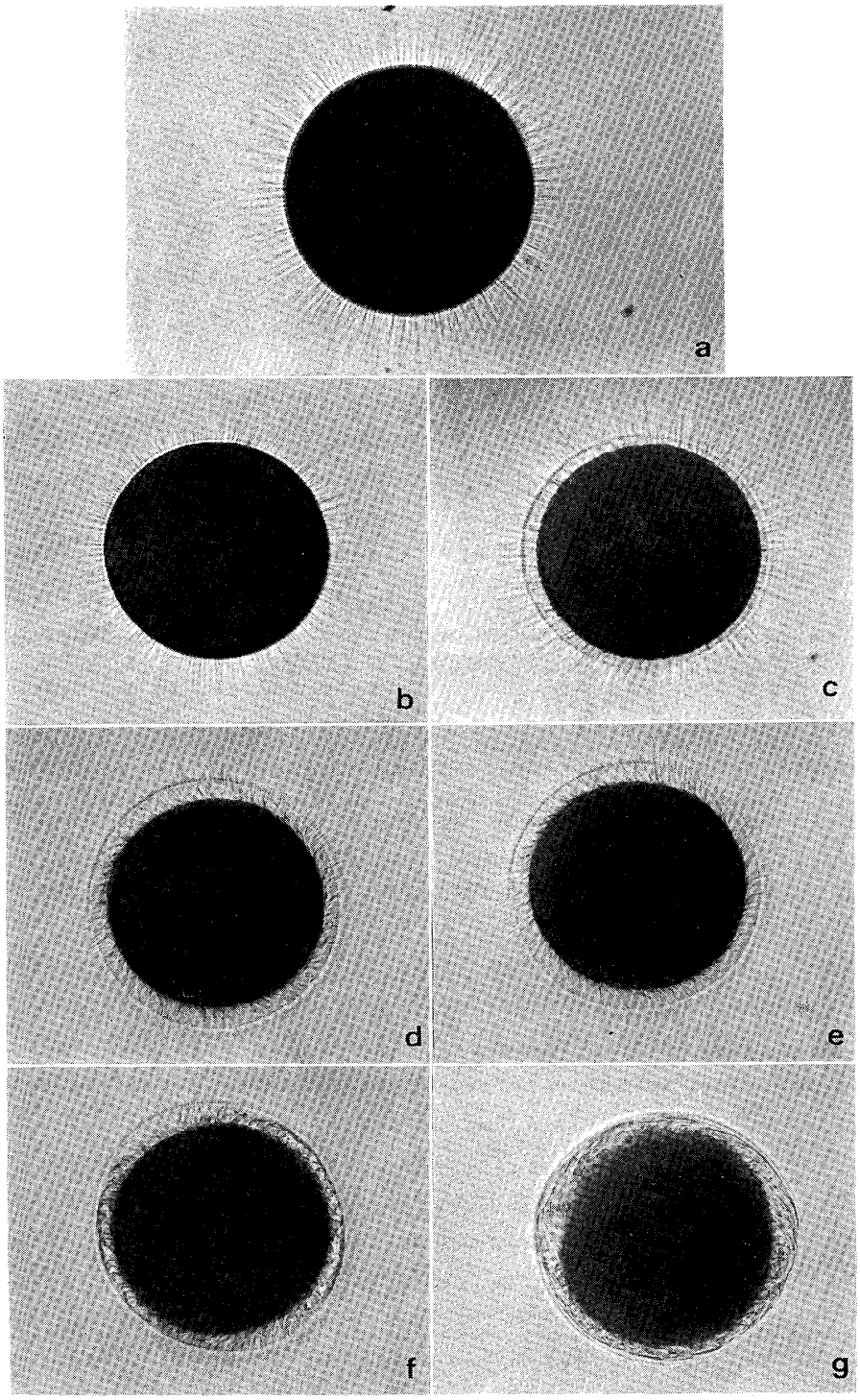


Fig. 7. a. Unfertilized egg of *T. japonica*. Radiating fiber bundles surrounded the egg. Diameter is 130 μm. b–g. Process of fertilization membrane elevation and egg rotation in *T. japonica*. (light microscope). b. 5 seconds after fertilization. c. 10 seconds after fertilization. Fertilization membrane elevated. d. Eggs begin to rotate, drawing the fiber bundles into the perivitelline space (15 seconds). e. 30 seconds after fertilization. f. The fiber bundles are within the perivitelline space except at the two poles (1 minute). g. Fiber bundles are completely drawn into the perivitelline space (4 minutes).

to the outside of the membrane and to change the nature of the bundles of the two poles.

After the radiating fiber bundles have been drawn inside of the fertilization membrane, the fertilized agg adheres to the substratum, apparently by means of some adhesive substance secreted on its surface. The substance seems to change the nature of the fiber bundles inside and outside the perivitelline space and at the same time cause the egg to adhere to the substratum. The adhering place on the egg is not certain, but since adhesion has an important influence on development, there might be a definite part of the egg for adhesion, determined by gravity or some other factors.

(2) Cleavage

The fertilized egg adheres to the substratum and begins to develop, first becoming rather flat like a bun. That must be because the eggs have heavy yolk and tension of the egg surface is weak. In *T. serica*, this phenomenon is more conspicuous than in *T. japonica*.

In *T. japonica*, at an hour after fertilization the first cleavage takes place vertically to the surface of the substratum to which the egg adheres (Fig. 9a). In one more hour after the first cleavage, the second one also segments the egg vertically and crosses the first one at right angles (Fig. 9b). The third cleavage segments each blastomere at an oblique angle to the substrate plane (Fig. 9c). After the 16-cell stage, cleavages sometimes take place horizontally to the adherence plane, but this seldom occurs in the early cleavages. It is considered that the inhibition of horizontal cleavage in the early stage is significantly influenced by the adhesion of the egg, and the heavy yolk prevents the spindle from forming vertically to the adherence plane in the cell division.

(3) Pinacoderm formation

In *T. japonica*, the egg becomes a morula by 16 hours after fertilization (Fig. 9d). From this time, the cells near the surface of the solid cell mass are observed to migrate into the perivitelline space (Fig. 10a). As development proceeds the cells in the perivitelline space increase in number (Fig. 10b). These cells project pseudopodia to the surface of the fertilization membrane, and still more they project filopodia to the outside of the membrane (Figs. 10c, 10d).

The cells attach to the fertilization membrane at the upper surface of the larva, flattening its shape, and adhere on the inner surface of the fertilization membrane (Fig. 11), forming the monolayer of the pinacoderm (Figs. 10d, 11, 12). The cell differentiation has not yet taken place in the morula stage. There is no difference between the outer cells, which will become pinacytes, and the inner cells which remain in the center of it. The pinacoderm which is formed here is not the epithelium of the larva, but

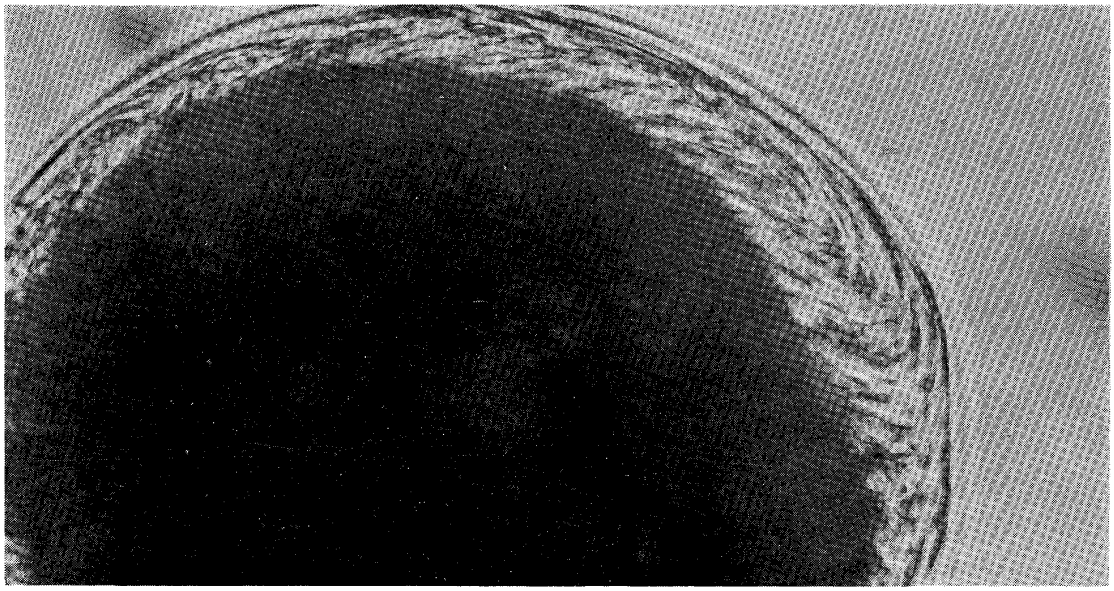


Fig. 8. The egg of *T. japonica* 7 minutes after fertilization. Fiber bundles in perivitelline space show zigzag foldfng. (light microscope)

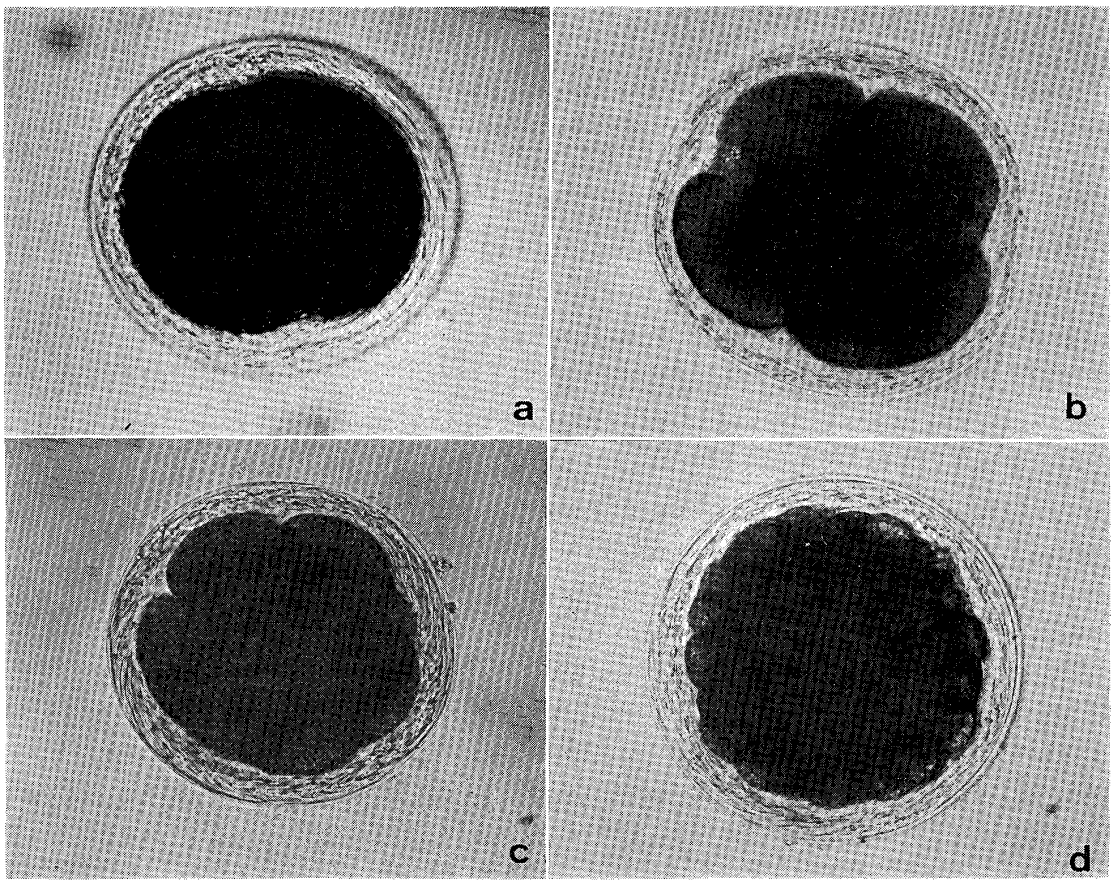


Fig. 9. Cleavage of *T. japonica*. (light microscope)
a. 2-cell stage, 1.5 hours after fertilization.
b. 4-cell stage (3 hours).
c. 8-cell stage (4.5 hours).
d. morula stage (16 hours).

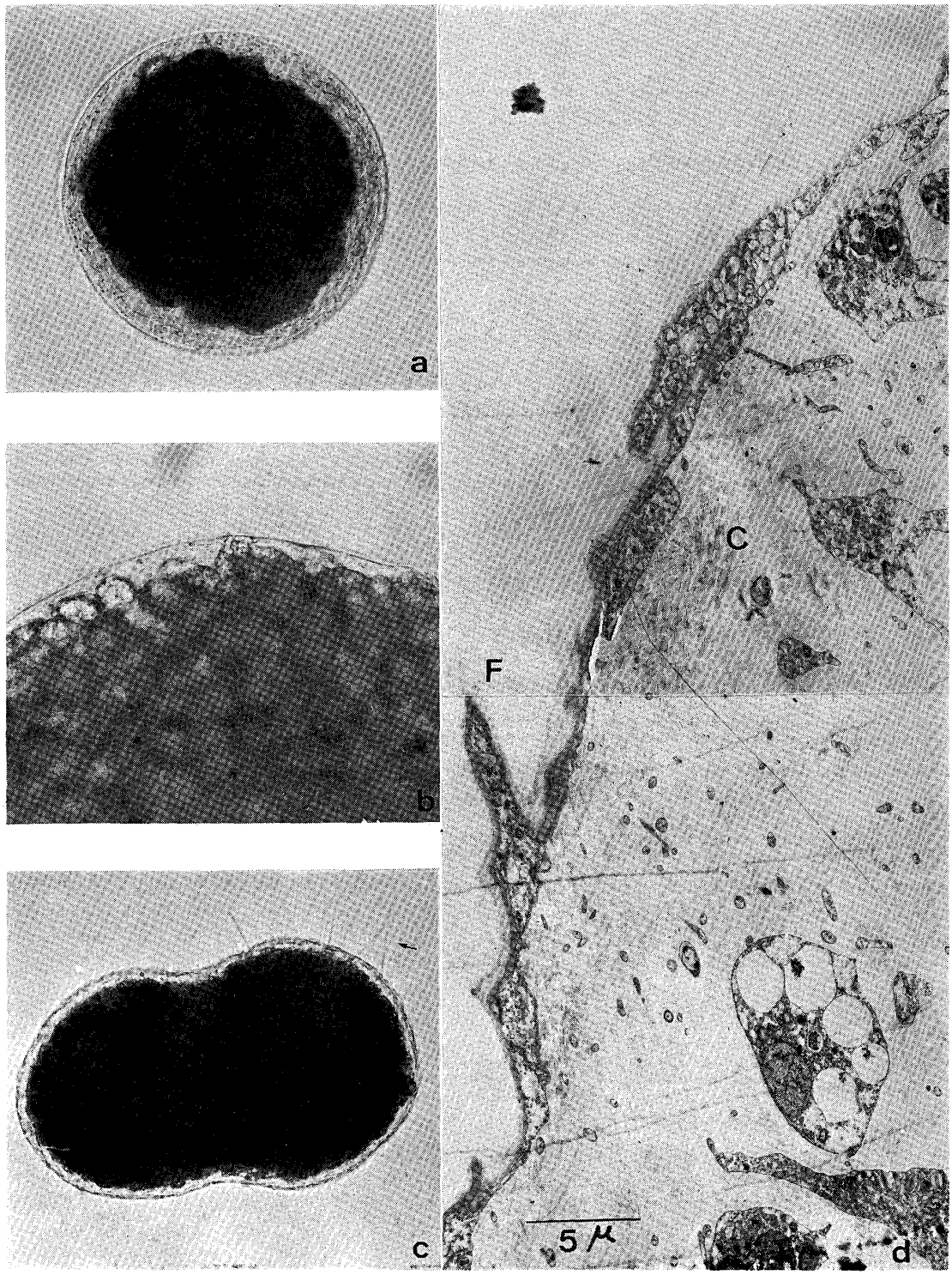
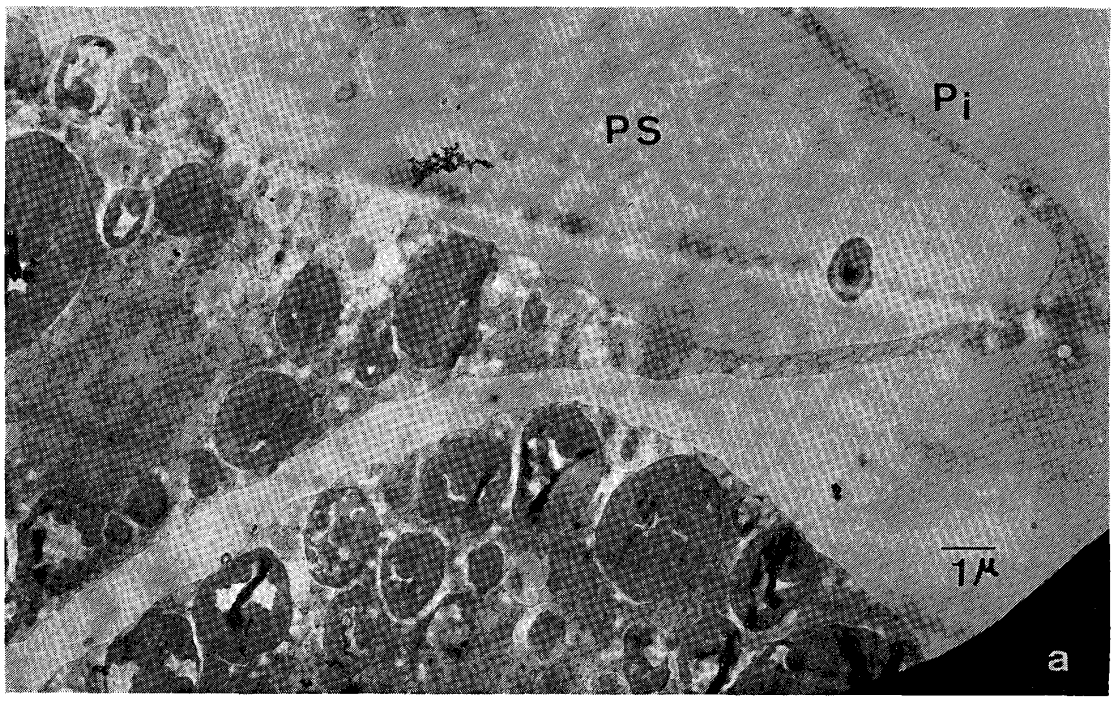


Fig. 10. Process of pinacoderm formation.
a. Morula cells migrate into perivitelline space (16 hours).
b. Migrated cells adhere to inner surface of fertilization membrane to form pinacocytes (32 hours).
c. Pinacocytes project filopodia (arrow) to the outside. Morula formed by two fused eggs, 2 days after fertilization. (light microscope)
d. Pinacoderm in 4-day larva. A pinacocyte extends a filopodium (F). Beneath the pinacocyte, collagen fibers (C) are seen.



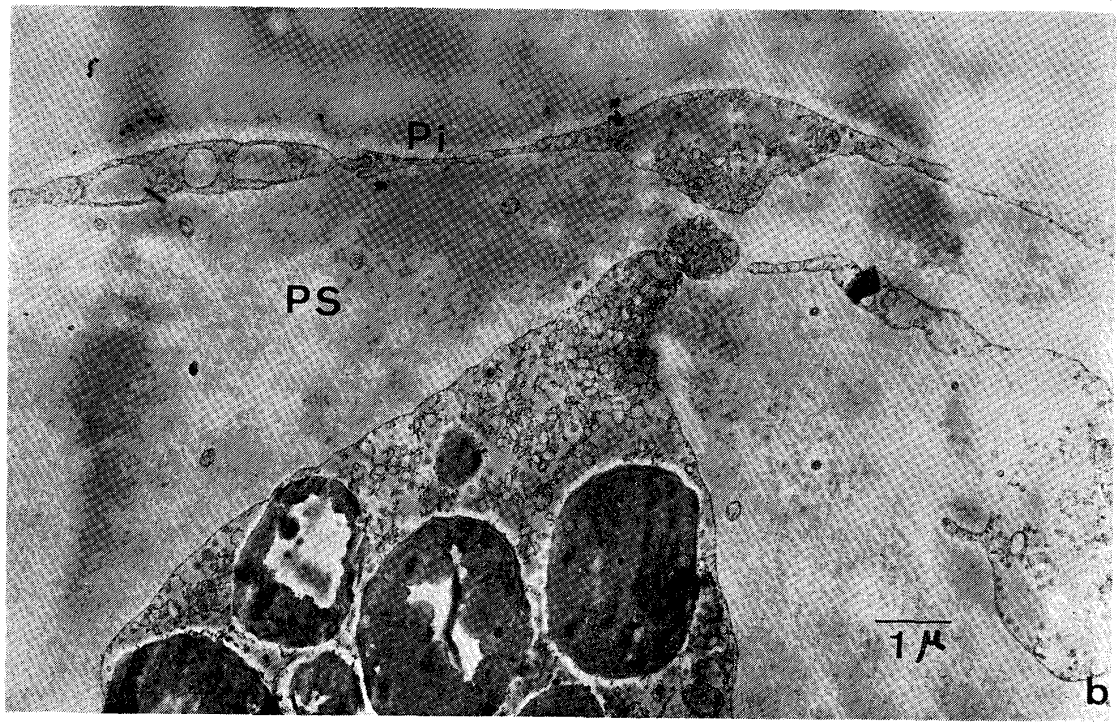


Fig. 11. Sections through pinacocytes of morula larva (27 hours).
a. A cell projects a pseudopcdium into the perivitelline space (PS) and spreads on the inner surface of the fertilization membrane to form the pinacoderm (Pi).
b. A similar section.

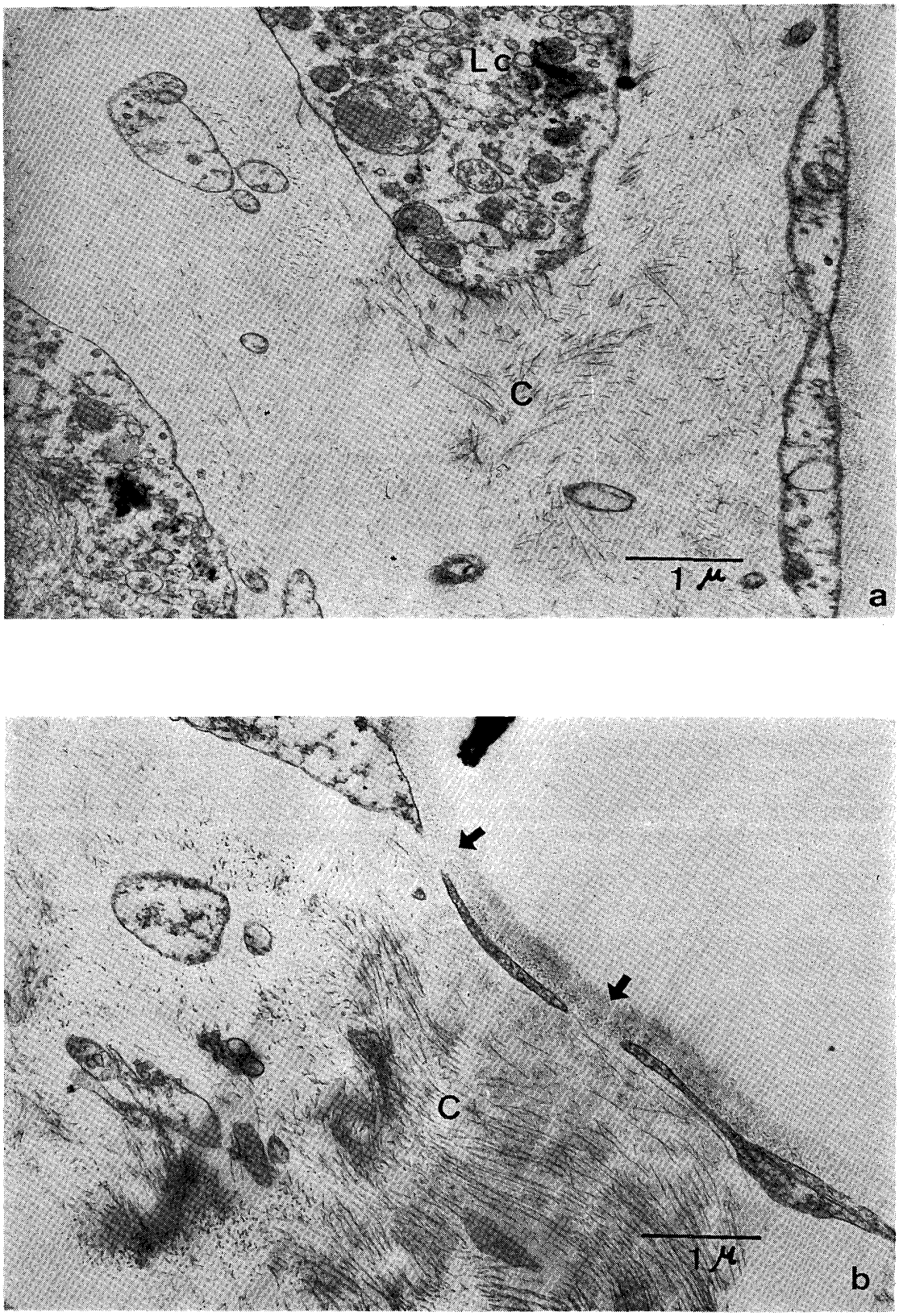


Fig. 12. Pinacoderm in 4-day larva of *T. serica*.
a. Mesohyle collagen (c) is newly formed by a lophocyte (Lc).
b. Two ostia open in the pinacoderm (arrows).

that of the adult. Though other sponges at first form a larval epithelium and then through metamorphosis form a pinacoderm which is the epithelium of the adult, *Tetilla* has a very unique mode of formation. The thickness of the pinacocyte is only 1 μm, so it is difficult to distinguish the existence of these cells with light microscopy. The formation of this layer brings the substance in the perivitelline space completely inside the larva. Active migration of the cells is observed in this part. At the edge of the site of larval attachment, projected filopodia attach flat to the substratum and extend themselves on the substratum to form the outgrowth (Figs. 13a to 13d).

(4) Discussion

The fertilization of a viviparous sponge was first observed by Gatenby (1927). He discovered that the choanocytes mediated fertilization in a calcareous sponge. After that Duboscq and Tuzet (1937, 1942, 1944) reported in detail about the fertilization of calcareous sponges mediated by carrier cells. After these studies, several researchers reported that among the Demosponges, a carrier cell mediated the fertilization in *Cliona viridis* (Tuzet. 1930; Sarà, 1961), *Reniera elegans* (Tuzet, 1932), *Hippospongia communis* (Tuzet and Pavans de Ceccatty, 1958), *Octavella galangaui* (Tuzet and Paris, 1964). These materials in which fertilization has been observed are all viviparous sponges.

Since the first description about fertilization by Gatenby (1927), the "carrier-cell system" of fertilization has become established by the later investigation. But, Reiswig (1976) had doubt about this phenomenon, because of the difficulty of the observation of the ordinary fixed specimens. In the same view, Fell (1974) indicated in his work, "Reproduction of Marine Invertebrates" the problem of drawing conclusions about dynamic events from looking at static sections in fixed material. Except for *Tetilla,* nothing has ever been observed directly with living material in connection with the fertilization of either viviparous or oviparous sponges.

Lévi (1956) reported the following sponges to be oviparous; *Tethya aurantium, Polymastia mammilaris, Cliona robusta, Ficulina ficus, Axinella damicornis, Adreus fascicularis, Raspailia hispis, R. pumila* and *R. ramosa.* But, it is not known whether fertilization takes place before or after extrusion of the oocytes from the parental tissue. Borojević (1967) observed the spawning and early development of *Polymastia robusta* and stated that eggs are fertilized outside the body, but he reported that he was unable to observe the process of fertilization. Reiswig (1976) observed spawning in several tropical Demosponges, and he also observed early development in *Agelas sp.* and *Hemectyon ferox,* but he could not observe the process of their fertilization. In comparison with these sponges, the fertilization of *Tetilla* cleary takes place outside the body, and the elevating fertilization membrane was observed following fertilization. This phenomenon is similar

to the process of fertilization of marine invertebrates such as the sea urchin. But, verifying the later development, the nature of the fertilization membrane of *Tetilla* is different from that of other invertebrates.

In *Tetilla*, the radiating fiber bundles are taken into the perivitelline space, and the substances in this space are later taken into the larva as part of the developmental process. The larva of *Tetilla* thus finally incorporates the accessory material around the egg itself into its body. Incorporating accessories around the egg into the inside of the larva is often seen in several sponges. In the oviparous *Cliona celata*, the eggs are released from the maternal osculum after fertilization, and they carry small amoeboid cells clinging to their surfaces These cells, derived from parental somatic cells, are observed to be incorporated into the larva in the course of development (Warburton, 1961). Reiswig (1976) reported that released eggs of *Hemectyon ferox* have the cells derived from parental somatic cells, which are incorporated into larvae in the process of development. In many viviparous sponges, parental somatic cells are observed within their larvae (Duboscq and Tuzet, 1942, 1944; Vacelet, 1964). The same phenomenon is expected in *Verongia*. Gallissian and Vacelet (1976) reported about the accessories around the mature egg, but they have not yet reported about fertilization or development. Bergquist et al. (1970) suggested that in some species the unfertilized diploid oocyte might develop into a larva.

Thus, various phenomena different from those occurring during the development of other Metazoa have been reported in the process of development of sponges. The peculiar structure of the *Tetilla* egg and the incorporation of its accessories during embryogenesis seem to show that in the oviparous sponge, something (cells or fibrous substance) derived from the maternal body may be incorporated into the larva.

In general, sponges with a free-swimming larval stage have simple columnar flagellated epithelial cells. This is not found in *Tetilla*, which undergoes direct development, and in which the adult pinacoderm appears precociously in the morula larva. It is not known which cells of the morula develop into pinacocytes, but since there may be no evident differentiation among the morula cells, *Tetilla* may be considered to skip the swimming larval stage. According to Reiswig (1976), the most advanced stage of *Hemectyon ferox*, in his observation, is a solid ball of 100 to 150 cells, entirely lacking in evidence of cellular differentiation and thus lacking the columnar flagellated cell layer characteristic of sponge parenchymella. However, as the development of the larva has not been observed after that, it is not known whether adult pinacocyte is formed directly in it as in the *Tetilla* larva or not.

Sect. 5. Later development

(1) Outgrowth formation

When the pinacoderm is formed, filopodia extending from the upper

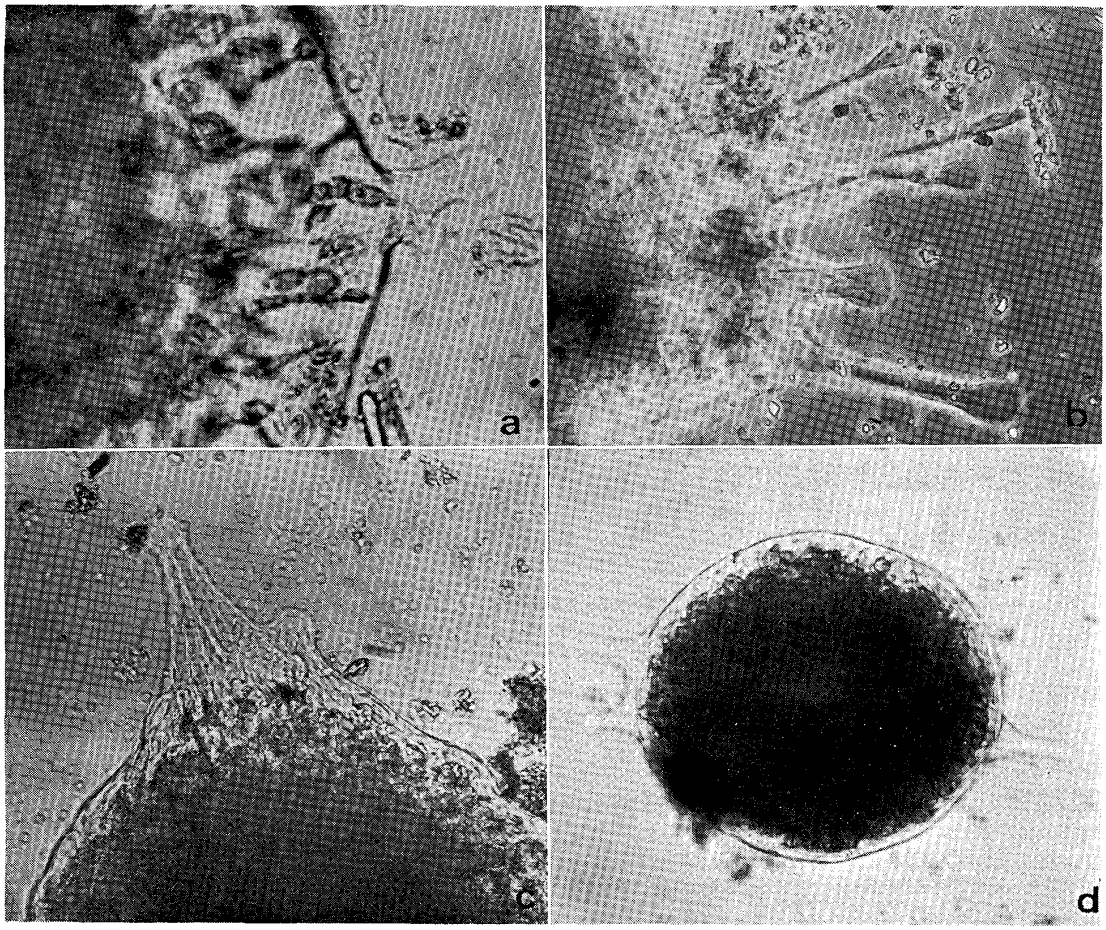


Fig. 13. Outgrowth formation in *Tetilla*. (light microscope)
a. 5-day larva of *T. serica:* 2 lobes of outgrowth begin to project.
b. 5-day larva of *T. serica:* the tip of an outgrowth sticks to the substratum.
c. 6-day larva of *T. japonica*; the cells migrate into the outgrowth.
6. 6-day larva of *T. japonica*; 3 lobes of outgrowth are extending on the substratum.

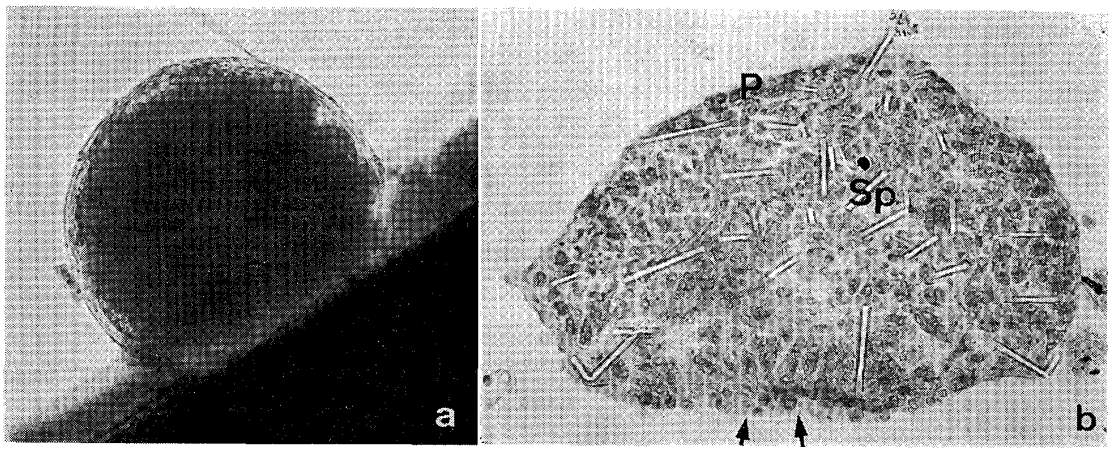
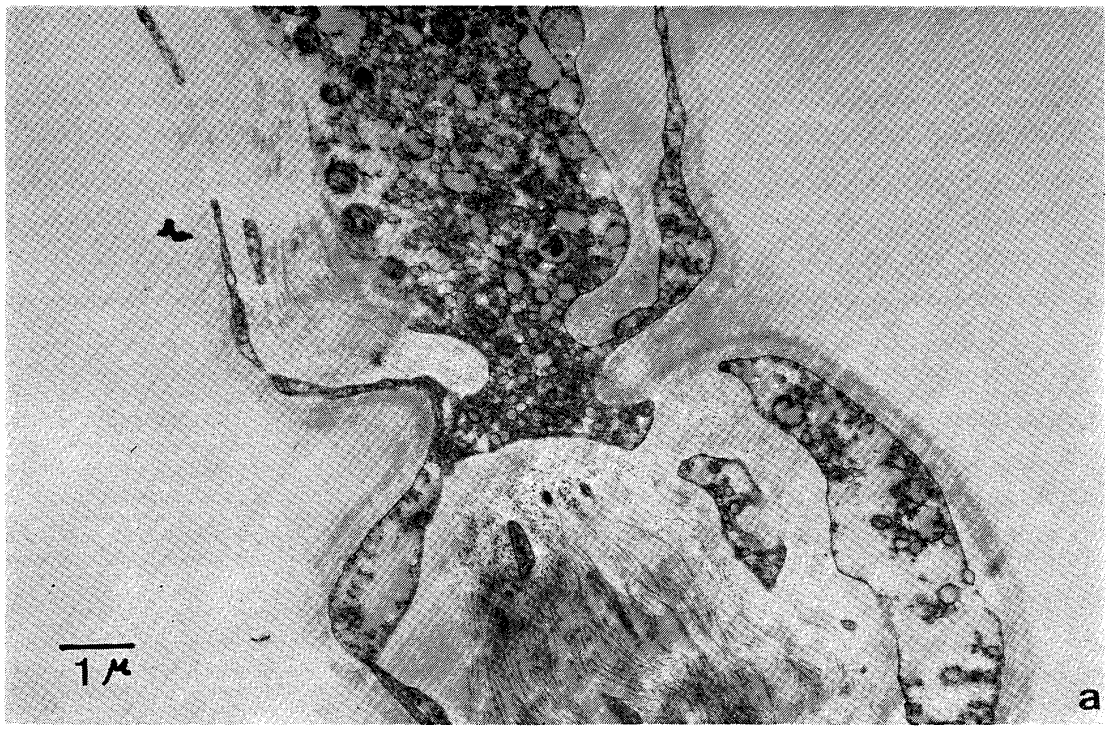


Fig. 14. Process of invagination. (light microscope)
a. Side view of 3-day larva of *T. japonica*.
b. Longitudinal section through 4-day larva of *T. serica*. Inward migration of cells is seen at center of attached area (arrows).
P: pinacoderm, Sp: spicule



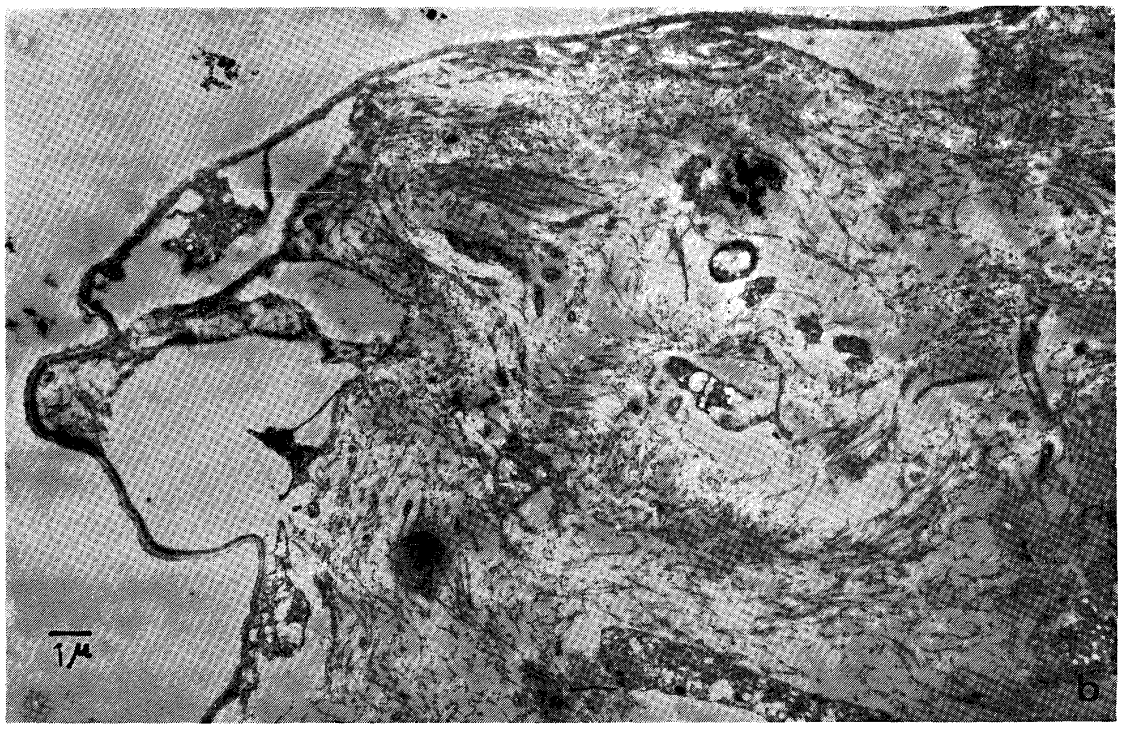


Fig. 15. Electron micrographs of outgrowth in 4-day *T. serica*.
a. Basal part of outgrowth.
b. Distal part of outgrowth.

surface of the larva are drawn in. At the larval edge of the site of adhesion, migrating cells from the inside spread on the inner surface of the fertilization membrane to form the pinacoderm, and still more conspicuously they extend themselves, conducted by filopodia, and migrate outward on the substratum (Fig. 13). Wilson (1935) called this part the "outgrowth". Since the outer cells of the outgrowth are very thin (Figs. 13, 15), even its existence was not observed under the light microscope and I could not describe its characteristics clearly in my earlier papers (1957, 1967). Using the electron microscope, the outgrowth is observed to extend itself, drawn by the pinacocytes (Fig. 15). It is observed that the cells in the center of the larva move into the outgrowth with the shape of spindles (Figs. 13a, 13c). Lobes of the outgrowth are projected in various directions from the edge of adhesion of the larva (Figs. 13b, 13d), so comparing this stage of *Tetilla* to the developmental stage of other Demosponges, it may correspond to metamorphosis, and forms a flat, projecting outgrowth.

(2) Formation of ectosome and choanosome

As mentioned before, active cell migration is observed in the flat larva projecting an outgrowth. The cell migration of this stage is seen, not only in the outgrowth, but also in sectioned preparations, in the other direction at the inner part of the body. That is, at the larval surface of adhesion, longitudinal cells in the center of the adhering area are seen to migrate to the inner part (Fig. 14b). In this stage, the cell migrations in both inward and outward directions are observed in the larva (Figs. 13c, 14b). The movement of the cells into the inner part at the surface of adhesion is to be considered a kind of invagination.

After the extension of the outgrowth ceases, the flattened larva again increases in height (Fig. 14a). This means that the migration toward the outside has ended and inward migration has become more active. Under the electron microscope, a collagenous layer of mesohyle is noticed under the pinacoderm in the outgrowth (Fig. 15) and the outer part of the larva (Fig. 12). At the inner part, the initial choanocytes are seen to aggregate (Watanabe, 1978). At this time ectosome and choanosome must be differentiated. In general sponges with a free-swimming stage, the inner and outer cell layers exchange places at this stage. The outer epithelial cells of the larva form choanocyte chambers, and the inner cell mass forms the ectosome. In *Tetilla*, however, such phenomena are not seen; only migration is seen in the cells of the larva as they form the ectosome and choanosome.

(3) Formation of spicules and aquiferous system (canal system)

Inside the 3-day larva the sclerocytes are differentiated (Fig. 16). The spicules that develop at first are monoaxonic (oxae), and each spicule develops independently (Endo et al., 1967). Finally these spicules extend outwards from the center (Fig. 16c) and come to be arranged radially in bundles

(Figs. 18c, 18d). A little after formation of oxae, the protriaene, spicules peculiar to *Tetilla*, develop (Fig. 18). Until the larva reaches 8 days, its only spicules are of these two types. Later anatriaene and sigmas develop only in the root tuft (Fig. 16b).

Simultaneously with the differentiation of the sclerocytes, choanocyte chambers begin to appear inside the larva, where incomplete canals are formed (Fig. 17a). As the skeletal framework takes shape in the larva, vestibules (subdermal cavities) appear between the radial spicule bundles under the pinacoderm (Figs. 18c, 18d), and these closely communicate with the outside through the ostia (pores). Inside the larva, canal and choanocyte chambers are joined to each other and the aquiferous system is completed (Fig. 18).

About 1 week after fertization, the aquiferous system is completed in both species of *Teilla*, and a current of sea water is observed to flow out through an exhalant pore which is formed temporarily at the base (Figs. 18a, 18c, 18d, 19b, 19c, 19b′, 19c′). This stage may be called rhagon larva as compared with other sponges, i.e., 8 to 10 days after fertilization (Figs. 18a, 18c). The atrium (gastral cavity) and osculum of the adult are formed some time after this (Fig. 18b).

(4) Change in body plan

At 3 to 4 days after fertilization, the larva is flattened by the development of the outgrowth; it rounds up again as the skeletal framework develops to form a hemispherical rhagon larva (Figs. 18a, 18c, 19b, 19b′). As the larva enters this stage, the extended lobes of outgrowth are gathered and united into a single stalk under its lower surface. In *T. serica* the stalk is located excentrically, and the unattached part of the larva separates from the substratum and assumes a vertical position a little to one side of its first location (Figs. 18a, 18b, 19c). After rising up, the front of rhagon larva is flat, and the other sides have the round shape. At this stage, the rhagon larva of *T. serica* has bilateral symmetry (Figs. 19c, 19d). In *T. japonica*, the point of attachment is under the center of the larva, which gradually separates from the substratum around this point until only a small root tuft remains attached (Figs. 18d, 19c′).

After the completing the root tuft, the rising rhagon larva of *T. serica* is bilaterally symmetrical, but *T. japonica* retains its radial symmetry.

(5) Discussion

As mentioned above, the substance in the perivitelline space is taken into the larva at the time when the pinacoderm is formed. The radiating fiber bundles around the fertilized egg have been drawn into the perivitelline space; therefore, all the substance around it is entirely incorporated into the sponge body. Thus, *Tetilla* does not cast off the fertilization membrane as most other marine invertebrates do at the time of hatching. Endo et al.

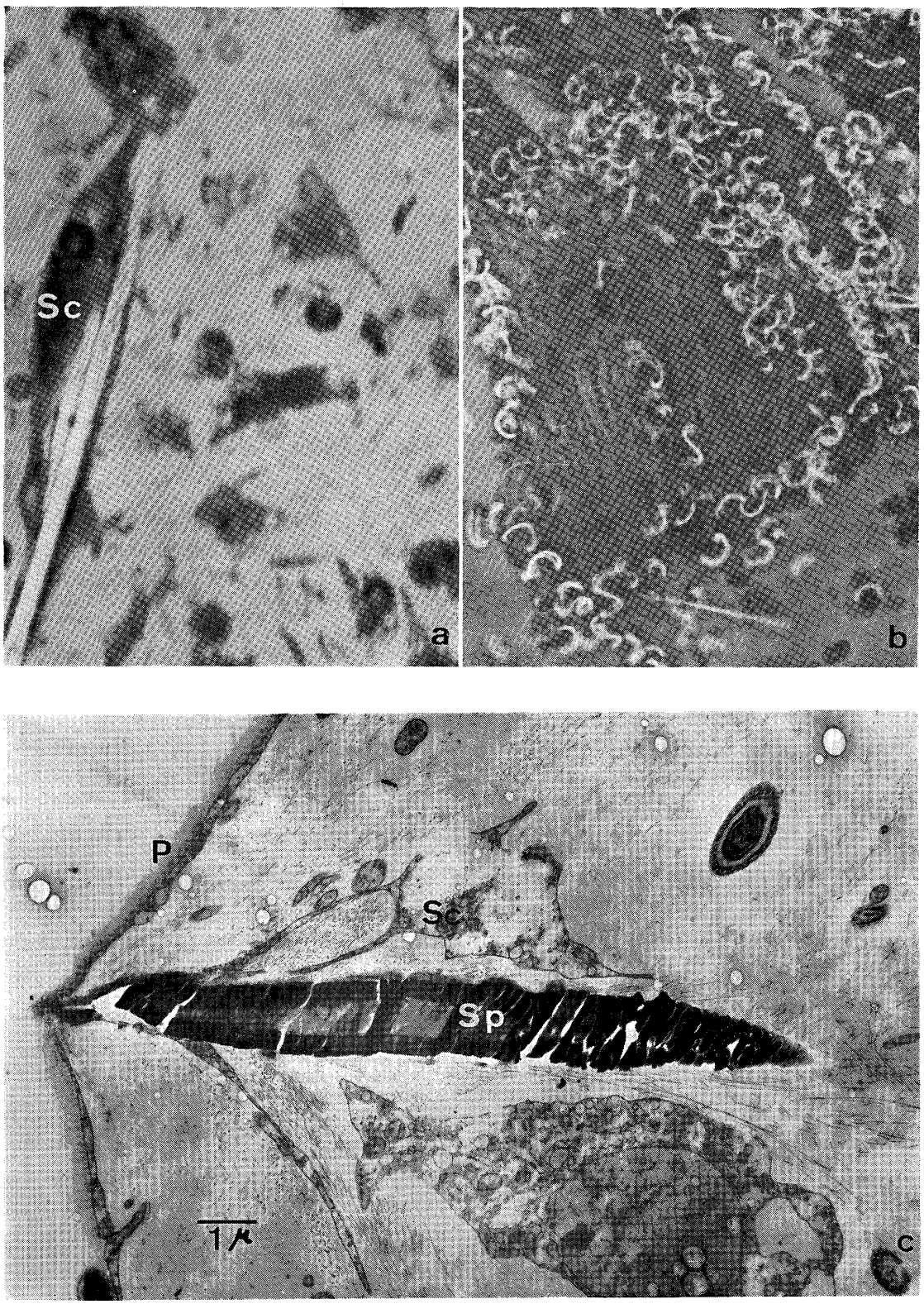


Fig. 16. Spicule formation.
a. Sclerocyte forming a spicule (light microscope).
b. Sigma microscleres in root tuft (light microscope).
c. Electron micrograph of a spicule extending outward from center of larva.
Sp : spicule; P: pinacocyte; Sc: sclerocyte

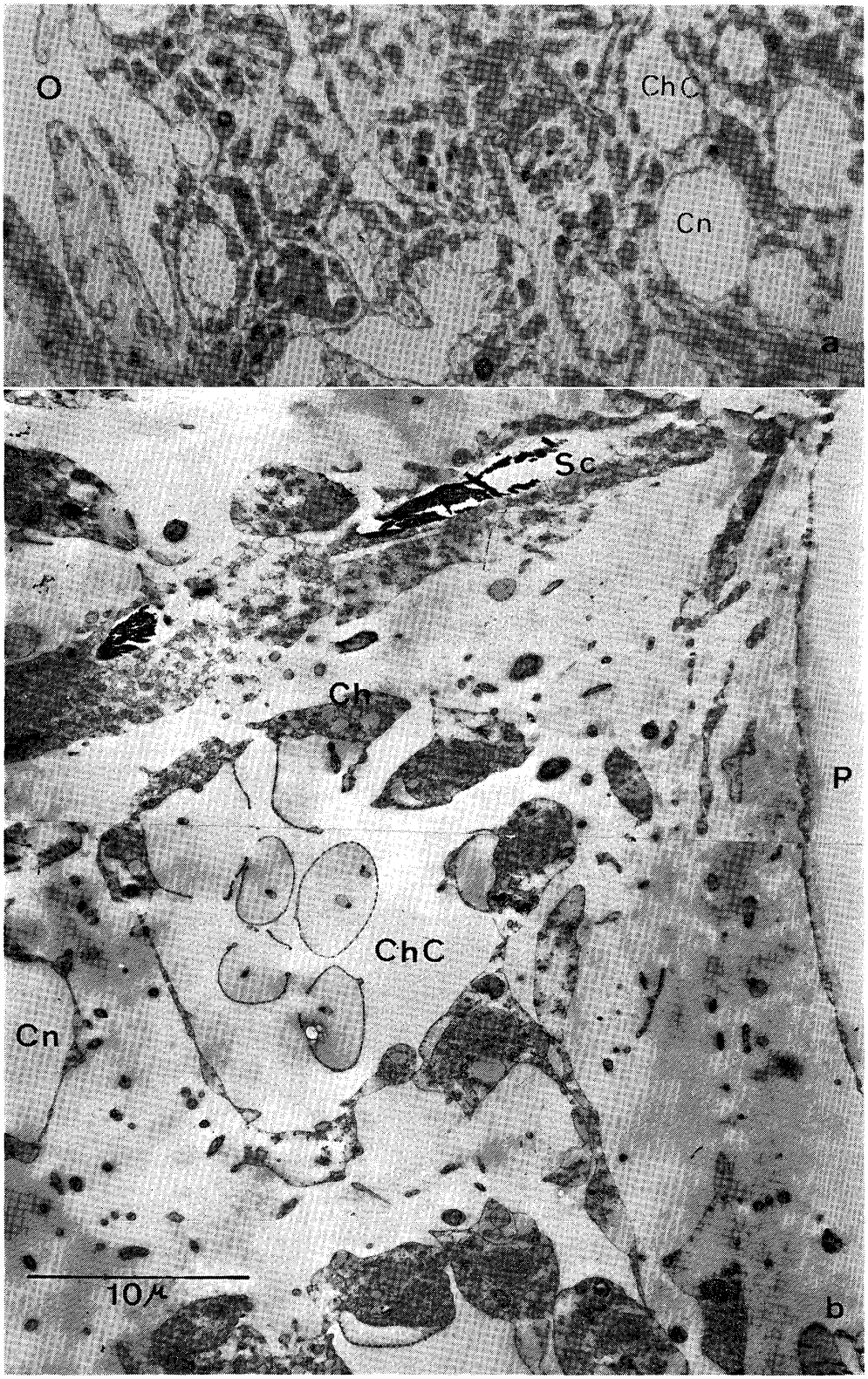


Fig. 17. Aquiferous system.
a. Cross-section of 5-day larva of *T. serica*, showing choanocyte chamber and canal (light microscope).
b. Electron micrograph of 5-day *T. serica* larva.
Ch: choanocyte; ChC: choanocyte chamber; Cn: canal; P: pinacocyte; O: ostium; Sc: sclerocyte

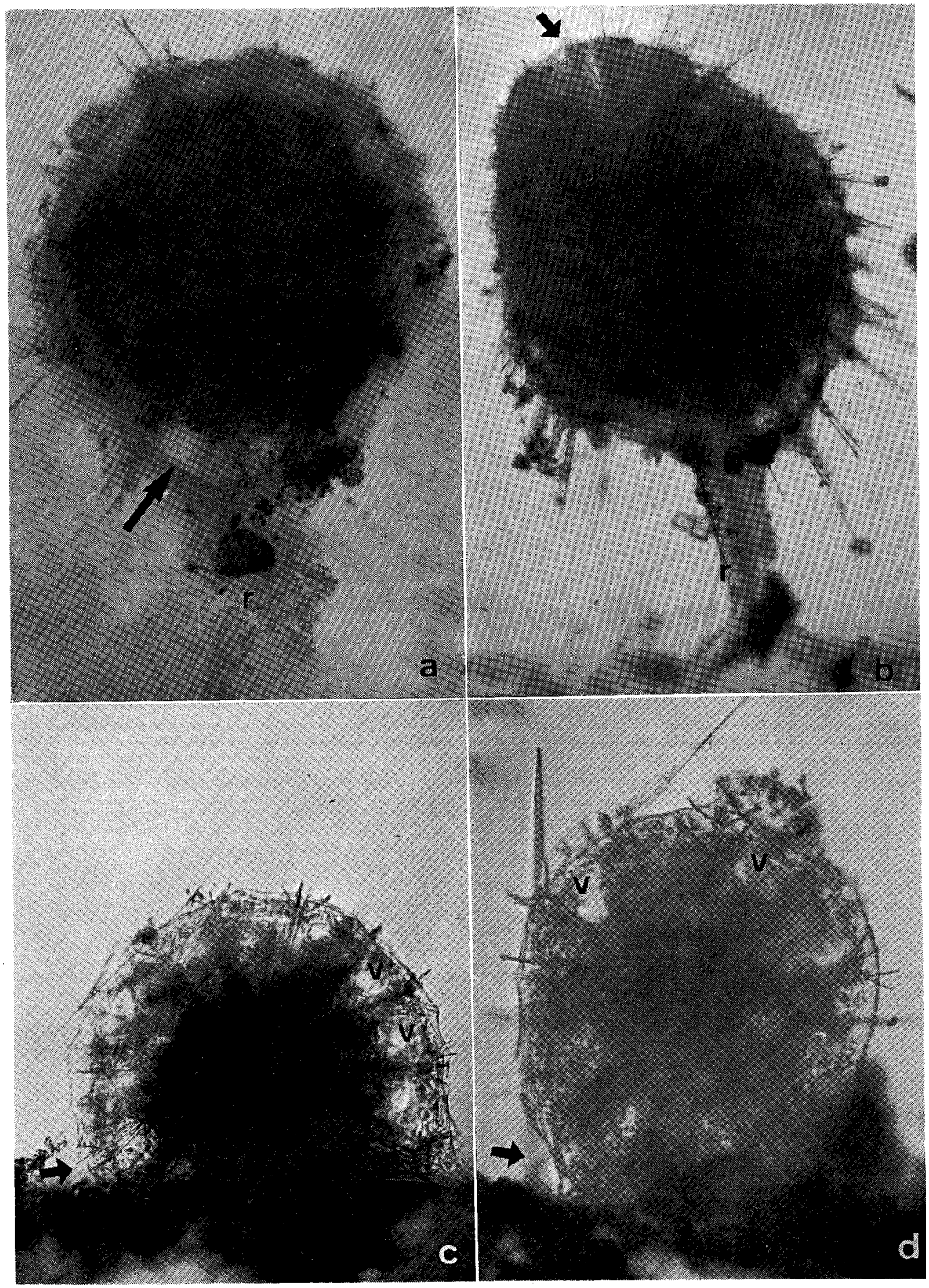


Fig. 18. Rhagon larvae of *T. serica* and *T japonica*. (light microscope)
a. 14-day larva of *T. serica*. Exhalant pore is seen at the base (arrow).
b. 63-day young sponge of *T. serica*. Osculum is formed in apical part (arrow).
c. 20-day larva of *T. japonica*. Hemispherical rhagon larva with exhalant pore at the base (arrow).
d. 45-day larva of *T. japonica*, gradually increasing its height.
r: root tuft; v: vestibule

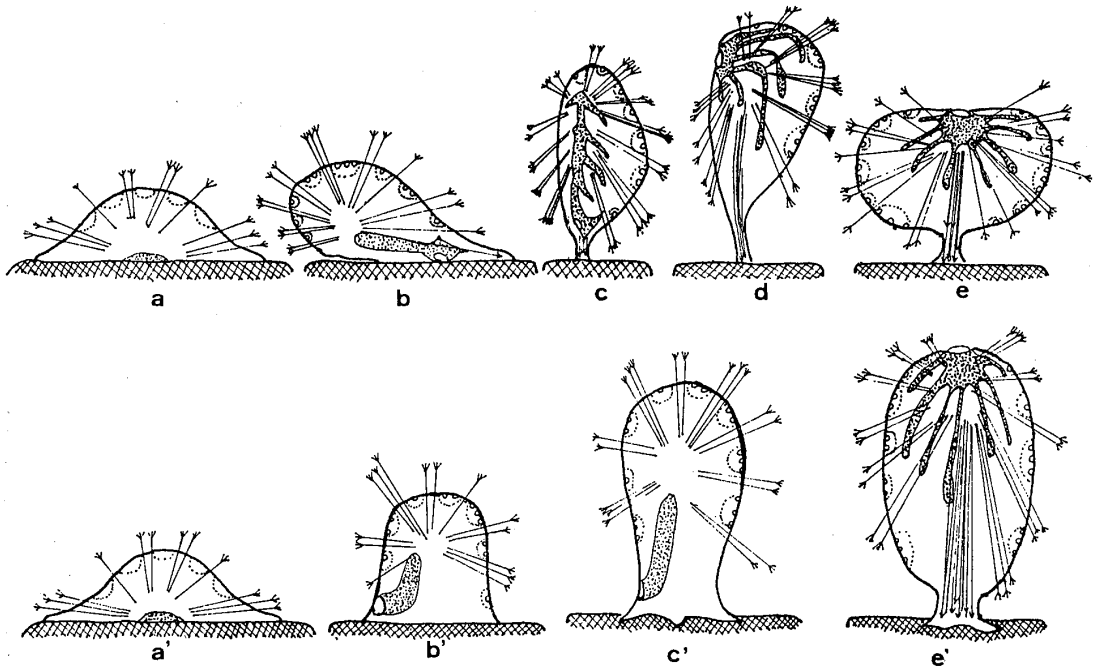


Fig. 19. Schematical diagram of change in body plan in *T. serica* (upper row) and *T. japonica* (lower row).
a. & a'. Flattened stage of larva caused by development of the outgrowth (radial symmetry).
b. & b'. The larva round up again to form a hemispherical rhagon larva. In *T. serica*, the root tuft is located excentrically (b), but in *T. japonica*, it is formed under the center of the larva (b').
c. In *T. serica*, the unattached part of larva separates from substratum and stands up vertically (bilateral symmetry).
c'. Rhagon larva of *T. japanica* gradully separates from substratum but does not change its body plan.
d. Bilaterally symmetrical rhagon larva of *T. serica*. Osculum is located at one side.
e. Adult of *T. serica*. Body plan changes again from bilateral symmetry to radial symmetry.
e'. *T. japonica* does not change its symmetry.

(1967) showed in an electron micrograph that the fiber bundles of the unfertilized egg have collagen-like striations. Gallissian and Vacelet (1976) reported that there is a collagen layer around the mature egg of *Verongia*. They did not report about the fate of this accessory after fertilization, but I have great interest in its structure as compared with the accessory on the egg of *Tetilla*.

In the flattening stage of the larva, mesohyle that is rich in collagen fibers is observed cleary, lying closely under the pinacocytes of the outgrowth (Fig. 15). This suggests that the radiating fiber bundles on the surface of the unfertilized egg are the source of the collagenous substance provided by the maternal sponge to the larva.

Tetilla develops directly and has no swimming larval stage, and without

differentiating an inner and outer cell layer of the larva. It directly differentiates into adult pinacoderm and choanoderm. When it forms this adult tissue, violent cell migration is observed.

The stage of spicule formation in sponges is different from species to species. *Hymeniacidon sanguinea, Pronax plumosa* and *Gellius angulatus,* which belong to the Demosponges, are known to have spicules in the parenchymella larva released from the maternal body (Lévi, 1956). In Hexactinellidae, the released larva already has a megasclere framework and hexaster microscleres (Okada, 1928). In these species the incubation period within the maternal body may be long.

T. serica, with the development of its shape, changes from radial symmetry to bilateral symmetry and back again to radial symmetry (Endo et al., 1967). In *T. japonica,* since the root tuft is formed at the center of adhesion and the larva rises to develop, the rhagon larva does not change its symmetry. Such symmetrical alternation is interesting as it is compared with Jägersten's report (1955). In his "bilateral blastea theory", he infers the possibility of changing its form from bilateral symmetry to radial symmetry in the sponge.

CONCLUDING REMARKS

The author has related the development of two species of sponges that are oviparous, which is very unusual for Demosponges. Their peculiar characteristics have some points in common with the development of other marine invertebrates. External fertilization, elevation of a fertilization membrane and blastoderm formation are common phenomena in other Metazoa. In the blastoderm formation, which is the main factor to distinguish the sponges as Parazoa from Eumetazoa, it is shown that two species of *Tetilla* are more similar to Eumetazoa than other sponges. Both in fertilization and development, their different characteristics from those of other sponges seem more similar to those of the lower invertebrates rather than Porifera.

Recently gonochoristic sponges have been receiving attention and several studies have been reported (Lévi, 1956; Sarà, 1961; Tuzet and Paris, 1964; Fell, 1970; Gilbert and Simpson, 1976). But, very little is known concerning the development of gonochoristic sponges. Comparing the development of general Demosponge with what is known of oviparous sponges, it is intended to consider the development of *Tetilla* in Demosponge for phylogenetic position in Porifera. Lévi (1956) and Sarà (1974) reported that oviparous sponges with gonochorism are mostly found in Tetractinomorpha.

Demosponges are divided into two subclasses, Ceractinomorpha and Tetractinomorpha, depending upon the spicule construction with or without spongin. The sponges which belong to Tetractinomorpha are divided into three groups from the view point of developmental pattern (Lévi, 1956, 1957).

1. Homosclerophorida type; typical group of *Oscarella* with similar development to calcareous sponge and develops asconoid rhagon through incubated amphiblastula larvae.
2. Clavaxinellida type; typical group Hadromerida and Axinellida, which belongs to oviparous sponge and develops syconoid rhagon larva through parenchymella.
3. Tetractinellida type; Lévi (1957) described that sexual reproduction of it was still unknown. *Tetilla* belongs to this group.

As stated before, there are many peculiarities in the development of *Tetilla* different from those of other sponges, but from the viewpoint of sexuality, its development has many things in common with gonochorism or oviparity in many kinds of Clavaxinellida type or some kinds of Ceractinomorpha. In the point of accessories around the eggs, it has more in common with Ceractinomorpha than Tetractinomorpha. By Reiswig (1976), *Hemectyon* which belongs to Axinellida is shown to be able to develop directly without the stage of larva. These studies show that the development of *Tetilla* has something in common with some species of Axinellida.

These characteristics of the development of *Tetilla* may constitute also the basic patterns of all the Demosponges including Ceractinomorpha. These factors are not specialized as in the development of general sponges, but are suggested to be common to Eumetazoa. I have not yet enough evidence to determine, in the evolution of Demosponges, whether the developmental pattern of *Tetilla* is progressive or primitive. If more information can be obtained with respect to direct development in sponges, it may be possible to clarify some of the phylogenetic relations among the Porifera, and of the Porifera to other Metazoa. The developmental pattern in *Tetilla* suggests the phylogenetic proximity of the Porifera to Eumetazoa.

SUMMARY

1. The fertilization and development of two species of oviparous sponges, *Tetilla serica* and *Tetilla japonica*, were described. The results of this study show that these two species have common features in their development, but this is very unusual as compared with that of other sponges.

2. The mature egg is released through the maternal osculum and fertilized outside the body. After fertilization, it immediately adheres to the substratum without a free-swimming larval period and develops directly; this is not observed in other sponges.

3. These species have radiating fiber bundle on the egg surface, which are taken into the perivitelline space after fertilization. At this time the egg performs a sepcial type of rotation which is not known in any other eggs.

4. The radiating fiber bundles taken into the perivitelline space and the

space itself will be incorporated into the larva as the development proceeds.

This accessory covering of the egg is imagined to be the structure which carries substance to the larva from the maternal body, as has observed in other sponges.

5. The larvae of two species of *Tetilla* do not differentiate the columnar flagellated cell layer, characteristic of parenchymella larva, but differentiate directly adult ectosome and choanosome from undifferentiated cells of morula.

6. When the larva forms an aquiferous system and skeleton and develops to be a rhagon larva, alternation of body symmetry is seen in *T. serica*, not in *T. japonica*.

7. At present stage of study, the development of *Tetilla* seems very unique among the sponges, but with the progress of research on oviparous sponges, the relation within the Demosponges or the phylogenetic position in the Porifera of these species may possibly have to be reconsidered.

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