Electron Microscopy on the Polyp of Staghorn Corals with Special Reference to its Skeleton Formation*

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The formation of skeletons is one of the most conspicuous characteristics of Scleroactinian corals. Naturally, many scholars have worked on this problem using either classical histological methods, such as Fowler (2), Bourne (1), Krempf (11), Hayashi (4), or modern methods of electron microscope or of isotopes, such as Wainwright (13), Sato (12), and Goreau (3).

In our electron microscopic studies on the Scleroactinian corals we also examined both polyps and skeletons from various kinds of corals. However, the skeleton is too hard to get good results for the elucidation of the skeleton formation. This fact is more pronounced when we use materials from the coasts of the main islands of Japan where the corals grow very slowly. The processes of spicule formation in a Gorgonian coral (5) and Alcyonarian corals (*Heteroxenia* (9) and organ-pipe coral (10)) are observed within scleroblasts which usually originate in the ectodermal cells and are embedded in the mesogloea.

In some rapidly growing corals. calicoblastic cells are found around the growing portion of the skeleton, but seldom in the old or resting portion. This fact suggests that the skeleton is formed by calicoblastic cells which are derived from the ectodermal cells and which accumulate calcareous substances within them, thus turning into a skeleton.

Material and Method

Materials used in this study were three species of staghorn corals, *Acropora formosa*, *Acropora nasuta*, and *Acropora sp.* The first two were collected at Kikaijima, one of the Amami Islands, and the last one came from Shirahama, Wakayama Prefecture. *A. formosa* grows rapidly and forms a large colony. The present sample was collected from a colony growing in a tide pool as is shown in Fig. 1. This species bears a brown color, showing practically no greenish hue. The sample of *A. nasuta* was collected from a small colony of purple color growing on the outer slope of a reef.

Apical portions of the branches of each species were used for the experiment. They were cut off from the colony just after collection. In some cases, however, small

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branches were brought back to the laboratory for further treatment.

For light microscopy, tip portions were fixed either in 7% formalin in sea water or in Bouin's solution. The samples fixed with formalin were immersed in a saturated EDTA (ethylene diamine tetra acetic acid disodium salt) for decalcification for 4 days. The EDTA solution was renewed two or three times during the period. These samples were treated by a usual method and the sections were stained with azin triple staining. For the calcium test of the sections, formalin-fixed samples were used without decalcification. The sections were examined by the von Kossa silver test for calcium.

For electron microscopic observations the apical portion of each branch was cut off and fixed in 5% glutaraldehyde in buffered sea water for 1.5 hours. The samples were fixed again in 1% osmium tetroxide in buffered sea water for 1 hour. They were treated by the routine method, dehydrated and embedded in araldite or epon. In some cases the samples were decalcified by keeping them in a saturated EDTA for 5 days. The sections in araldite were stained with 1% $KMnO_4$ alone or with 1% $KMnO_4$ and lead citrate, while those in Epon 812 were stained with uranyl acetate and lead citrate.

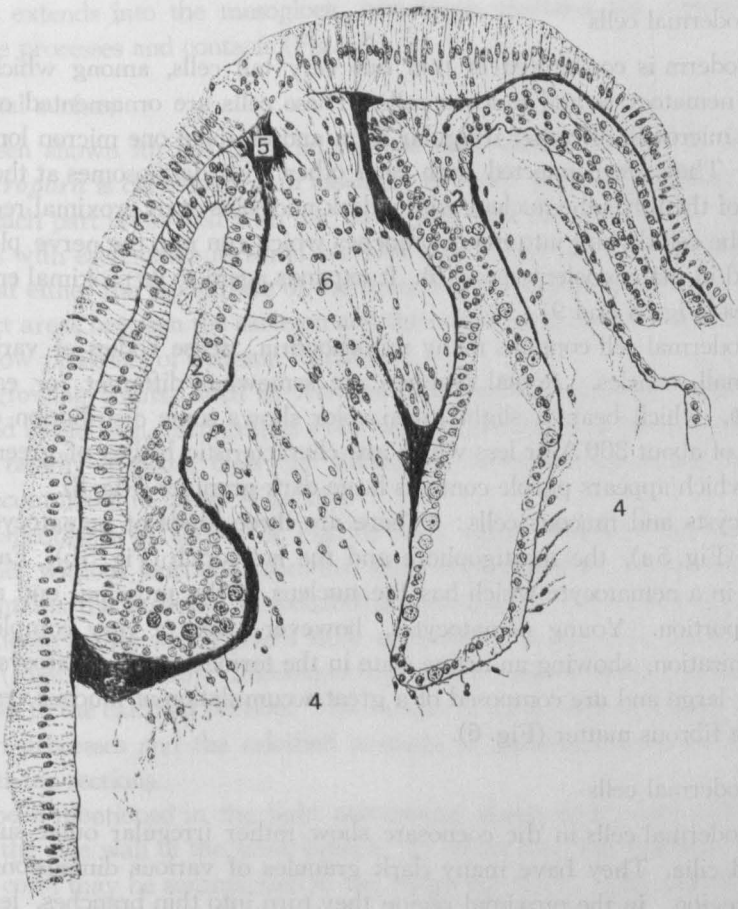
Results

A. Light microscopic observation

Fig. 2 shows part of a cross section of the apical portion of a branch from *Acropora formosa*. The outer layer of both the coenosarc and the polyp is covered with a thin tissue which is composed of the ectoderm and the endoderm connected by the mesogloea in the middle. However, the gastrodermic layers in the middle portion show very complicated forms. Such differences between the polyp-proper and the coenosarc may be due to an extremely contracted state in the former. The skeletal portions which were removed by decalcification and so appear as empty spaces are shaded with dots in Fig. 2 in order to indicate clearly the interrelations between the skeleton and the soft portions.

The skeleton is covered with the mesogloea and the endoderm. In some cases, especially in the surface region of the skeleton, there are some remaining scattered cells. These cells are frequently found abundantly in the growing apical portion of the branch as is shown in Fig. 3 a. A drawing of part of Fig. 3 a is given in Text-Fig. 1. Serial sections of such a portion show a diffuse accumulation of these cells throughout the portion. There is no definite lining of cells along the mesogloea to form an ectodermal layer. That is, the tissue of the skeleton formation is different from that of the mollusks where a single layer of the mantle epithelium faces the shell.

These scattered cells are derived from the ectodermal cells and are destined to accumulate a calcareous matter within them and to turn into the skeleton. They may be called scleroblasts or calicoblasts. Calicoblasts are found in all three species examined. However, they are more abundant in *A. formosa* which grows rapidly and has rather a soft skeleton than in the other two.



Text-Fig. 1. Diagram showing groups of calicoblasts at the skeletal portion of a branch tip from *Acropora formosa*. Drawn from the section of Fig. 3.

Calcium tests for the sections from a similar portion were successful as are shown in Fig. 3 b. Calicoblasts are observed abundantly only in a limited part of the apical portion of the branch, usually within less than 0.5 mm from the apex, even in a rapidly growing species, *Acropora formosa*. They are very scanty or completely absent in the old portion of the skeleton. These circumstances will explain why previous scholars had controversial results concerning the tissues of skeleton formation in reef corals. Calcification processes in the calicoblasts seem to be very rapid.

B. Electron microscopic observation

An electron micrograph of a part of a cross section through the coenosarc in a distal portion of a branch of *Acropora sp.* is given in Fig. 4. The coenosarc is composed of two layers, the outer ectoderm and the inner endoderm with a thin mesogloea between them.

1. Ectodermal cells

The ectoderm is constituted of thin but very tall cells, among which there are many large nematocysts and mucous cells. These cells are ornamented on the outer surface with microvilli of rather irregular form and of about one micron long and have a few cilia. They are connected with each other with desmosomes at their distal regions. Each of them has the nucleus with a dark nucleolus at its proximal region. Below the nucleus the cell divides into many branches which run into the nerve plexus as has been reported in many coelenterates (6). It expands again to its proximal end and faces the mesogloea (Figs. 4 and 9).

The ectodermal cell contains many mitochondria, dense bodies of various dimensions and small vesicles. Actual features are somewhat different for each species. *Acropora sp.* which bears a slight green color shows some distribution of spherical dense bodies of about 300 A or less which are characteristic bodies of green corals (7). *A. nasuta* which appears purple contains large dark granules (Fig. 8).

Nematocysts and mucous cells: There are three kinds of nematocysts, that is, the spirocyst (Fig. 5a), the mastigophore and the holotorich (Fig. 5b). Each nematocyst is found in a nematocyte which has the nucleus at the proximal end and a small cytoplasmic portion. Young nematocytes, however, have a very complicated cytoplasmic organization, showing an active state in the formation of nematocysts. Mucous cells are very large and are composed of a great accumulation of mucous vesicles which contain a thin fibrous matter (Fig. 6).

2. Endodermal cells

The endodermal cells in the coenosarc show rather irregular outer surfaces with microvilli and cilia. They have many dark granules of various dimensions especially in the distal region. In the proximal region they turn into thin branches, leaving large interspaces. They expand again into a thin film lining on the mesogloea. The cilium is usually palisaded with ten long microvilli as is shown in Fig. 7.

Zooxanthellae (Figs. 7 and 11) are found in some of the interspaces between the endodermal cells. They are spherical in shape and are covered partly or completely with an endodermal cell or cells. Each of them has a chain of chloroplasts in its peripheral region, and the nucleus, mitochondria, and pyrenoids in the central portion. The general appearance of the zooxanthellae is the same as that described in the previous papers (8). Zooxanthellae are comparatively few in the endoderm covering the skeleton, especially in the inner ones where the intensity of illumination will be greatly reduced (Fig. 2).

3. Mesogloea

The mesogloea is very thin and sometimes measures only about 1 micron. It is composed of irregular arrangements of thin filaments (Figs. 12—16). The two sides of the mesogloea are covered with the ectoderm and the endoderm. Close to the mesogloea there are small bundles of muscle fibers in the cells of the coenosarc. Here and

there, the cell extends into the mesogloea, sometimes reaching the opposite side to form nerve-like processes and contacts (Fig. 10).

4. Skeletal surface

As has been shown for the light microscope observation in Figs. 2 and 3, the skeleton of *Acropora* is composed of an assembly of small parts of irregular form and dimensions. Each part is covered with the mesogloea and the endoderm. These parts are continuous with each other in serial sections and finally they are in contact with the ectoderm at either the top surface of the branch or the side surface of the coenosarc. In such contact areas between the skeleton and the ectoderm there are some calicoblastic cells which show processes of skeleton formation.

In slow-growing corals, such as *Acropora sp.* and *A. nasuta*, calicoblasts are found scattered in electron micrographs even in the top surface of the branch (Figs. 12 and 13). In rapidly growing ones (*A. formosa*), however, there are many small calicoblasts accumulated in layers (Fig. 16).

Young calicoblasts as shown in Fig. 16 have many branches but are separated from each other without junctional portions, such as desmosomes, between them. Each calicoblast contains the nucleus, mitochondria, endoplasmic reticula, vesicular bodies and electron dense bodies which give a dark appearance to the cell. In a slightly advanced stage (Figs. 14 and 15), calicoblasts turn darker and decrease in number. They are found close to the calcified portion. The sample in these cases was prepared without decalcification processes and the calcified portions or skeletons were removed during the treatments for sections.

As has been mentioned in the light microscope observation, such calicoblasts are not found in the side wall of the same branch. In conclusion, the skeleton formation of the staghorn coral may be summarized in the following way: The calicoblasts which are derived from the ectoderm at the growing surface of the skeleton or branch rapidly accumulate calcareous substances within them. These cells are deposited on the surface of the skeleton, resulting in its growth.

Summary

1. Electron microscopic observations were made on the coenosarc of three species of staghorn corals with a special reference to the skeleton formation.

2. The skeleton is composed of small portions which are enclosed with the mesogloea and the endoderm. These portions are connected with each other to form a porous skeleton. The outer boundaries are growing points where calicoblasts are found.

3. Calicoblasts are derived from the ectoderm. They accumulate calcareous matter within them and turn into calcareous particles which are deposited on the skeleton, resulting in its growth.

References

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Abbreviations in Figures

1. ectoderm
2. endoderm
3. tentacle
4. skeletal portion
5. mesogloea
6. scleroblasts
7. damaged portion

A, microvilli
B, cilia
CAL, calicoblast
ECT, ectoderm
END, endoderm
G, mucous cell
MES, mesoderm
N, nucleus
Nh, nematocyst, holotorich
Nm, nematocyst, mastigophore
Ns, nematocyst, spirocyst
NT, nematocyst
P, nerve plexus
Z, zooxanthella

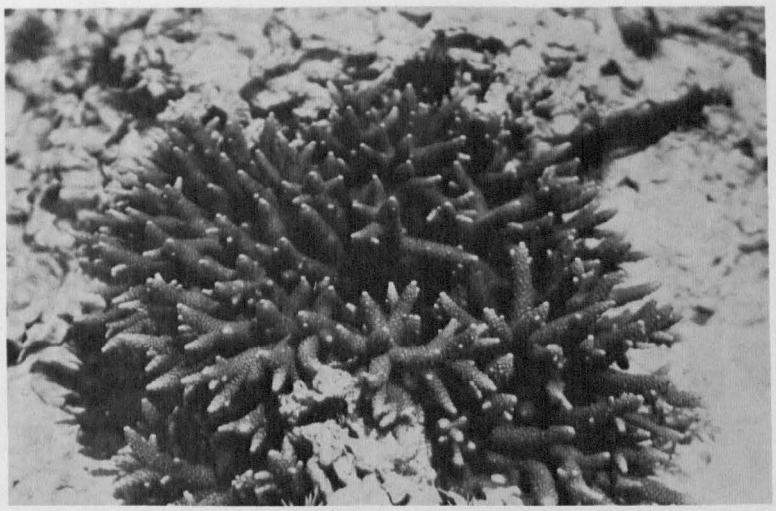

Fig. 1. *Acropora formosa* growing in a tide pool at Isago, Kikaijima. Apical portions of branches appear rather pale brown.

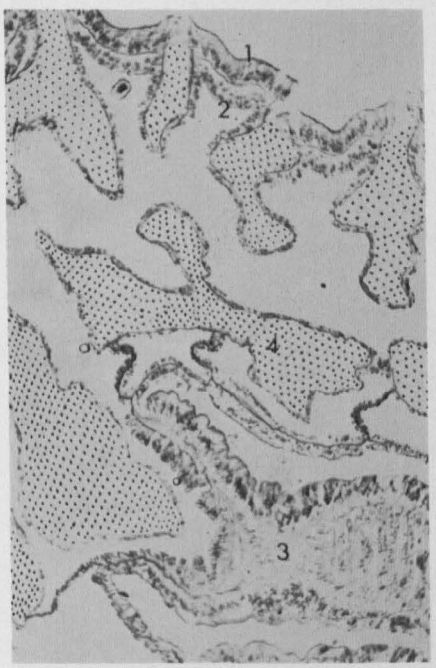

Fig. 2. Photomicrograph of a part of a cross section through the apical portion of a staghorn coral, *Acropora formosa*, after decalcification. Skeletal portions are shaded with dots in order to show interrelations between soft tissues and the skeletons.

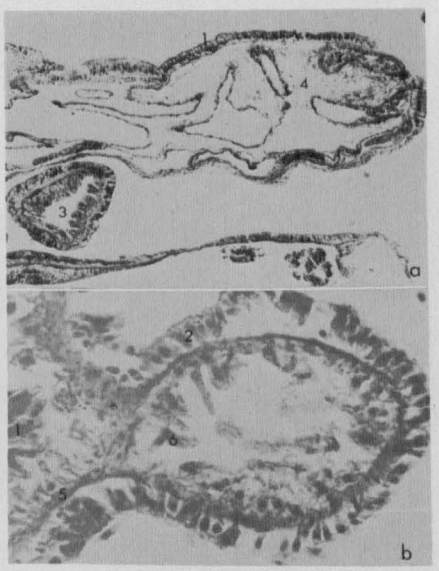

Fig. 3 a. Part of a longitudinal section of the tip of a branch of *A. formosa*.
Fig. 3 b. A similar portion indicating the presence of calcium in the calicoblasts by von Kossa method.

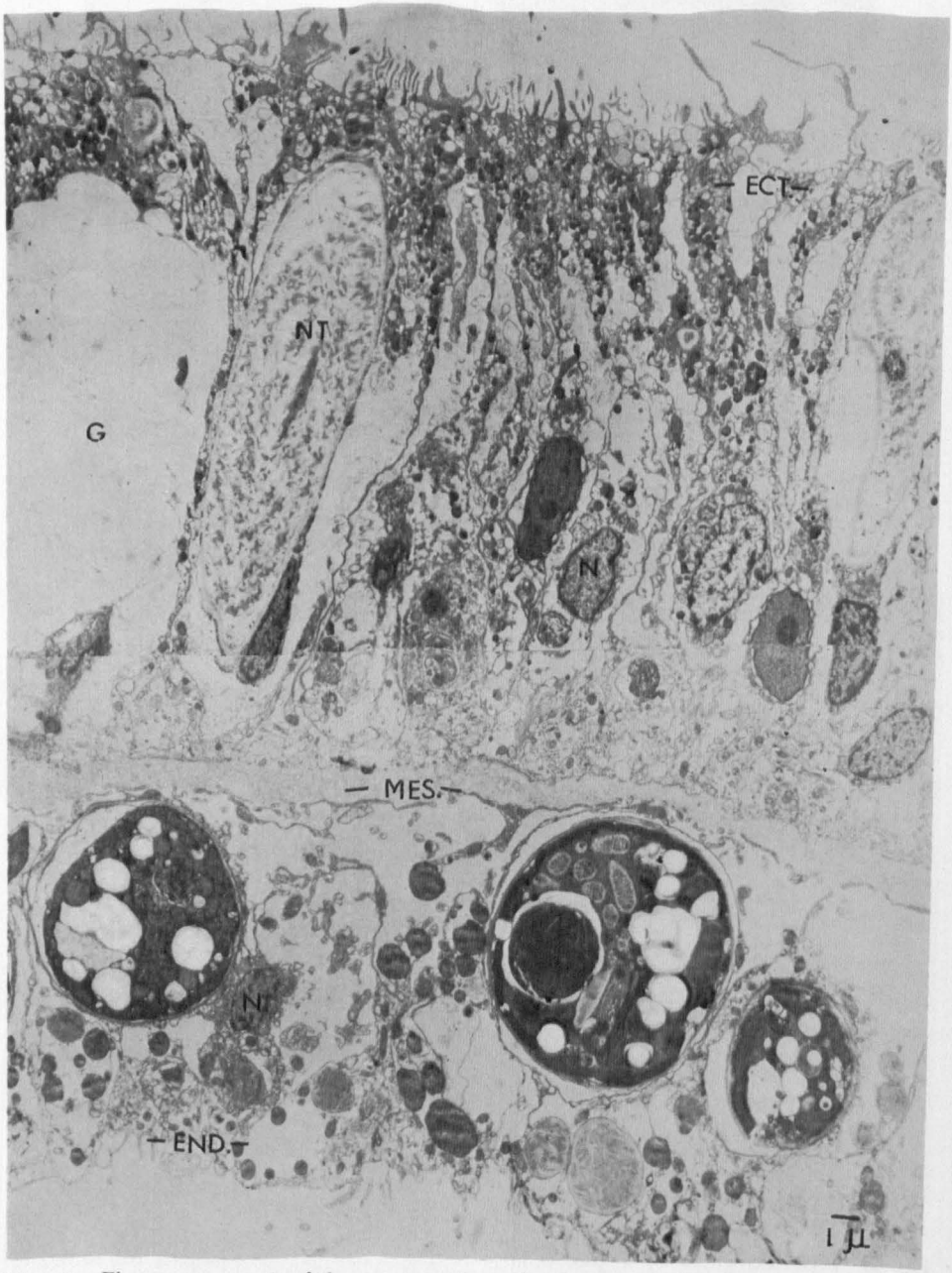

Fig. 4. Electronmicrograph of the coenosarc from the tip portion of a branch of *Acropora sp.* × 4000

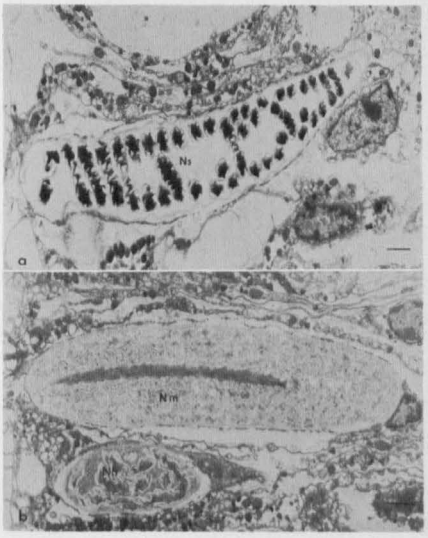

Fig. 5. Longitudinal cuts through nematocysts in the ectoderm of *Acropora sp.*
a. spirocyst × 3000
b. mastigophore × 3000

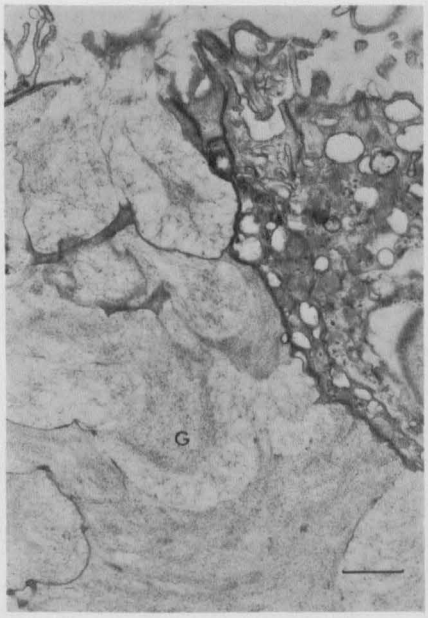

Fig. 6. Part of the mucous cell in the ectoderm discharging mucus through the apical pore (*Acropora sp.*). × 8000

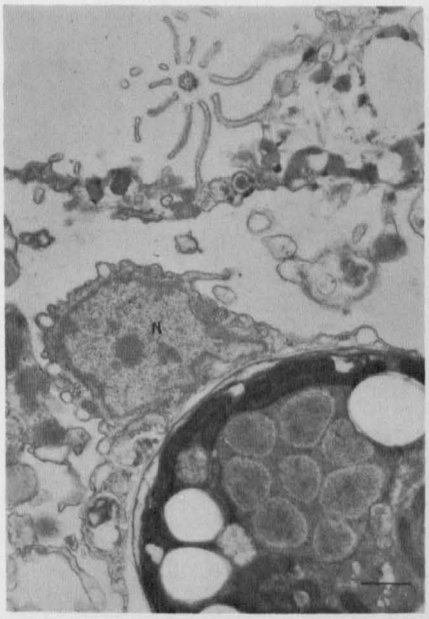

Fig. 7. Part of the distal region of the endodermal cell of *Acropora sp.* Note a cross section of a cilium palisaded with 10 microvilli on the outer surface × 6500

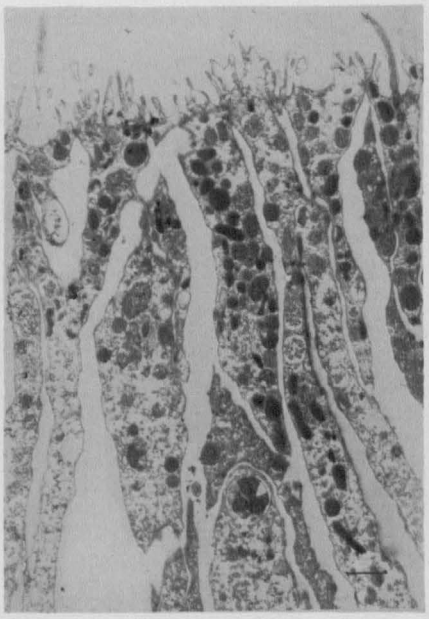

Fig. 8. Part of the surface region of the ectoderm from *A. nasuta.* × 5400

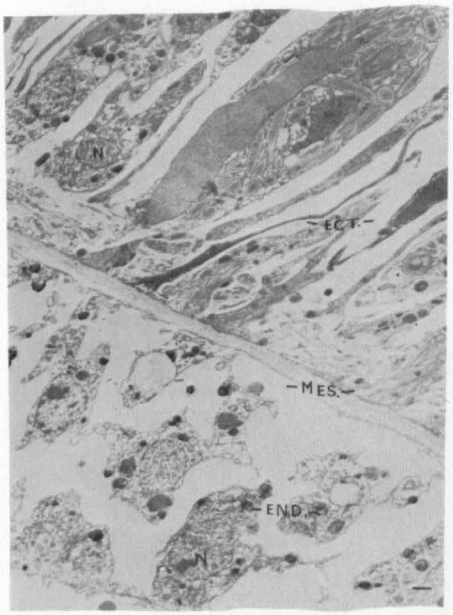

Fig. 9. Electron micrograph of the proximal portion of the ectoderm and the endoderm of the coenosarc of *Acropora nasuta*. × 2500

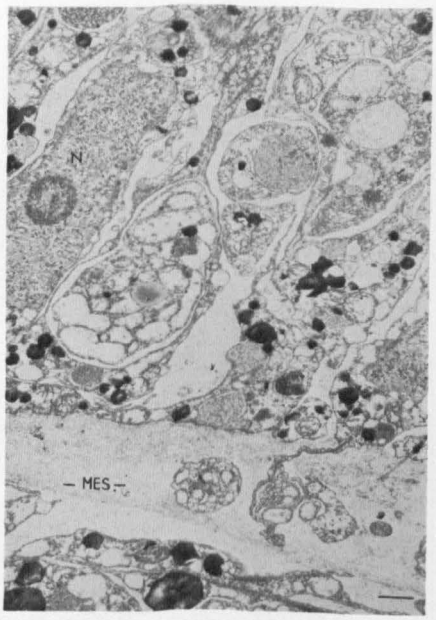

Fig. 10. Part of the proximal regions of the ectodermal cells with nervous processes in the mesogloea (*A. formosa*). × 4600

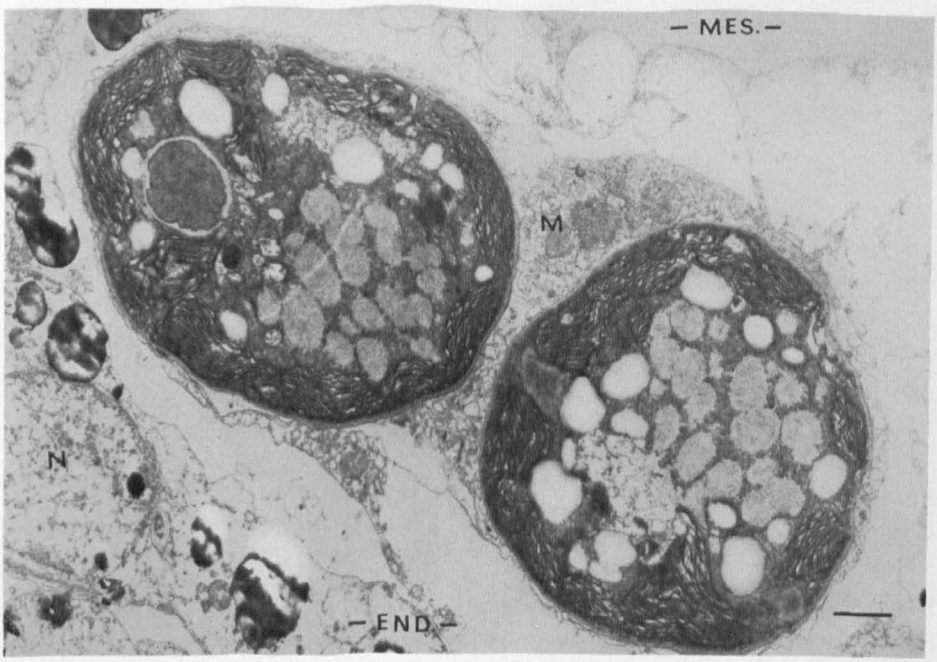

Fig. 11. Zooxanthellae in the endoderm of the coenosarc from *A. formosa* fixed with osmium. × 8000

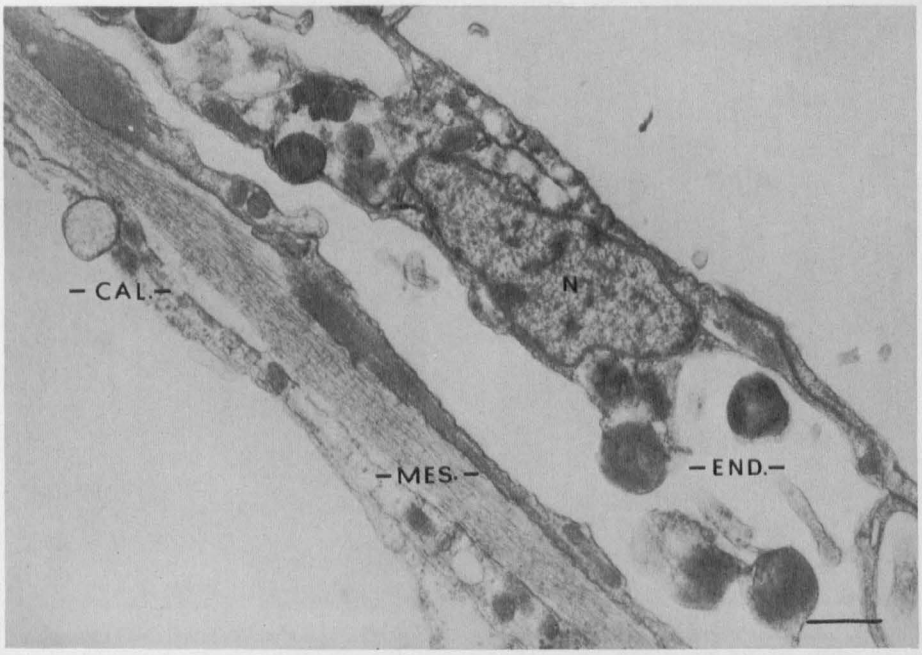

Fig. 12. Part of the endoderm and calicoblasts on the skeletal surface from *Acropora sp.* The sample was decalcified by EDTA before dehydration. × 10000

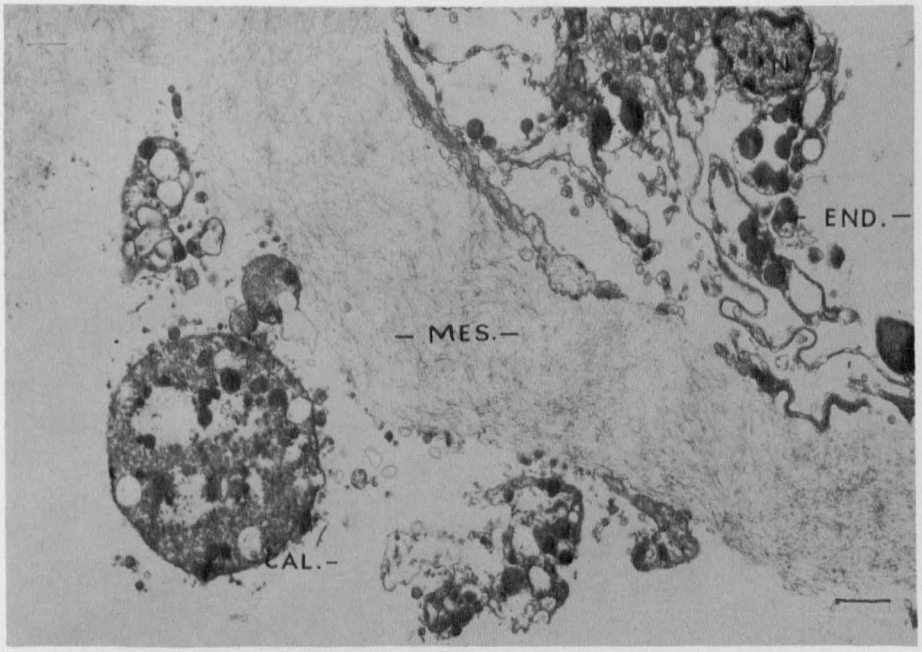

Fig. 13. Another view of calicoblasts from the surface region of the skeleton of *Acropora sp.* and an inner endoderm with the mesogloea. × 7000

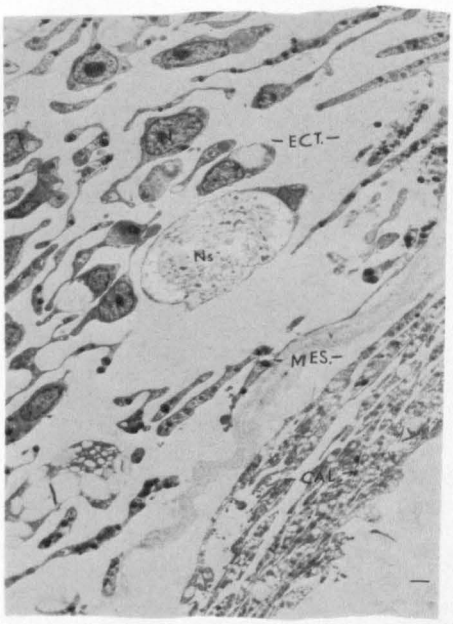

Fig. 14. Electron micrograph of the cortical region of the branch fixed with glutaraldehyde and osmium showing an accumulation of calicoblasts and the remainder of the skeleton at the right bottom corner (*A. formosa*). × 2500

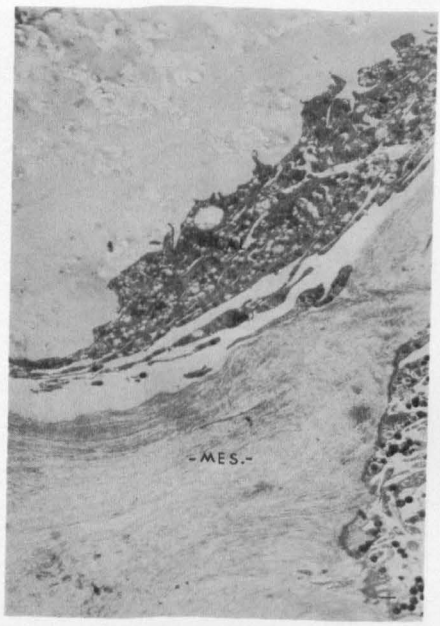

Fig. 15. Another example of calicoblasts and the remainder of the skeleton from *A. formosa*. × 2500

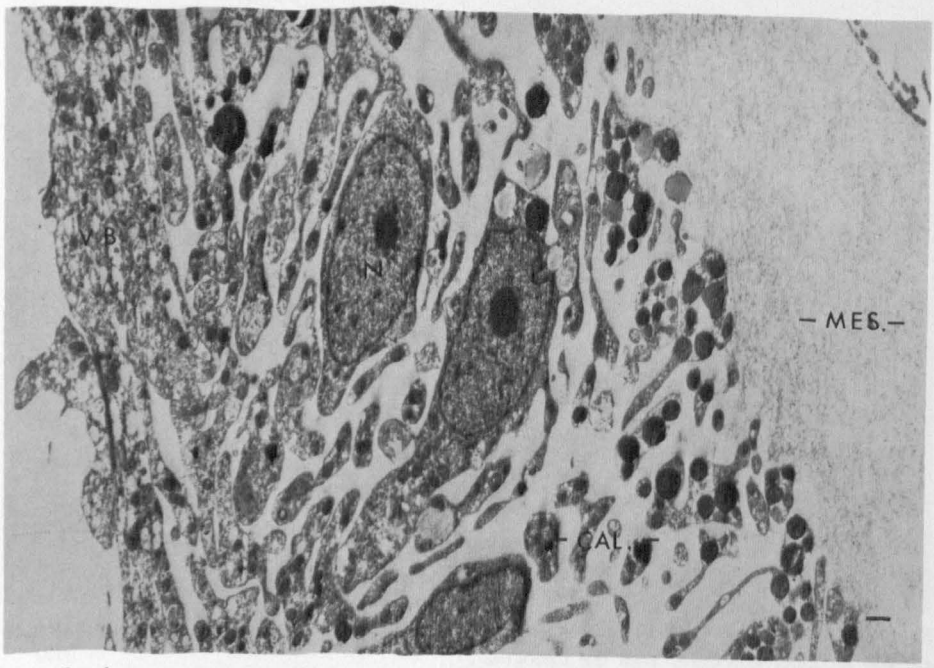

Fig. 16. A layer of young calicoblasts from *A. formosa* fixed with glutaraldehyde and osmium. × 3000