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Studies on the Gregarines in Japan

(Part I)

The fine structures of some gregarines

By

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Introduction

The cephaline gregarines belonging to the class Sporozoa are unique and interesting animals in the following points.

1) They are all parasitic without exception. The species of their hosts are various and extend through almost all phyla of the invertebrates except Polifera and Coelenterata, and also in the Tunicata and Enteropneusta of the phylum Chordata. Some gregarines parasitise throughout their hosts' life, from the larval stage to the adult stage, but others parasitise only at the larval or the adult stage. Most gregarines live only in the digestive tract, but rarely a few gregarines change their parasitic positions from the digestive tract to the body cavities and grow up to be sporadins there.
2) The gregarines are very large unicellular animals. For example, *Hoplorhynchus polyhamatus* measures over 3mm and *Nina gracilis* measures about 4—5mm.
3) Their bodies show characteristic differentiations. Each body is divided into three parts—epimerite, protomerite and deutomerite.
   The epimerite is the sticking organella, so the body adheres to the host with this epimerite sticking in the wall of the digestive tract. The types of the epimerite are varied, such as the simple globular type, hooked type, fine needle type and so on. This organella is considered very important from the point of view of classification.
   The shapes of protomerite and deutomerite are very simple. Between them the septum is observed, but a few species like Lecudinidae, have no septum. The septum looks like a cellmembrane, but nothing is clear about its significance. Any other characteristic organella except the nucleus can't be observed in the protomerite and deutomerite with the light microscope.

From these points mentioned above many basic and biologically interesting problems are contained in the cephaline gregarines. For a long time lots of studies about

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them have been made by many investigators such as Watson, Léger, Dubosq, Théodoridès and others. Many remarkable and useful works have been done by them. Moreover various new species and genera are now found every year. But there is no objection to saying that many problems which need greater investigations are still left undone. The present author wants to resolve these problems in this report.

This paper consists of two parts. One is on the fine structure of gregarines investigated with the electron microscope and with the scanning electron microscope. The other is on the taxonomic studies of cephaline gregarines found in Japan. In this paper the author adds new species to the species which are already known and then describes all of them in his suggested new way.

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The history of the study on the cephaline gregarines in Japan

The study on the cephaline gregarines in Japan was begun by S. Ishii. He published 3 papers on the gregarines. In his first paper published in 1911, he reported the existence of the three species of gregarines—*Gregarina polymorpha*, *G. cuneata* and *Steinina ovalis*. By using sectioned preparation he observed that *Gregarina polymorpha* lives an intracellular life. Then in 1914 he reported the four species of gregarines—*G. cuneata*, *G. minuta*, *G. crassa*, *Steinina obconica*—from *Tribolium ferrugineum*. He reported that one of them, *G. cuneata*, was an already known spescies but the others were new species. Moreover he observed for the first time *Spirosoma caudata* from Diplopoda, *Fontaneria coarctata* in 1915.

In 1923 S. Iitsuka reported *Lecudina fluctus* from Echiuroidea, *Urechus unicinctus*.

T. Hukui published 6 papers from 1939 to 1952. In 1939 he reported 3 species of gregarines from *Siphonosoma cumanense*. As for one of them, *Filipodium ozakii*, observing its life cycle in detail, he found that the trophozoite sticking in the intestinal cannal of its host changes its position to the coelomic cavity in the sporadine stage. At the same time he observed that the animal has interesting filamentous projections on its body surface. In 1951 he reported *Stenophora triangula* from Diplopoda, *Nedyopus patrioticus patrioticus*; in 1952 *Hoplorhynchus bouruiensis*

from Chilopoda, *Otocryptopus rubiginosus*. At the same time in 1952 he reported *H. ozakii* from the same host and *Hoprorhynchus aratoensis* from Chilopoda, *Crhyptopus japonicus* ; *Stenophora nematoides* and *S. ozakii* from Diplopoda, *Orthomorpha gracilis* in 1952. (The former was the species already known in France.)

From 1944 to 1954 H. Hoshide wrote 15 papers, in which he reported 50 species of cephaline gregarines including 44 new species. Most of their hosts are insects, but occasionally some other animals belonging to Polychaeta, Chilopoda and Diplopoda are included as their hosts. From 1956 to 1958 he published a series of papers. In the first paper on the subject of "Relation between cephaline gregarines in their early stage of growth and the host cells" he described the following points.

1) The species with the traceable or simple shaped epimerites in structure have an intracellular life. For example, the families Lecudinidae, Stenophoridae and Cephaloidphoridae can be put under these types.
2) The species with the complex shaped epimerites in structure don't pass into the intracellular stages. For example, the families Stylocephalidae and Dactylocephalidae are these types.
3) Some species with the epimerites which are intermediate shaped described above have an intracellular life, but others don't, for example, the families Gregarinidae and Actinocephalidae.

   But there are some exceptions in this classification. Some species like Coronoepimeritus pass into the intracellular stage though they have complex epimerites.
4) Besides H. Hoshide reported the differentiation of the body segment in its early stage of growth and also reported on whether they cause damage to the structures of their hosts or not by their parasitism.

In the paper, part II, he listed up the cephaline gregarines taken in Japan and classified them into 10 families, 41 genera and 115 species. And then he added 7 new genera and 40 new species to Japanese gregarines fauna. Among these species *Triseptata fungicola* and *Tintinospora soroniae* belonging to Family Gregarinidae, *Umbracephalus longicollis*, *Urnaepimeritus spathiformis* and *Acanthoepimeritus jimukade* belonging to Family Actinocephaildae, *Coronoepimeritus japonicus* and *C. monospinus* belonging to Family Acanthocephalidae are very interesting species from the morphological point of view.

From 1959 to 1968 no reports on the studies of gregarines taken in Japan can be uncovered.

From 1968 to 1973 K. Hoshide in co-operation with H. Hoshide and others reported 18 species of cephaline gregarines including 12 new species. Moreover K. Hoshide observed the fine structure of *Ferraria cornucephala iwamusi* by using the electon microscope.

Part I

The fine structure of Sporozoa has gradually been made clear with the development of the electron microscope and of the techniques of preparation. But Sporozoa is still the most enigmatic group in Protozoa. Sporozoa is considered to be a polyphyletic group and it includes many groups of organisms from different origins. Gregarines are one of the big groups belonging to Sporozoa, and study on the gregarines is a backward field in that on the Sporozoa. By studying the characters of the fine structure of the gregarines, the author wants to assemble the knowledge on the gregarines, to clear up the relationship of various groups among them and to define the position of the gregarines in Sporozoa. The fine structure of the gregarines is one of the few keys left by which the origin and the interrelation of each group can be traced. It is a huge-scale program to make clear the relationship of each gregarine. As the first step he wants to clear up the difference and the similarity of the fine structure of some gregarines. He tried to investigate the fine structure of the gregarines invarious stages but could observe only the gamont of gregarines which belong to Cephalinoidea. Up to now he has not succeded in observing the other stages such as the cyst or the spore. Since the spore is too small to make efficient preparations and the cyst is too hard to infiltrate the epoxy resin inside of itself. Nobody has described the fine structures of the cyst nor the spore of gregarines, and so they remain unknown. Hereafter, the author wants to continue studies on gregarines and to solve these problems by introducing new devices and techniques.

Materials and Methods

The materials and their hosts used in this study were as follows :

Parasite	Host
1. *Pyxinoides kamenote* (H. Hoshide) (11)	*Mitella mitella* (Linne)
2. *Gregarina acantholobae* H. Hoshide (13)	*Acrydium japonicum* de Hann
3. *Gregarina ovata* Dufour (10)	*Anisolabis maritima* Borelli
4. *Hoplorhynchus orthetri* H. Hoshide (14)	*Orthetrum albistylum speciosum* Uhler
5. *Ancyrophora mutabilis* n. sp.	*Copera anulata* Selys
6. *Nina japonica* H. Hoshide (12)	*Scolopendra subspinus multilans* Koch

Mitella mitella is a common stalked cirriped which inhabits areas between rocks in and near the Seto Inland Sea. *Mitella mitella* was torn off with a knife or a small shovel and carried into the aquarium in the laboratory. *Acrydium japonicum* is a small grasshopper, which is caught from March to October on grassy plains in or around the campus of Yamaguchi University. *Anisolabis maritima* is a common

earwig inhabiting places under rubbish, or sea weeds washed ashore on sandy beaches. *Anisolabis maritima* was caught at Murozumi or Aio, Yamaguchi Prefecture. *Orthetrum albistylum speciosum* is one of the typical dragonflies in Japan. *Copera anulata* is a small damselfly and a lot of them were easily caught near the Fushino River in Yamaguchi City during early summer.

Scolopendra subspinus multilans is a big centipede inhabiting rotten stumps or areas under stones in the shade.

The hosts, except *Mitella mitella*, were dissected in a petri dish with Ringer's solution, but *Mitella mitella* was done in that with sea water. In both cases the heads of the hosts were cut off and their digestive tracts were pulled out into the solution. In some cases the intestines with the parasites were fixed. In other cases the intestines were cut open, and the sporadins were washed out. The sporadins were gathered with a fine pipet and removed to 5% glutaraldehyde with 0.1M cacodylate buffer (pH 7.3) for prefixation. After two-hour prefixation they were rinsed several times with cacodylate buffer and were transferred into 1% buffered O_8O_4 (pH 7.3, 0.1M cacodylate buffer) for postfixation. They were dehydrated with ethylalchol series (70, 80, 90, 95, 100%)—in each of 70—95% for five minutes and in 100% for one hour. After dehydration the specimens for the scanning electron microscope were put into isoamyl acetate and then frozen in liquid nitrogen. They were sublimed quickly and dried on a metal holder. In some cases the specimens adhered to the holder by themselves but in the other cases they did not. In the latter cases the specimens were gathered and set on the holder with cellophane tape. All the specimens were coated with carbon and gold. Then they were examined with the JSM S—1.

On the other hand the specimens for a transmitted electron microscope were transferred into propirendioxide for 15 minutes after dehydration. They were embedded into Epon 812 or Alardyte (18). The sections 1—2μ thick were made and stained with toluidine blue for orientation before thin sectioning. The thinner sections were made with a glass knife. The ultramicrotomes used in this study were an LKB ultratome 1 and a Porter-Blum MT-1 microtome. The photographs were taken with an Akashi electron microscope TRS-50E, a JEM-5HS and a JEM-T6. The sections were stained with lead acetate and uranyl acetate (22).

Observation of the whole body with the scanning electron microscope

The whole body of the three species, *Ancyrophora mutabilis* n. sp., *Nina japonica* and *Gregarina ovata*, were observed with the scanning electron microscope.

Ancyrophora mutabilis : When it was washed out and was set free in Ringer's solution, the protomerite ordinarily shrunk and the epimerite invaginated into the protomerite. But in the natural condition the gregarine sticks to the host's intestine

with the epimerite. The electron micrograph (Fig. 1) shows its almost natural condition. The deutomerite is cylindrical and its anterior part slightly swells, from the center of which the swelling protomerite extends. The epimerite and the anterior part of the protomerite thrust into the tissue of the host's intestine. At the boundary between the protomerite and the deutomerite a deep constriction and several radial grooves appear. The grooves run posteriorly from here on the surface of the deutomerite.

Nina japonica : The whole body is elongate cylindrical in shape. The anterior part of the protomerite irregularly shrinks. There is a constriction at the septum. The deutomerite widens at the shoulder, and thence tapers gradually to the well-pointed posterior end. In its living condition the protomerite is bilaterally symmetrical and consists of two lobes. At one extremity the protomerite separates and forms two horn-shaped projections. The other extremity doesn't separate and is obliquely upturned. Each margin of the lobes has a row of short spines from which long slender filaments come out (15).

On the other hand in its fixed condition (Fig. 7, 8) the protomerite loses the filaments. It shrinks irregularly and both extremities are involved, between which a distinct concave surface comes out. The part just below the separate extremity mentioned above often swells. Many transverse furrows and projections are observed on the surface of the concave surface. A deep longitudinal groove separating the two lobes is also discernible there. It continues to the basement of the horn-shaped projections.

G. ovata : Matured sporadines leave the wall of the host's intestine and move freely in it. The two individuals in the intestine often make a syzygy. Each individual is rather slender in the early stage of the sporadin. With the maturation it becomes obese. The primite is nearly ovoidal and a little compressed. Its protomerite is semispherical. The part near to the bottom of the deutomerite in the primite is widest. The satellite is also ovoidal and more compressed than the primite. The posterior part of the deutomerite in the satellite is broadly round. The micrograph (Fig. 10) shows a sporadin which does not make a syzygy. It is ovoidal. The middle of the body is widest. The protomerite is semispherical and its anterior end is flat and irregular ; the posterior end of the deutomerite is broadly round. There is a slight constriction at the septum. There are a lot of transversal undulations on the surface of the deutomerite and a lot of swelling projections on the surface of the protomerite.

The surface structure

The mechanism of the movement of gregarines must closely relate to their surface structure. Gregarines have two types of movement (25, 30) : one is a bending movement and the other is a gliding movement. The latter movement is characteristic

of gregarines. Gregarines move without changing the shape of their bodies. Since the gliding movement was first described by Kölliker, numerous observations (4, 25, 26, 30, 31, 32) have been done with the light and the electron microscope and numerous theories have been published. The theories can be divided roughly into two groups as Vavra mentioned—1) some contraction of elements produces the movement 2) the ejection of mucus or water causes the gregarines' propelling power. On the surface structure longitudinal striations are often, and networks of transversal fibrillal structures are sometimes, observed with the light microscope. *Ancyrophora mutabilis* n. sp., *Nina japonica* and *Gregarina ovata* were observed with the scanning electron microscope. All three of these have many longitudinal folds on the surface of their bodies. The whole body from the top of its anterior part of the end of its posterior part, is covered with these folds. The length, the width and the shape of the folds differ from one species to another. The characters of the folds of each gregarine are as follows.

* *Ancyrophora mutabilis* (Fig. 1—6)

The surface of this protozoa is covered with a lot of longitudinal folds. Apparently the folds seem to continue from the top to the end of the whole body, but in fact they consist of a lot of short folds. At their end each tapers. The length of the folds is 25—66μ, the width is 800—900mμ, and the interval between them is 800—1000mμ. The interval is wider in comparison with the width. The folds are rather straight, but at some parts of the body they are gently undulate.

The high magnification of a micrograph (Fig. 4, 5) makes it clear that the surface of the folds and the grooves consists of many fine fibrils of the same size.

* *Nina japonica* (Fig. 7—9)

The surface of the body is covered with longitudinal folds similar to those of *A. mutabilis*. The length of the folds is 5—10μ, the width is 100—160mμ, and the interval between them 60—80mμ. The interval is much narrower, compared with that of *A. mutabilis*, so the surface seems to be packed with folds. All the folds wave transversely in parallel on a 2—2.3μ cycle.

* *Gregarina ovata* (Fig. 10—12)

The surface of the whole body is covered with a lot of longitudinal folds and the folds themselves consist of fine fibers like *A. mutabilis*. The length of the folds is 20—50μ, their width 300—450mμ, and the interval between them is 300—600mμ. The size of the folds is between that of *A. mutabilis* and of *N. japonica*. The ratio of the width of the folds to the interval between the folds is similar to that of *A. mutabilis*.

However the appearence is different ; the folds are rather straight without undulation and have a lot of branches connecting with the adjacent folds. In the branched parts the width is much narrower. A lot of transversal wrinkles are also observed on the surface ; these are irregular and seem not to be permanent.

The surface structure observed with the transmitted electron microscope

The surface structure of gregarines observed with the scanning electron microscope is mentioned above. In this chapter it is observed by means of sectioned preparations and with the transmitted electron microscope.

With the light microscope the body of gregarines, except the nucleus, seems to consist of cytoplasm composed of two parts—ectoplasm and endoplasm. The former is hyaline and homogeneous and the latter has many granules. On the other hand with the electron microscope, in the cytoplasm three parts are distinguishable—one part with a lot of vacuole-like structures, a 2nd part without such structures, and a 3rd with an epicyte. But there is no special membrane-like structure to divide these three parts. The epicyte folds, running longitudinally from the top to the end of the body, are observed with the scanning electron microscope. In a crosssectional view the size and the shape of all the folds are almost equal to one another. But the longitudinal or oblique-sectional view shows that the shape of the folds is varied. The facts above indicate that on the surface the folds are arranged at regular intervals.

The cross sectin of *Pyxinoides kamenote*
(Fig. 13, 14, 15)

The epicyte consists of two parts—a basement layer and folds. The thickness of the basement layer is 65mμ. Between the epicyte and the ectoplasm, the electron-density is completely different in each ; it is much higher in the former part. The cross section of the folds were observed. The size and the shape of the folds are as follows. The height is 680—700mμ ; the width is 130mμ and almost equal from the top to the basement though the folds sometimes become narrower at the extreme basement. The tops of the folds are round.

The folds and the grooves are covered with two lamella units—the outer and the inner membrane. The electron-density of the inner membrane is much higher than that of the outer. The outer is the tri-partite unit membrane, which consists of two parallel high electron-dense layers and one low electron-dense layer situated between the two. This membrane is equal to the cell membrane in general cells. The inner is a pentalaminar membrane, which consists of three high electron-dense layers and two low electron-dense layers situated among them. The two membranes are 16—20mμ apart. At the top of each fold, between the two membranes five or six high electron-dense fibers are observed and they are attached to the outer surface of the inner membrane. At the very middle of the basement of each fold there is one high electron-dense fiber.

The longitudinal section of *Pyxinoides kamenote*
(Fig. 16, 17, 18)

The shape of the folds, that is, their length and width varies ; some of them are long and slender, and others short and stout. But the height of the folds, from the top to the basement layer, is constant, and thus the line linking the tops of each fold parallels the basement layer. The outer and the inner membrane were observed. The outer is the thin and low electron-dense layer and the inner is the rather thick and high electron-dense layer. At the tops of the folds one or two fibrillar structures run in parallel with the basement layer between the inner and the outer membrane. No high electron-dense basement fiber can be observed, but in the basement layer the fine low electron-dense fiber is sometimes inside of the inner membrane.

The oblique section of *Gregaria acantholobae*
(Fig. 25, 26, 27, 28)

The anterior surface of the protomerite is often on a large scale, concave or convex. The size of the convex surface is about 2.5μ in width and 1.6μ in height. The surface of the whole body is covered with folds. The characteristic feature of the folds in this species is their swollen top part. The folds consist of the stalk-like part and the swollen bulb-like top parts. At the protomerite and the anterior part of the deutomerite, folds of various heights are observed. They are roughly classified into three types : (1) high folds consisting of a long stalk-like part and a bulb-like top part ; (2) folds of medium height consisting of a rather short stalk-like part and a bulb-like part ; (3) low folds only with a short stalk-like part. In some parts, high and low folds appear alternately, but in other parts they appear at random. According to observations with the scanning electron microscope, at the end of the folds their height seems to become less and at the anterior part of the protomerite a lot of low folds are observed. These facts indicate that there are a lot of ends of folds in that part of the protomerite.

The oblique section of *F. cornucephala iwamusi*
(Fig. 39, 40, 41)

F. cornucephala doesn't have a distinct septum but the species is classified as a Cephalinoidea. It is the characteristic species of the Cephalinoidea. So far as the arrangement of the folds is concerned, it is not different from the other species. The surface structure of the body is covered with longitudinal folds ; the height of the folds is 600—900mμ, and their width 300—500mμ. The folds have gentle undulation on a 0.5—1μ cycle. The folds and the grooves are covered with two membranes—the outer and the inner membrane. They are the same as the membranes of the other gregarines ; the outer is tri-partite and the inner pentalaminar. At the

basements or at the tops of the folds no fibrillar structure can be observed.

The longitudinal section of *Hoplorhynchus orthetri*
(Fig. 29, 30)

In the longitudinal section there are 3—6 long longitudinal layers on the outside of the surface of the deutomerite. In some parts of the layers they continue rather straight, and at others they wave up and down. On the surface of the protomerite the folds are sectioned not longitudinally but obliquely. So the sectional view of the protomerite and that of the deutomerite differ from each other. The structure of these folds is shown in the schema (Fig. 47). The folds become higher at the septum ; there the folds are 1.5 times as high as those at other parts.

The longitudinal section of *Nina japonica*
(Fig. 31, 32, 34, 35, 36)

The surface structure of the small trophozoite is completely different from that of the matured sporadin.

On the surface of the small trophozoite no folds can be observed. In most parts of the deutomerite the surface is smooth, but around its anterior part the surface presents complicated coagulation and deep-invaginate structures are observed. On the surface there are two membranes which especially on the smooth surface are conspicuously observed. A comparison between the two membranes shows the outer to be of higher electron-density than the inner.

On the other hand, examination with the scanning electron microscope shows the matured sporadin to be covered with longitudinal folds. In most parts of the detomerite there are a lot of undulate folds. But the folds at its anterior part are rather straight and they are four times as high as the folds in the other parts. The protomerite is complex in shape and its surface is rough. One of the extremities comprises the two horn-like projections. The surface of these projections is also complex ; it is extremely rough without any regularity, and uncommon shapes of projections and invaginations are seen.

The ectoplasm other than the epicyte

Seen with the light microscope the outer zone of the body, the ectoplasm, comprises an epicyte, a sarcocyte, and a myocyte, and observation with the electron microscope proves that the epicyte consists of the folds and the basement layer as described above. In this chapter the sarcocyte and the myocyte are described. With the electron microscope no difference can be observed between these two parts ; in other words, between the basement layer and the endoplasm there is only one layer

from the viewpoint of structure. In most species the boundary between this layer and the basement layer is clear because of the considerable difference of electron-density, but the boundary between this layer and the endoplasm is not clear. In this layer, other than the part of the many granules and some membranous structures, the substance is uniform in electron-density.

Pyxinoides kamenote
(Fig. 13, 14, 16)

The layer, viz., the ectoplasm other than the epicyte, surrounds the whole body. It is 2.3—4.5μ thick and is thicker at the anterior part of the protomerite and at the septum. This layer is directly connected with the septum without any boundary between them. With the exception of the anterior part of the primite protomerite, it contains many granules ; these are ellipsoidal or circular in shape, and are divided in size (230—250mμ). The smaller granules are often circular and the larger ones are often ellipsoidal. This is a crosssectional view and the granules themselves are sectioned at various angles. So it can probably be said that most granules have the shape of an ellipsoid. The layer at the anterior part of the primite protomerite lacks the granules but it contains very small vacuole-like structures surrounded with a membrane. At the joining surfaces of a syzygy many ellipsoidal granules appear and sometimes high electron-dense fibrils stretch inward from the basement of each hold. The fibrils seem to exist only in the layer.

Hoplorhynchus orthetri
(Fig. 29, 30)

The layer is rather thick : it is 2—3.5μ in thickness. The endoplasm contains many vacuole-like structures and large, high electron-dense bodies, but in the layer there is no such structures. So it is easy to distinguish this layer from the endoplasm. This layer and the septum connect with each other without any boundary. The layer is about 1.5 times as thick as the septum and it does not increase its thickness at the septum. The component of the layer is the same as that of the septum : they are both composed of small granules and homogeneous substance.

G. acantholobae
(Fig. 27)

The thickness of the layer is 0.5—1.5μ. There are a lot of ellipsoidal granules and small vacuole-like structures in it. Especially at the top of the protomerite a lot of vacuole-like structures are recognized. In several specimens the epimerite is observed. The inside of the epimerite is filled with the same component as the

layer—and more than half of the epimerite is occupied by small vacuole-like structures. Joining surfaces of the satellite are especially interesting in this species. The protomerite of the satellite is compressed to decrease its volume, and it seems to be depressed into the top of the deutomerite ; the deutomerite seems to be protruding its shoulder. At the joining surfaces the layer, which comprises the protruding part, is extremely thick. The layer is distinguishable from the endoplasm because of its lower electron-density.

Nina japonica
(Fig. 31, 32, 33, 34)

The situation of the layer in the matured sporadin is different from that of the trophozoite. In the trophozoite the differentiation of each part has hardly advanced yet, and no longitudinal folds can be observed on the surface. The number of the vacuole-like structures in the endoplasm is much smaller than that in the adult sporadin. There isn't any difference of structure between the endoplasm and the ectoplasm. Only on the surface membrane, the electron-density is higher than in the other parts. In the case of the matured sporadin, the layer is 0.5—3.5μ at its deutomerite, and it is very thick at the anterior part of the deutomerite, where vacuole-like structures are few in the endoplasm. It is difficult here to distinguish the boundary between the layer and the endoplasm. The electron-density of the epicyte and of the layer is equal ; there is no boundary between the two parts. In the layer small vacuole-like structures, small granules and a few rather big spherical bodies are observed but inside the folds only small vacuole-like structures are recognized.

Ferraria cornucephala iwamusi
(Fig. 39, 40, 41, 42)

In this species the differentiation of the layer is not recognized. With the light microscope one or two pseudoseptums are observed, but with the electron microscope neither septum-like structures nor any other basic difference are recognized in any area of the cytoplasm. Many various-sized vacuole like structures are partially dispersed in the cytoplasm.

The joining surfaces of the syzygy

Gregarines form a characteristic association called a syzygy before they form a cyst. In most species of Cephalina two sporadins mutually associate with the posterior end of the primite and the anterior end of the satellite, but in some species they associate with each other's anterior end. In both cases they never attach to the other parts of the bodies of their partners'. So it follows that gregarines can

recognize the part suitable for attaching. It is a very interesting question as to how they recognize the suitable part. The attached surface was first examined morphologically with the electron microscope. In this study only the syzygies of *Gregarina acantholobae* and *Pyxinoides kamenote* were used. The situation of these two kinds of syzygies is as follows. Some species, such as *Nina japonica*, *Ancyrophora mutabilis* and Tricoptera sp., are very difficult to effect their syzygy. For they associate just before making a cyst and the length of the syzygy is very short.

The joining surface of *Gregarina acantholobae*
(Fig. 26, 28)

Using the light microscope at the syzygy stage the protomerite of the satellite appears to become thin and a shallow dish-like structure exists at the joining surface. The end of the primite fits into the shallow dish of the satellite. At the center of the joining surface, which is the center of the anterior part of the satellite protomerite, there is a small transparent lens-shaped body corresponding to the thickened epicyte of the primite.

On the other hand the electron microscope shows the center of the joining surface of the primite and the satellite to be concave. There is no particular structure at the concave surface ; the concave surface is nothing but lens-shaped space. Around the space the primite and the satellite join. At the joining surface the folds of the satellite become lower in general, and they fuse with the folds of the primite. All the tops of these folds become flat and widened. The folds never adhere to the parts of the grooves. Each pair of joining folds still have cell membrane on each of their surface. Between them there is a narrow gap filled with high electron-dense cytoplasm.

The joining surface of *Pyxinoides kamenote*
(Fig. 21, 22)

The situation of the joining surface of *Pyxinoides kamenote* is very different from that of *Gregarina acantholobae*. In the case of *Pyxinoides kamenote* the joining surface presents two different types of appearance. In one type of appearance—losing their boundary membrane, the top parts of the primite's folds and those of the satellite's fuse with each other and the fused part makes a plate. The folds do not attach with each other at top to top but each top of the primite's folds is arranged in regular alternation between every two tops of the satellite's. As for the height of the folds there is neither extension nor compression at this joining surface. In the other type of appearance—the joining surface swells and the swollen part becomes 1.5—3 times as wide as the other type of the joining surface explained above. The folds at the swollen part have changed the shape irregularly

and the layer between them has extremely widened. A lot of complex structures are recognized. The sectioned face is spherical, ellipsoidal or orvoidal in shape.

The septum

A septum is the thin layer which separates the two portions of the body of a gregarine, viz., the protomerite and the deutomerite. It is the main characteristic of Cephalinoidea. With the light microscope the septum is observed as a transparent membrane-like structure and it has the same components as the ectoplasm. The electron microscope shows the same patterns. The observation of the septum of *Pyxinoides kamenote*, *Hoplorhynchus orthetri* and *G. acantholobae* is as follows.

The septum of *Pyxinoides kamenote*
(Fig. 20)

The septum invaginates from the part where the body constricts. It is thick at the start of the invagination, where the septum and the ectoplasm form the shape of a triangle in the longitudinalsection. Except for the triangled parts on both sides, the thickness of the septum is constant (1.3μ). The parts of the septum from the outer margin to about the middle of the triangled parts contain many granules, but the other part of the septum doesn't have any granules.

The septum of *Hoplorhynchus orthetri*
(Fig. 30)

The septum invaginates from the same part as *Pyxinoides kamenote*, but at the start of the invagination it is not thick like *P. kamenote*. The thickness is 1.4—1.7μ. The septum does not contain either the vacuole-like structure or the high eletron-dense body which exist in the endoplasm.

The septum of *Gregarina acantholobae*
(Fig. 25, 26, 28)

The septum of the primite is almost similar to that of *P. kamenote*. Morphologically the septum of the satellite is very different from that of the primite. Compressed into the top of the deutomerite, the protomerite of the satellite changes its shape : it is hemispherical, its anterior side is slightly concave and its posterior side is swollen. On the anterior side the protomerite is covered with thin ectoplasm, and on the posterior side it is separated from the endoplasm of the deutomerite with a thick septum. The septum is the same in quality as that of the primite ; it contains neither granules nor vacuole-like structures.

The epimerite of *G. acantholobae*
(Fig. 27)

Observed with the light microscope, gregarines have only a few organellae and a highly differentiated epimerite, organella for attaching. So in the classification of gregarines an epimerite is an important organella and it is expected to help in making the fine structure of an epimerite clear. But the chance to find an epimerite in the sections for use in the electron microscope is small because, compared with the whole body of a gregarine, an epimerite is small. In this study the fine structure of the epimerite of *G. acantholobae* alone is made clear.

According to the observation with the light microscope, the epimerite presents a simple spherical hyaline knob on a short slender stalk. Observed with the electron microscope it projects from the top of the protomerite. The shape is hemispherical or paraboloidal. Its surface is covered with the folds and the basement layer. The folds on the epimerite connect with those on the protomerite. There is a clear boundary between the basement layer and the inside part of the epimerite. As mentioned above, the inside part of the epimerite connects with the ectoplasm of the protomerite. The inside part contains many granules or small vacuole-like structures. In some specimens a lot of granules occupy more than the half of the epimerite, and in others a lot of vacuole-like structures do.

The nucleus of *Pyxinoides kamenote*
(Fig. 23, 24)

The nucleus of *Pyxinodes kamenote* alone is observed in this study. The nucleus is the only organella that is conspicuous in the cytoplasm and distinguishable with the light microscope. It consists of the nucleus envelope, the karyoplasm and the nucleolus. The nucleus of this species is spherical (30μ in diameter) and has one big nucleolus. The nucleus is surrounded with the nucleus envelope, which is a tripartit unit membrane : there is a low electron-dense layer between two high electron-dense layers. The thickness of the envelope is 150—250Å. It is irregular : at some parts it projects hemispherically and at others it is concave. A lot of pores are distributed on it. The characteristic nucleus envelope with a honeycomb layer, which is observed in some gregarines, is not recognized in this gregarine. The karyoplasm comprises two kinds of substances distinguished from each other by the difference in the electron-density—the higher and the lower electron-density. The former may be the chromatin bounded with DNA. With the electron microscope a large spherical nucleolus is observed just as with the light microscope. The nucleolus is homogeneous and its electron-density is much higher than that of the karyoplasm.

The endoplasm

The part inside the ectoplasm is filled with the endoplasm. The endoplasm is clearly distinguished from the ectoplasm. With the light microscope, in the endoplasm many large granules are observed and they are called paraglycogen granules because of their chemical composition ; as granules are deeply stained by iodine solution, and are thought to be made of glycogen. All the endoplasm observed with the electron microscope is composed of three elements—vacuole-like structures, granules, and a homogeneous substance. The vacuole-like structures correspond to the structures called paraglycogen granules in the observation with the light microscope. They are surrounded with a thin membrane of high electron-density.

The endoplasm of *Pyxinoides kamenote*
(Fig. 13, 14, 16, 17, 18, 19)

Observed with the light microscope, the endoplasm is dark brown, and with the electron microscope it comprises the vacuole-like structures, two kinds of granules and a homogeneous substance. The vacuole-like structures are circular and their diameter is varied (0.1—1.6μ in sectional preparation). In these structures fold-like shadows are often observed. There are two kinds of granules : granules of high electron-density and bigger granules of rather lower electron-density. The former ones are spherical and the latter ellipsoidal.

The endoplasm of *Nina japonica*
(Fig. 31, 32, 33, 35, 36, 37, 38)

The component of the endoplasm differs, which depends on the part of the body and on whether it is a trophozoite or a sporadin.

In the trophozoite the difference in the components in each part of the body is conspicuous. There are no vacuole-like structures in the protomerite nor in the anterior top of the deutomerite, where extreme accumulation of the granules is observed. There are two kinds of granules—big granules of high electron-density and very small granules of extremely high electron-density. The former granules are 4 times as big as the latter ones. At the anterior part of the deutomerite with no granules, a lot of longitudinal fibrils are recognized. At the anterior half of the deutomerite there are rather fewer vacuole-like structures and more granules than at its posterior half. In the posterior half a lot of large low electron-dense bodies are observed and epoxy resin does not readily penetrate into the bodies. So these often present a wrinkled appearance in the section.

In the sporadin the component of the body is dependent upon the part of the body. The endoplasm of the protomerite is very different from that of the deutomerite.

There are two types of vacuole-like structures in the sporadin : one type of structure is large-spherical (0.4—0.8μ in diameter) and is surrounded with a membrane ; the other type of the structure is small-spherical (0.1—0.2μ in diameter) and has no membrane. Except for its anterior part, the deutomerite is full of the larger vacuole-like structures, but only a few smaller ones are observed. On the contrary in the protomerite and in the anterior part of the deutomerite there are no larger vacuole-like structures, but a lot of smaller ones are observed. The high electron-dense bodies which are recognized in the anterior half of the trophozoite deutomerite are dispersed both in the protomerite and in the deutomerite, but the posterior part of the deutomerite has more such bodies than the other parts. There is no accumulation of the granules which is observed in the trophozoite. The accumulation of mitochondria is often observed in the deutomerite.

The endoplasm of *Gregarina acantholobae*
(Fig. 26, 27, 28)

Observed with the light microscope, the endoplasm is very dense. In the protomerite it is brownish yellow and much darker than in the protomerite. In the observation with the electron microscope, the endoplasm of the primite and that of the satellite differ much. In the primite the endoplasm of the protomerite and of the deutomerite is not much different. In both of them large spherical vacuole-like structures are dispersed rather sparsely. Their diameter is about 1μ. A lot of granules are observed. As for the satellite, the component of the endoplasm of the deutomerite is almost the same as that of the primite, but the endoplasm of the protomerite is much different from that of the primite. The protomerite of the satellite is compressed and changes its shape as mentioned in the description of the ectoplasm of this species. The endoplasm is filled with a lot of vacuole-like structures whose diameter is almost constant (0.3—0.4μ). There are some high electron-dense particles ; their size is various (0.01—0.2μ in diameter). No other granules are observed in the protomerite.

The endoplasm of *Hoplorhynchus orthetri*
(Fig. 29, 30)

Observed with the light microscope the endoplasm is very dense, opaque and dark brown. The protomerite is somewhat less dense than the deutomerite. Between these two, with the electron microscope not much difference in the components is observed. The endoplasm consists of spherical vacuole-like structures, granules and extremely high electron-dense substance.

The endoplasm of *Ferraria Cornucephala iwamusi*
(Fig. 39, 40, 41, 42)

This species has no septum, but the anterior part of the body seems to show some differentiation observed with the light microscope. The endoplasm of the anterior part, just below the top, is fine, granular and homogeneous. It is often extremely dense and looks brown. The main part of the body which presents no differenciation is dense and full of numerous vacuoles. With the light microscope and with the electron microscope, neither septum-like structures nor any basic differences are observed in the thin section all through the cytoplasm. The vacuole-like structures are partially dispersed in the endoplasm, and compared with the other gregarines examined in this study, they are fewer. The granules are also observed in the endoplasm. In the cytoplasm a lot of membranous structures, Golgi body-like structures, are observed, which is the characteristic feature of this species.

Discussion

Observed with the light microscope, the bodies of the cephaline gregarines except *Ferraria cornucephala* are divided by the transparent septum into two parts, the protomerite and the deutomerite. On the contrary in observations of the surface with the scanning electron microscope, all species of the three gregarines, *Ancyrophora mutabilis*, *Gregarina ovata* and *Nina japonica*, have either a slight or a conspicuous constriction at the septum, but between the two parts there is no fundamental difference on their surfaces, and no specific structures nor gaps are observed on the surface of the constricted part. Every fold and groove continues from the protomerite to the deutomerite without any boundary. By comparing the gregarines which attach to the host's tissue, with those released from it, it is found that ordinally their configuration is not so different from each other. But in some cases, when the gregarines leave their host's tissue, the configuration of the protomerite changes : the long-tube-like protomerite of *Ancyrophora sp.* shortens and swells ; the protomerite of *Nina japonica* coagulates.

The whole surface of the three gregarines observed in this study is covered with the longitudinal folds and apparently they continue all through the body. But in fact the surface consists of many short folds. The size and the configuration of the folds are varied, and depend on the species. Among the three, the folds of *A. mutabilis* are the longest, those of *G. ovata* the second longest, and those of *N. japonica* the shortest. As for the width of the folds, the order is just the same as that of the length. In the ratio of the space between the folds to their width, *G. ovata* is the largest, *A. mutabilis* the next and *N. japonica* the smallest. The folds of *N. japonica* present a lot of undulation ; those of *A. mutabilis* present a little ; those of *G. ovata* are straight.

The scanning electron microscope shows that the epicyte consists of longitudinal folds and a basement layer. The folds are the permanent structures and they exist in all the sporadins of the gregarines in Eugregarine and Schizogregarine (1, 3, 5, 6, 7, 8, 9, 16, 17, 19, 20, 21, 24, 27, 28). Their existence has already been proved in Schizogregarine *Selendium fallox*, *Ditrypanocystis cirratuli*, *Lankesteria culici*, etc., and Cephaline gregarine *Gregarina rigida*, *Pyxinoides balani*, *G. cuneata*, *G. rhyparobiae*, *G. deini*, *Pileocephalus blaberae* (3, 19, 21, 26, 28). Young trophozoites which attach to the host's tissue lack the folds and the surface of their body is smooth. Observed with the light microscope, very small trophozoites do not move actively even after they become free of the host's tissue. This fact shows that the folds have a close relationship with the characteristic movement of gregarines, that is, the gliding movement.

On the mechanism of the movement of the gregarines many hypothesis have been suggested, as mentioned before. Recently E. Vivier (28) reported that Lecudina has two kinds of folds. Every other fold remains stationary while the interposed folds are undulating. J. Vavra et al. (26) also hypothesize that gregarines move by undulating their pellicular folds judged by the regularity of the undulate pattern of the folds. The mucus which is excreted between the folds and the bubous rim enhances the effect of the gliding movement. The folds of *G. ovata* and *A. mutabilis* are straight or slightly undulate. Judging from the appearance of the folds it is unthinkable that the undulation of the folds is the main source of the propulsion. In *G. ovata* and *A. mutabilis* a lot of transverse wrinkles are recognized on the surface of the longitudinal folds. The wrinkles do not seem to be permanent structures. The up-and-down waves of the wrinkles proceed posteriorly from the front end, and this progression plays an important role in the gliding movement. *N. japonica* has conspicuously undulate folds, but among the folds two appearances—that is, the stationary and the undulate folds reported by Vivier—are not observed. The folds are close. The hypothesis of J. Vavra may be applicable, but that of E. Vivier is not applicable, to the mechanism of the movement of *N. japonica*.

The surface of the epicyte is covered with the two membranes, the outer and the inner membrane. The outer is a tri-partite unit membrane and the inner is a pentalaminar membrane. The inner membrane is thicker and of higher electron-density than the outer. These membranes are widely known all through the gregarines (8, 16, 19, 21, 29). Young trophozoites with no folds also have the same membranes. In some species, on the outside of the inner membrane at the top of the folds, 3—6 longitudinal and high electron-dense fibrils are recognized, and at the center of the basement of the folds rather thick and high electron-dense fibrils run longitudinally.

The folds play very important roles in syzygies. The observations of syzygies with the electron microcope have been done in a few previous papers (3, 7, 8). At first

the folds of both the primite and the satellite attach each to other. The tops of the folds widen and become flat. The cell membranes of both folds remain and at the surface they are in parallel with each other. Between the two membranes there is a narrow gap which is filled with high electron-dense substance. Later every adjacent fold fuses at the widened top and makes a plate. In this stage the cell membranes disappear. The cytoplasm exists in the folds and the plate without any boundary between them. The plate widens, the folds transform, and their structure collapses. A lot of complex structures appear at the widened plate, but unfortunately further observation has not been completed.

The bodies of the gregarines are filled with cytoplasm and they are divided into ectoplasm and endoplasm according to whether vacuole-like structures are contained or not. But there is no conspicuous boundary between the two parts. In some species the vacuole-like structures are few and it is hard to distinguish the ectoplasm from the endoplasm. *N. japonica* conspicuously assumes such a character. There is no vacuole-like structures in the protomerite. A young trophozoite especially has a lot of granules instead of vacuole-like structures in the protomerite. *Ferraria cornucephala* has a few vacuole-like structures and no difference is observed between the ectoplasm and the endoplasm (16).

The ectoplasm consists of the epicyte and the ectoplasm other than the epicyte. There is no specific structure between the epicyte and the ectoplasm other than the epicyte, but in many species the epicyte is distinguished from the ectoplasm except the epicyte because of the difference of the electron-density : the epicyte is of higher electron-density of the two parts. In *N. japonica* and *F. cornuceqhala* the electron-density of the two parts is equal.

The thickness of the ectoplasm except for the epicyte differs, according to the part of the body and to the species. Generally speaking, around the septum and the anterior part of the protomerite the ectoplasm is thick. In some species it becomes thick at a particular part : in *G. acantholobae*, at the anterior part of the satellite deutomerite, the ectoplasm thickens and is shaped like a disk. The vacuole-like structures which are contained in the endoplasm are not observed at the peripheral part of the body, with the exception of the small ones observed in some species. In stead of the vacuole-like structures, a lot of granules are contained in the ectoplasm. They are ellipsoidal and of high electron-density. Ordinally the same granules are contained in the endoplasm. Peculiarly, in the case of a young trophozoite of *N. japonica*, a lot of granules accumulate at the the protomerite. The reason for the accumulation is unknown. Except in some species, the typical mitochondria are not recognized. These granules play the role of energy metabolism or respiration instead of the mitochondria. In some species small vacuole-like structures appear in the ectoplasm, but they are entirely different from those in the endoplasm : those in the ectoplasm are much smaller and have no membrane.

The septum is not a membranous structure but a thick layer. It invaginates from part of the ectoplasm. There is neither a gap nor a boundary between it and the ectoplasm. Except the margin the thickness of the septum is constant and its component is the same as that of the ectoplasm : i.e. homogeneous and a high electron-dense substance. It does not contain either granules or vacuole-like structures. It is a clearly distinguished structure but its role or significance is unknown.

The epimerite of only *G. acantholobae* was observed. It is a knob-like projection at the top of the protomerite. Its surface is covered with the same folds that are observed on the surface of the protomerite and the deutomerite. The folds continue from the protomerite to the epimerite. The substance inside the epimerite connects with the epicyte of the protomerite. A lot of granules or small vacuole-like structures accumulate in the epimerite. Considered from its structure, the role of the epimerite may be not only attaching but the absorption in nutrition or the metabolism of substances.

It was reported in some previous papers that some gregarines had the characteristic nucleus envelope. The author wants to investigate many nucleus of various species, but the nucleus of only *Pyxinoides kamenote* is observed (2, 7, 27, 29, 30). It is composed of the nuclear envelope, the nuclear plasm and the nucleolus. The nuclear envelope is a tri-partite unit membrane and 150—250Å in thickness. It projects irregularly. The honeycomb layer, which attaches to the inner membrane and is 90mμ in thickness, is reported in some gregarines by Beam et al. and Grassé and Théodoridès (2, 9). In this species a lot of pores are distributed in the envelope but no honeycomb layer is observed. The nuclear plasm consists of the two parts of different electron-density. The higher electron-dense part might be the chromatin bonded with DNA.

Most of the body is filled with the endoplasm. The endoplasm consists of a lot of vacuole-like structures, granules and a homogeneous substance, and in some species mitochondria, Golgi body-like structures and high electron-dense bodies are included (7, 16, 21, 27, 29, 30). The endoplasm is characterized by the existence of vacuole-like structures. Observed with the scanning electron microscope, they are spherical and are surrounded with a membrane whose inside is low electron-dense. They may correspond to the structures called paraglycogen granules in the observations made with the light microscope. The number of the vacuole-like structures depends on the species : they are rather few in *F. cornucephala* and a young trophozoite of *N. japonica*. Their role is unknown.

A lot of granules are contained in the endoplasm and they are of high electron-density. The granules are the same as those which are observed in the ectoplasm. The role of these granules is not definite, but is thought to fi.l the role of the energy metabolism because in some species in which there are no mitocondria only these granules are recognized.

N. japonica and *H. orthetri* have high electron-dense bodies in the endoplasm, and *F. cornucephala* has Golgi body-like structures. The significance is completely unknown.

By comparing the fine structures of various gregarines with one another, several similarities and differences are made clear.

The similarities :

1) The surface of every species except that in the young stage is covered with the longitudinal folds.
2) The folds and the grooves are covered with the two membranes : the outer and the inner membrane.
3) Every species contains vacuole-like structures and ellipsoidal granules in the endoplasm.

The differences :

1) The shape and the size of the folds.
2) The number of the vacuole-like structures and granules.
3) The thickness of the ectoplasm.
4) The boundary between the epicyte and the ectoplasm other than the epicyte.
5) The existence of the typical mitochondria, the Golgi body-like structures and the electron-dense bodies in some species.

Summary

The fine structures of some gregarines, *Pyxinoides kamenote* (H. Hoshide), *Gregarina acantholobae* H. Hoshide, *Gregarina ovata* Dufour, *Hoplorhynchus orthetri* H. Hoshide, *Ancyrophora mutabilis* n. sp., *Nina japonica* H. Hoshide, are investigated with the scanning electron microscope and with the transmitted electron microscope. The observations are described on each species and on each part of the body, the surface, the epicyte, the ectoplasm, the endoplasm, the epimerite and the nucleus.

The surface structure of the three species of gregarines, *Ancyrophora mutabilis*, *Nina japonica* and *Gregarina ovata*, is observed with the scanning electron microscope. The surface of each gregarine is covered with longitudinal folds. The size and the feature of the folds differ with the species.

The four species of gregarines, *Pyxinoides kamenote*, *Gregarina acantholobae*, *Hoplorhynchus orthetri* and *Nina japonica*, are observed with the transmitted electron microscope. Each matured gregarine of these four species has longitudinal folds on its surface and contains a lot of vacuole-like structures in the body.

The young gregarines, trophozoite, have no folds. The folds develop as the gregarines grow older.

Each fold consists of two membranes, the outer and the inner membrane. The outer is the tri-partite unit membrane and the inner is the pentalaminar membrane.

This membranous system is the same that is observed generally in gregarines reported until now.

Pyxinoides has several high electron-dense longitudinal fibers at the top of the folds and one thick fiber at the basement of the folds. These fibers may relate to the characteristic movement of the gregarines.

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Explanation of Figures

Ancyrophora mutabilis

1. A sporadin attaching to the host's tissue.
2. Another sporadin.
3. The middle part of a deutomerite of a sporadin freed from the host's tissue.
4. Rather high magnification : each end of the folds becomes tapered.
5. High magnification : the surface consists of a lot of fibers.
6. The broken face of a deutomerite : a lot of spherical structures are observed.

Nina japonica

7. A sporadin.
8. The anterior part of a sporadin.
9. The middle part of a deutomerite.

Gregarina ovata

10. A sporadin not associated.
11. The middle part of a deutomerite.
12. Higher magnification of the folds : fine fibers are observed on the surface of folds.

Pyxinoides kamenote

13. Cross section of a deutomerite : endoplasm (end), ectoplasm (ect), vacuole-like structures (v), folds (f).
14. Rather higher magnification of the cross section of a deutomerite : a basement layer (b), high electron-dense fibers at the basement of folds (bf), granules (g).
15. High magnification of epicyte : an inner membrane (im), an outer membrane (om).
16. Longitudinal section of a deutomerite : low electron-dense fibers (lf).
17. Longitudinal section at the anterior part of a protomerite.
18. Higher magnification of Fig. 17 : small vacuole-like structures (sv), high electron-dense fibers (hf).
19. Endoplasm.
20. Cross section of a septum : septum (s).
21, 22. Cross section at the joining surface : swolln part (sp).
23. A nucleus : nucleus (n), nucleus envelope (ne), nucleolus (no).
24. High magnification of a nucleus.

Gregarina acantholobae

25. Oblique section at a septum : septum (s).
26, 28. Longitudinal section at the joining part : primite (pr), satellite (sa), lens-shaped space (ls).
27. Longitudinal section of an epimerite : epimerite (e), small vacuole-like structures (sv).

Hoplorhynchus orthetri

29. Longitudinal section of a deutomerite.

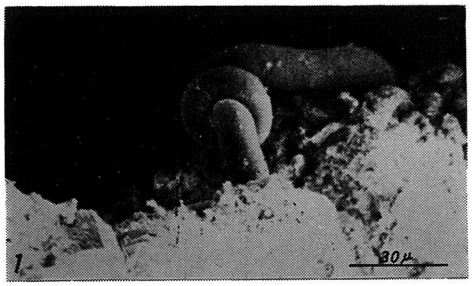
30. Longitudinal section at the septum : high electron-dense bodies (hb).

Nina japonica

31. Oblique section of a deutomerite : high electron-dense bodies (hb).
32. Longitudinal section at the anterior part of a deutomerite.
33. Cross section of a deutomerite : mitochondria (m).
34. High magnification of ectoplasm.
35. The anterior part of a trophozoite : electron-dense fibrils (fi).
36. Longitudinal section of a protomerite : inside of coagulated protomerite (ins), outside of surface (out).
37. Endoplasm of a deutomerite ; acumulation of mitochondria are observed : mitochondria (m).
38. High magnification of Fig. 37.

Ferraria cornucephala iwamusi

39, 40, 41. Oblique section of a body : Golgi-body like structures (go).
42. Longitudinal section of a body.
43. A cutaway view of *Pyxinoides kamenote*.
44. A three-dimensional view of epicyte of the *Pyxinoides kamenote*.
45. A cutaway view of the protomerite of *Gregarina acantholobae*.
46. A cutaway view of *Gregarina acantholobae* at the joining part between the primite and the satellite.
47. A three-dimensional view of the epicyte of *Nina japonica* at the anterior part of the deutomerite.


1
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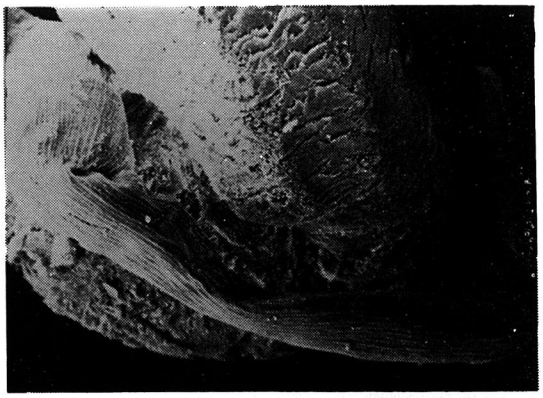

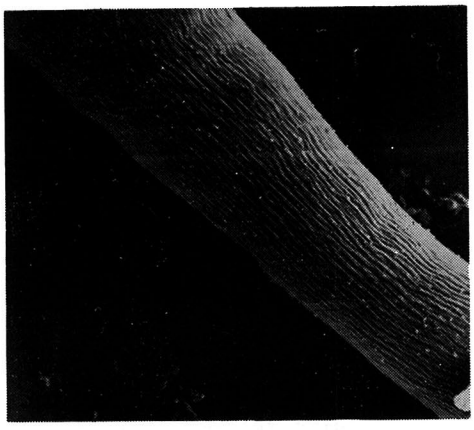

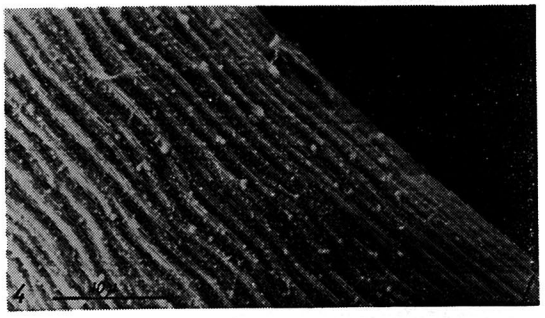
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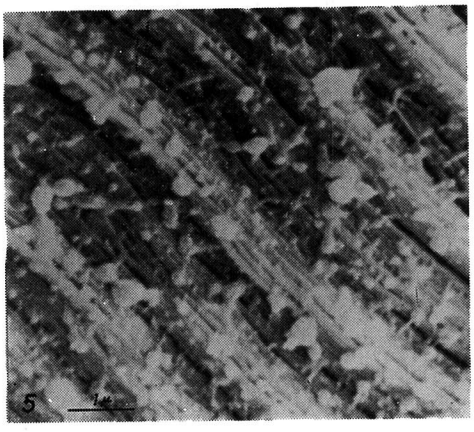
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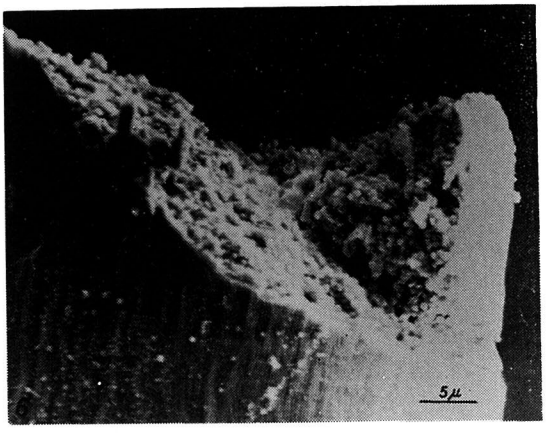
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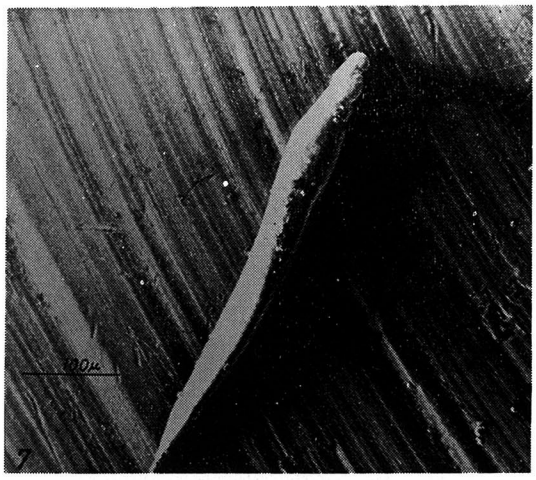
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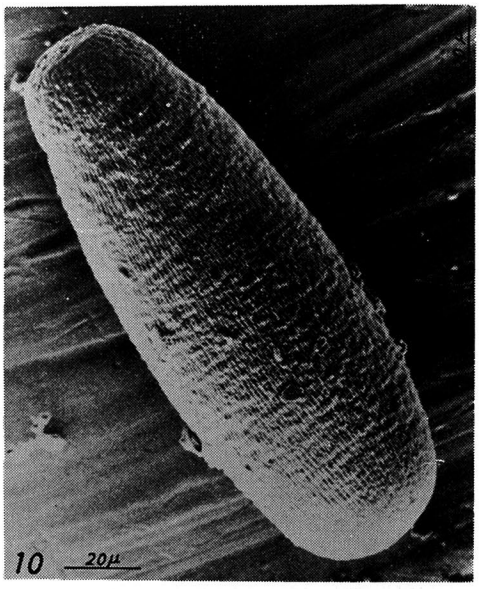
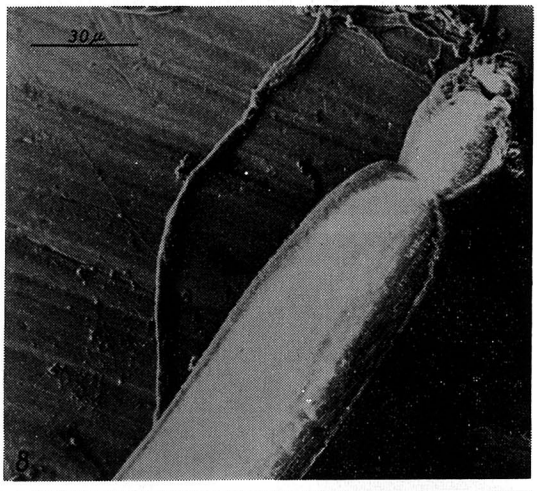

10
20μ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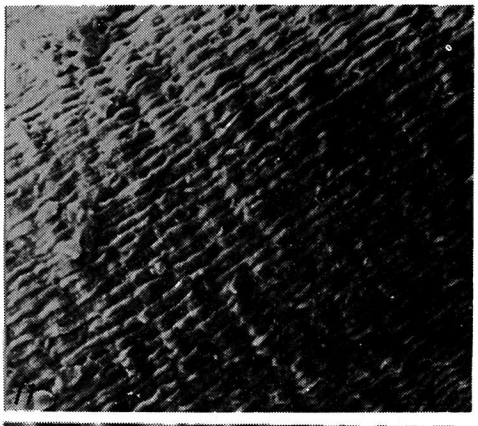
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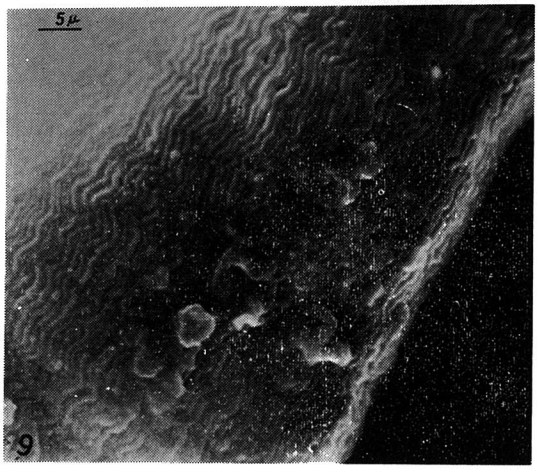
5μ
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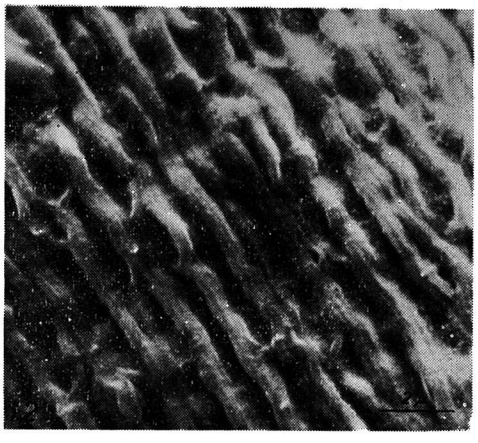
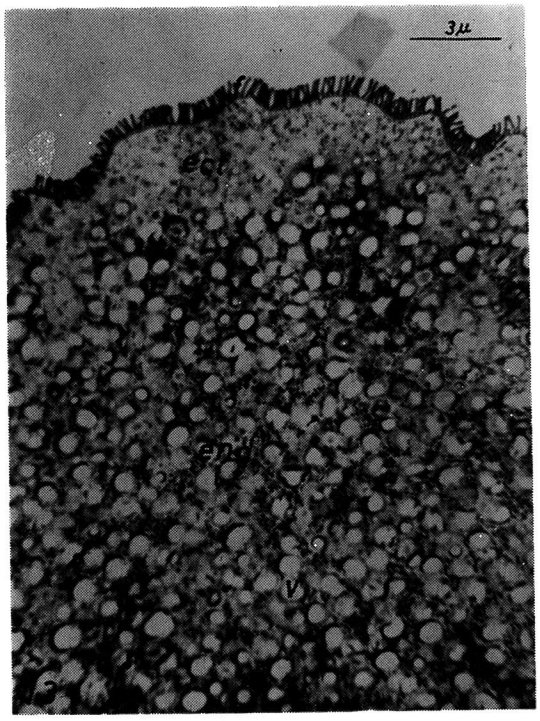
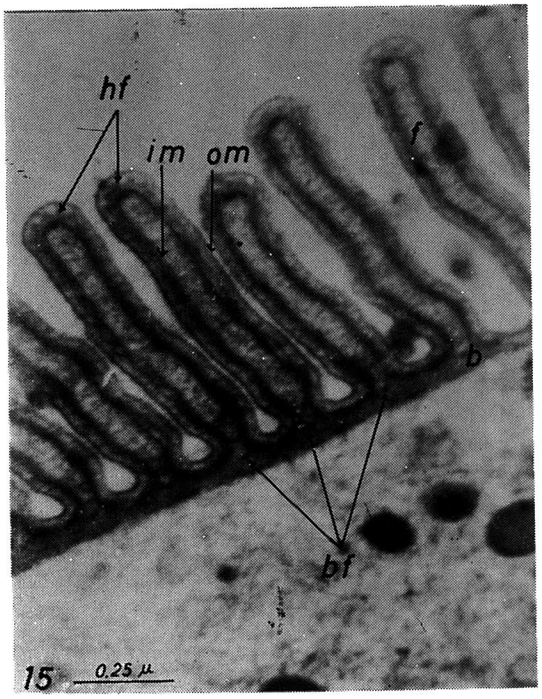
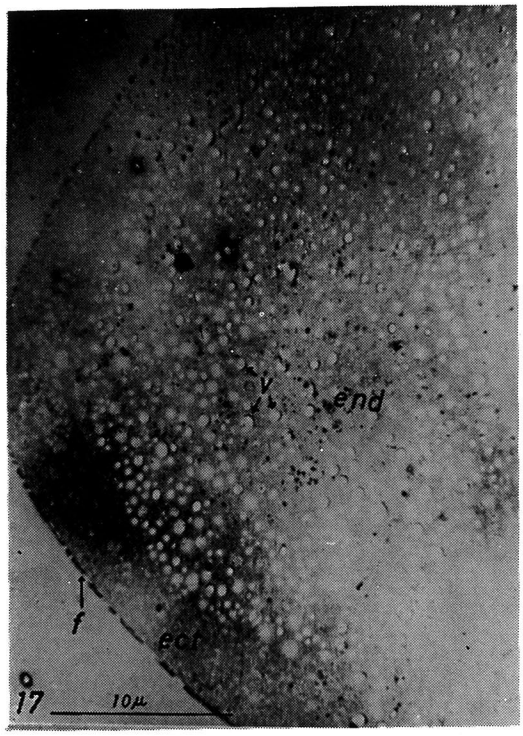


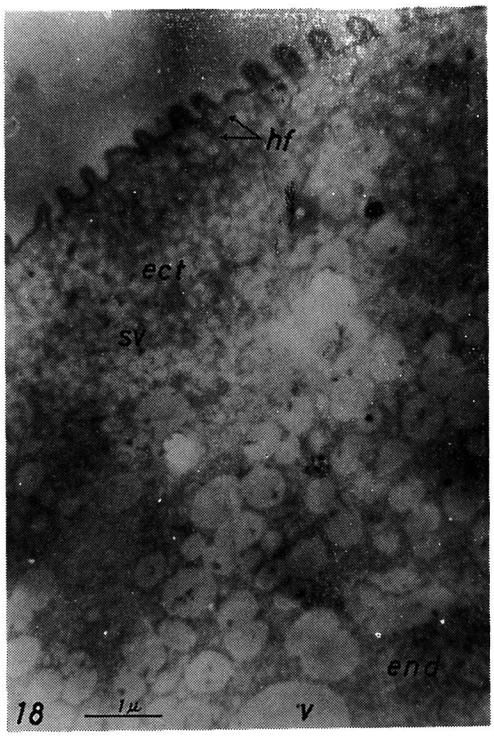

3μ
ect
en
v

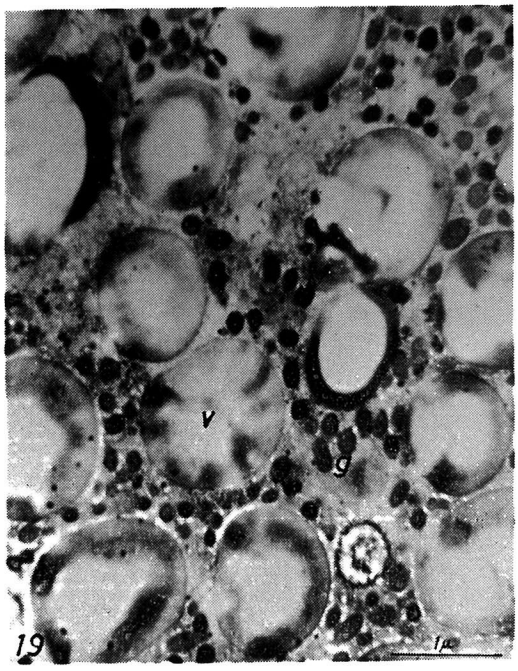

bf
f
ect
v
1μ
14

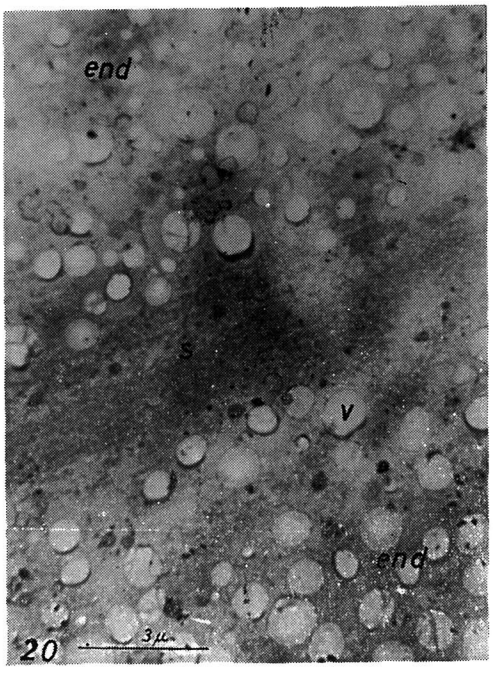

hf
im
om
f
b
bf
15
0.25μ


v
ect
f
lf
1μ
16


v
end
f
ect
17
10μ


hf
ect
sv
end
18
1μ
v


v
g
19
1μ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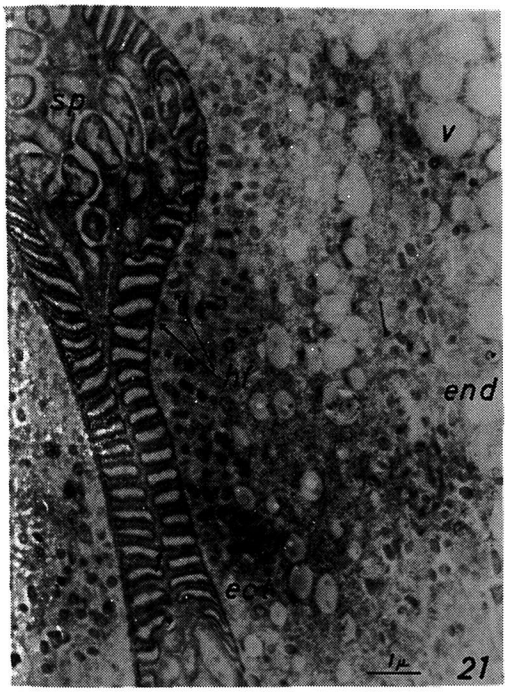
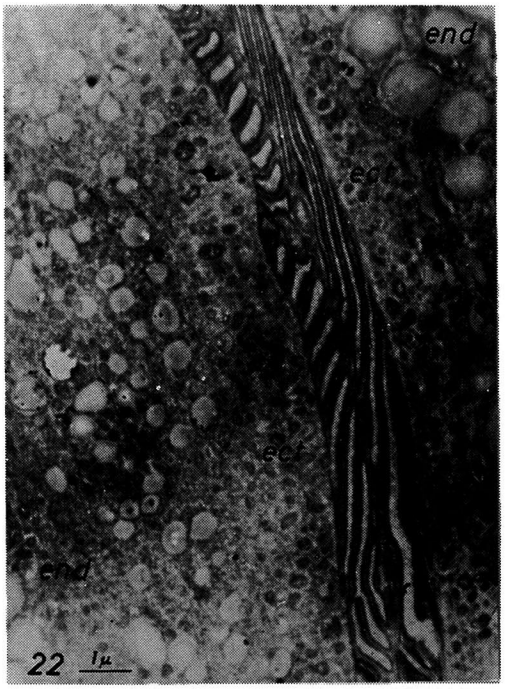
s
v
end
20
3μ


sp
v
end
ect
1μ
21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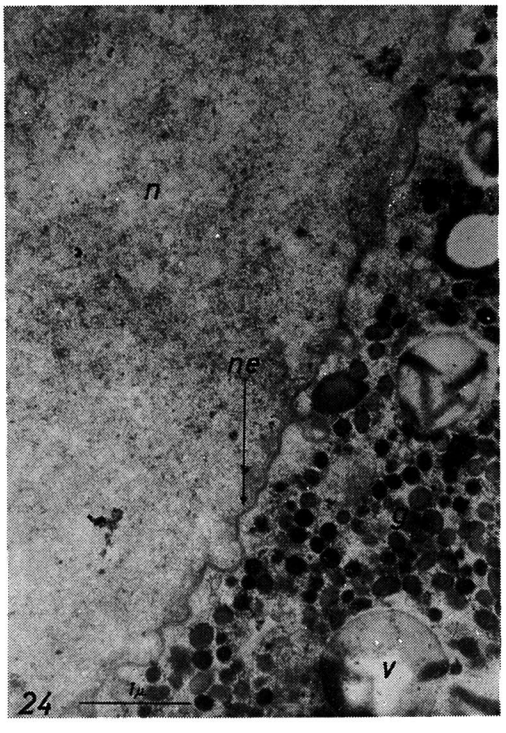
end
22
1μ


nc
ne
n
v
23


n
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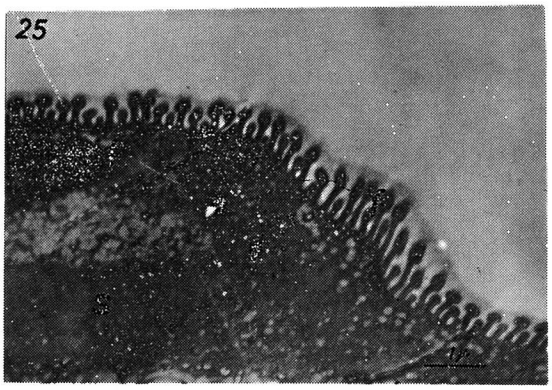
v
24
1μ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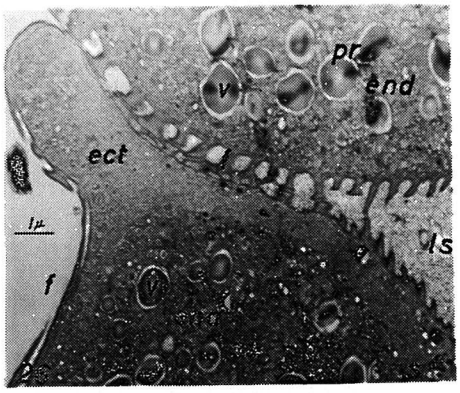
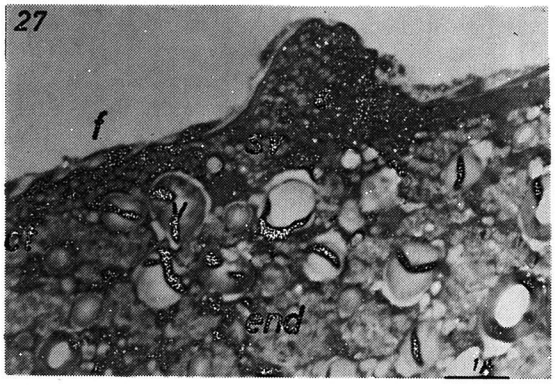
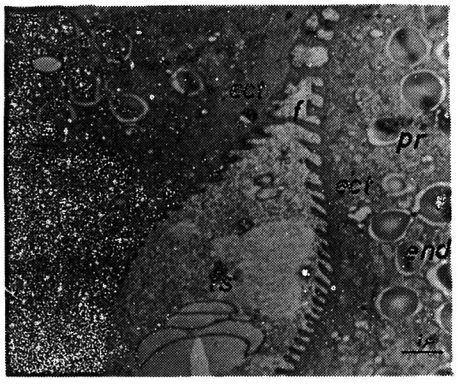

pr
end
v
ect
1μ
ls
f


27
f
v
ect
end
1μ


ect
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pr
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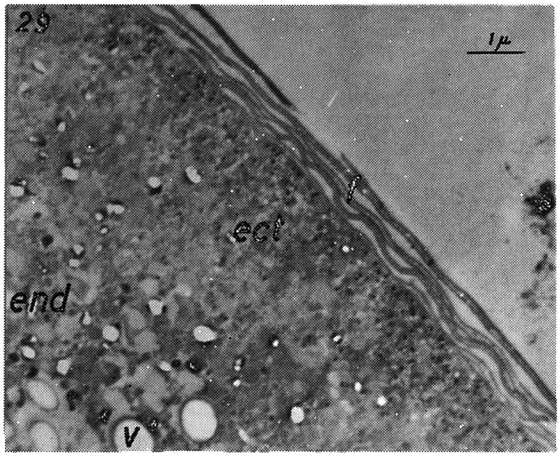
end
ls
1μ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f
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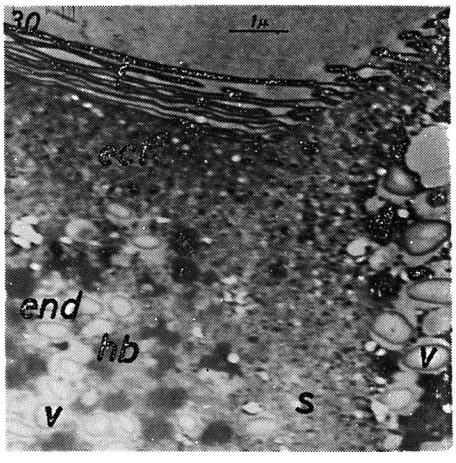
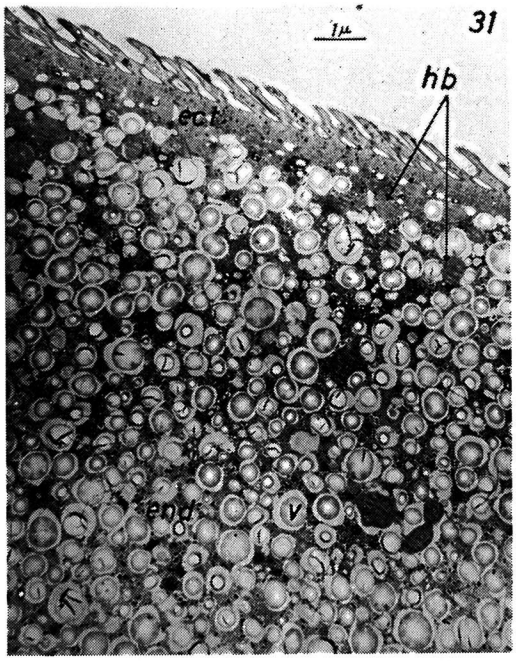
v


30
1μ
ect
end
hb
v
v
s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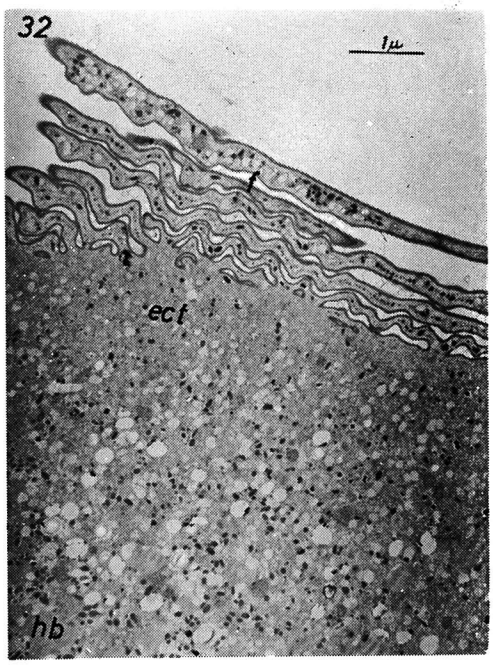
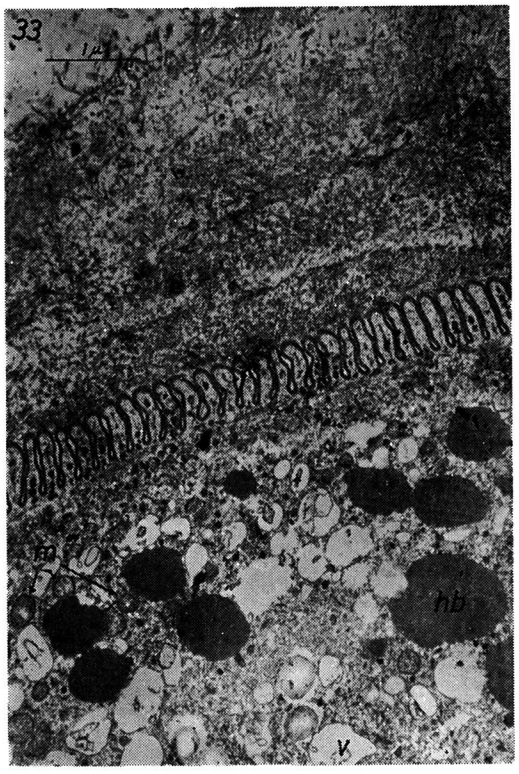
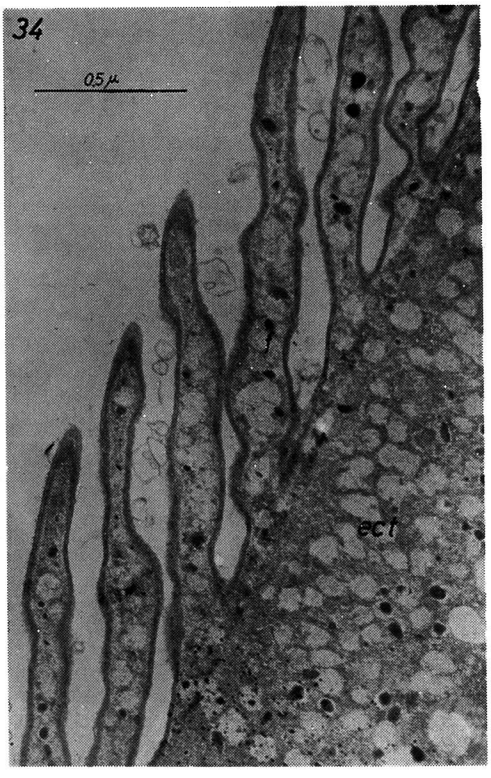
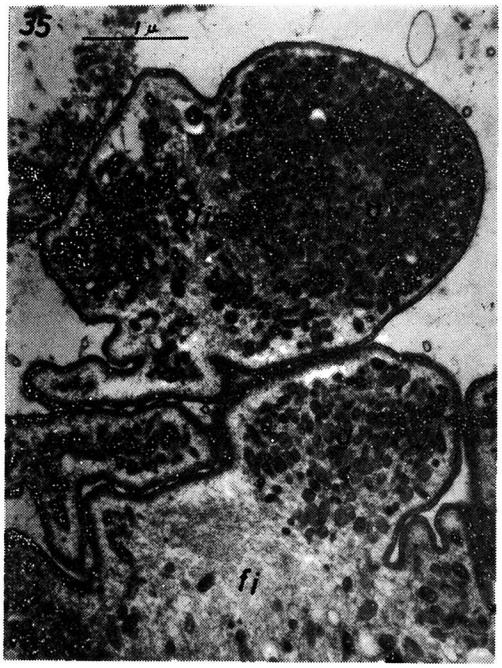
1μ
hb
ect
end
v


32
1μ
ect
hb


33
1μ
hb
v


34
0.5μ
ect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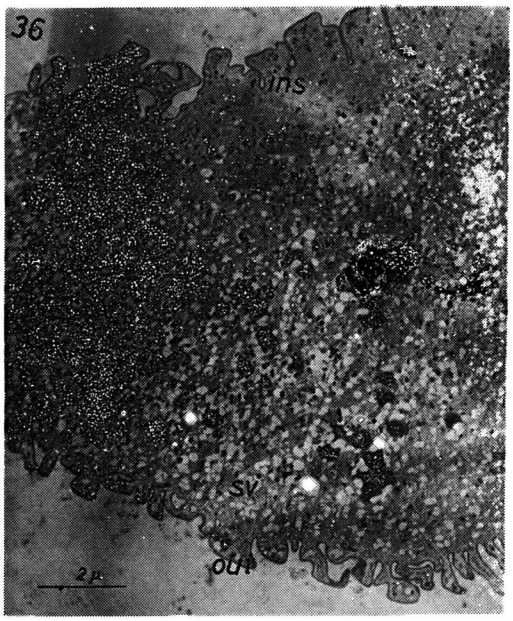
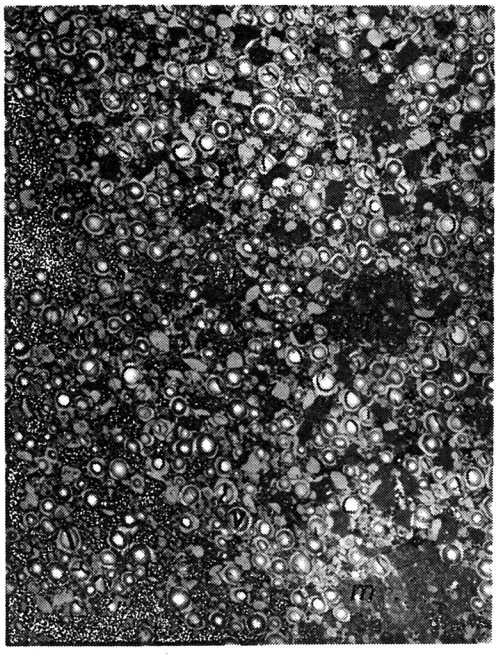
1 μ
fi


36
ins
sv
out
2 μ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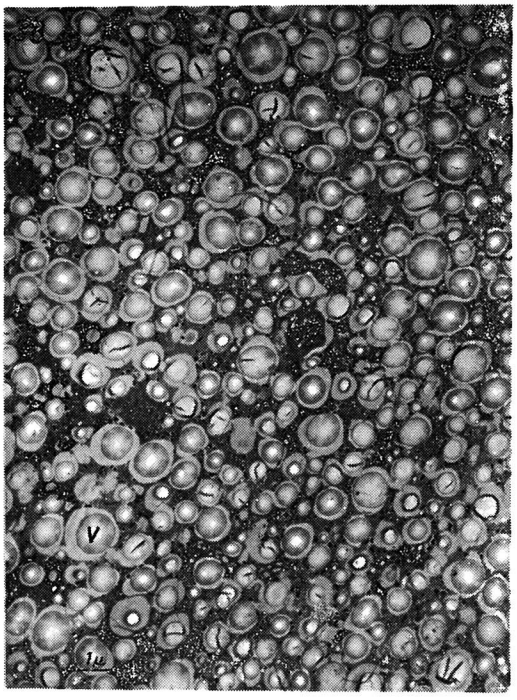
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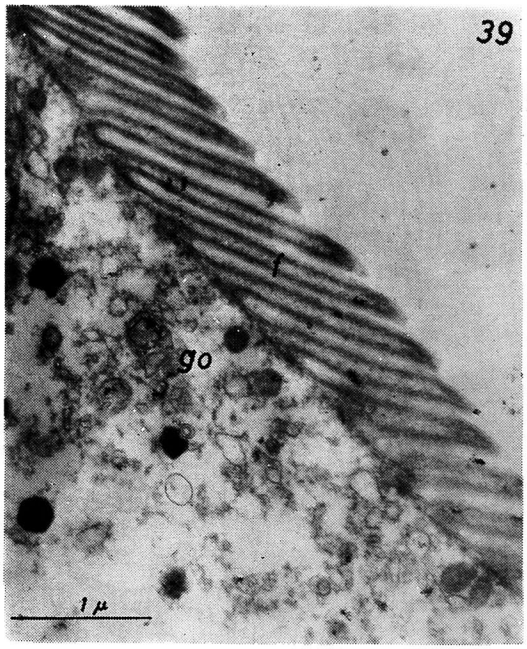
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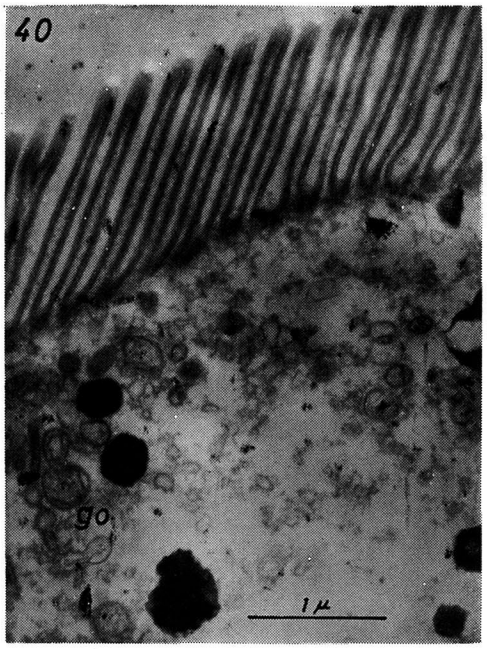
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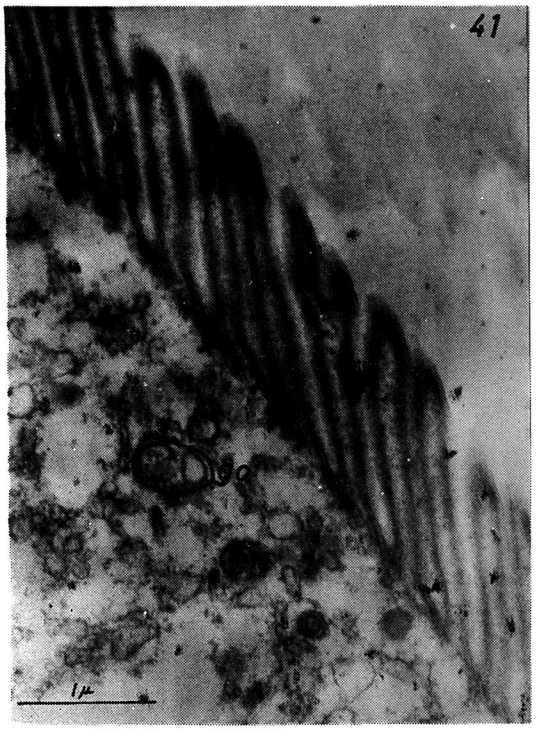
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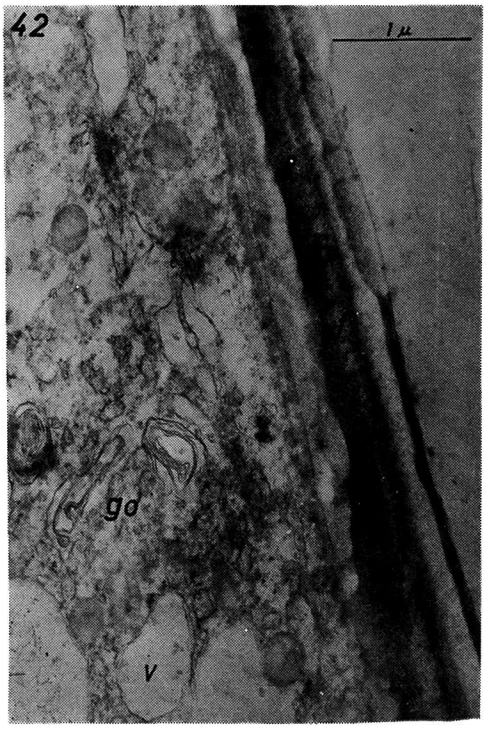
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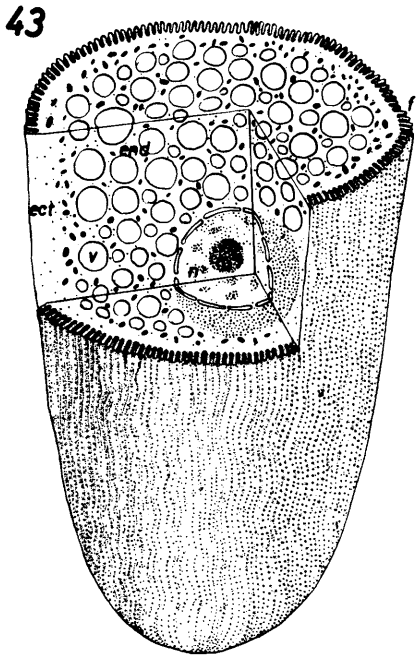
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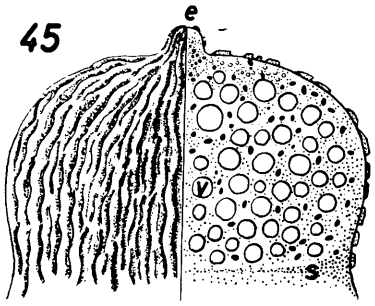
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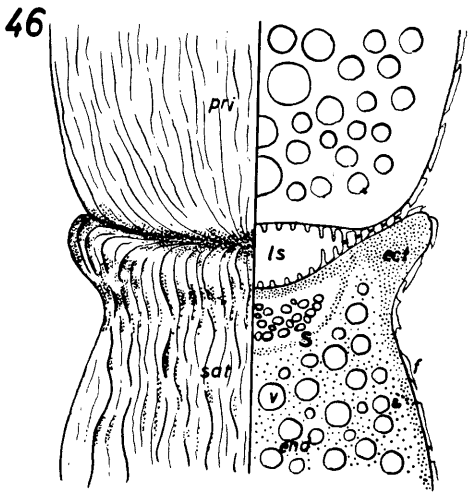
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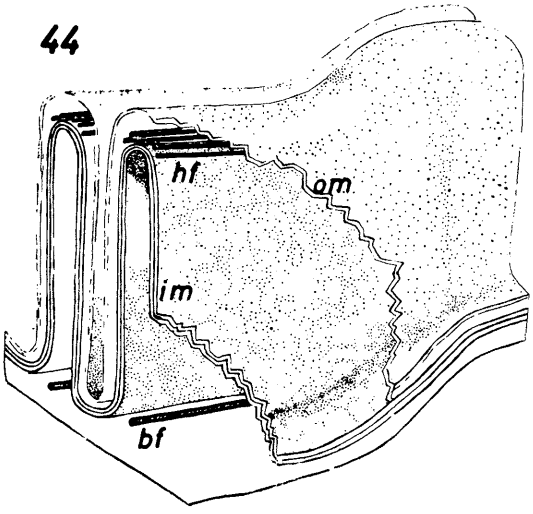
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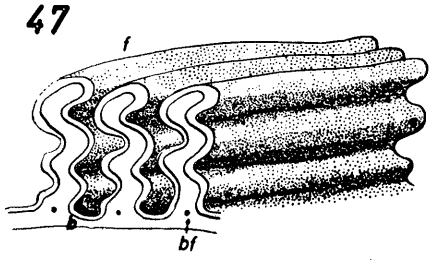
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