INFORMATION TO USERS

This material was produced from a microfilm copy of the original document. While the most advanced technological means to photograph and reproduce this document have been used, the quality is heavily dependent upon the quality of the original submitted.

The following explanation of techniques is provided to help you understand markings or patterns which may appear on this reproduction.

1. The sign or "target" for pages apparently lacking from the document photographed is "Missing Page(s)". If it was possible to obtain the missing page(s) or section, they are spliced into the film along with adjacent pages. This may have necessitated cutting thru an image and duplicating adjacent pages to insure you complete continuity.

2. When an image on the film is obliterated with a large round black mark, it is an indication that the photographer suspected that the copy may have moved during exposure and thus cause a blurred image. You will find a good image of the page in the adjacent frame.

3. When a map, drawing or chart, etc., was part of the material being photographed the photographer followed a definite method in "sectioning" the material. It is customary to begin photoing at the upper left hand corner of a large sheet and to continue photoing from left to right in equal sections with a small overlap. If necessary, sectioning is continued again – beginning below the first row and continuing on until complete.

4. The majority of users indicate that the textual content is of greatest value, however, a somewhat higher quality reproduction could be made from "photographs" if essential to the understanding of the dissertation. Silver prints of "photographs" may be ordered at additional charge by writing the Order Department, giving the catalog number, title, author and specific pages you wish reproduced.

5. PLEASE NOTE: Some pages may have indistinct print. Filmed as received.

Xerox University Microfilms

300 North Zeeb Road
Ann Arbor, Michigan 48106

73-20,161

ZUCKER, William H., 1943-
FINE STRUCTURE OF PLANKTONIC FORAMINIFERA
AND THEIR ENDOSYMBIOTIC ALGAE.

The City University of New York, Ph.D., 1973
Biology

University Microfilms, A XEROX Company, Ann Arbor, Michigan

© COPYRIGHT BY

WILLIAM H. ZUCKER

1973

FINE STRUCTURE OF PLANKTONIC FORAMINIFERA AND THEIR ENDOSYMBIOTIC ALGAE

by

WILLIAM H. ZUCKER

A dissertation submitted to the Graduate Faculty in Biology in partial fulfillment of the requirements for the degree of Doctor of Philosophy, The City University of New York

1973

This manuscript has been read and accepted for the Executive Committee in Biology in satisfaction of the dissertation requirement for the degree of Doctor of Philosophy.

February 16, 1973 Date	Chairman of Examining Committee Prof. J. Lee
February 20, 1973 Date	Executive Officer Prof. L.G. Moriber
Prof. L. Crockett	City College Institution
Prof. J. Tietjen	City College Institution
Prof. S. Goldstein	Brooklyn College Institution
Prof. F. Schuster	Brooklyn College Institution
Prof. A. Bé	Lamont-Doherty Geological Observatory Institution
Prof. R. Anderson	Lamont-Doherty Geological Observatory Institution
Prof. K. Pokorny	Osborne Laboratories of Marine Sciences Institution
Prof. A. Bennett	Brookhaven National Laboratory Institution

ACKNOWLEDGEMENTS

I wish to thank Professor J. J. Lee for his advice and assistance. I also thank Professors F. L. Schuster, L. J. Crockett, J. H. Tietjen, S. Goldstein, A. W. H. Bé, O. R. Anderson, and T. Saito. Electron microscope facilities were provided by Professor A. McIntyre at Lamont-Dougherty Geological Observatory. I am also grateful to the Captain and crew of the R/V Panulirus II. This research was supported by U.S. Atomic Energy Commission Contract number AT(30-1) 3995; reference number NYO 3995-22 (J. J. Lee) and National Science Foundation Grant number GB-1205 (A. W. H. Bé).

TABLE OF CONTENTS

ACKNOWLEDGEMENTS iv

INTRODUCTION 1

MATERIALS AND METHODS 6

OBSERVATIONS 8

I. PLANKTONIC FORAMINIFERA 8

Test Microstructure
Fibrillar System
Vacuoles
Nuclei
Reticulopodia
Mitochondria
Vacuolar System
Fibrous Bodies

II. SYMBIOTIC ALGAE 16

General Description
Nucleus
Chloroplasts and Associated Structures
Other Reserve Bodies
Mitochondria
Flagellar Apparatus
Other Organelles
Host - Symbiont Interface

DISCUSSION 22

I. PLANKTONIC FORAMINIFERA 22

Nuclei
Pores and Associated Structures
Exothalamous Protoplasm (Ectoplasm)
Reticulopodia
Fibrillar System

II. SYMBIOTIC ALGAE 40

Diagnosis

BIBLIOGRAPHY 48

LIST OF FIGURES 53

FIGURES 58

INTRODUCTION

Planktonic members of the sarcodine order Foraminiferida are widely-distributed and abundant in the zooplankton. They are major contributors to marine sediments, especially in the deep sea, where the rate of inorganic deposition is slow (Phleger, 1960). They are significant as indicators of water masses (Bé, 1959 a; Jones, 1969), and their coiling patterns provide evidence of events which took place during Pleistocene glaciation (Ericson, 1959). Independent of coiling, oxygen isotope ratios have also been used to determine the age and paleotemperature (at time of $CaCO_3$ deposition) of deep-sea cores containing foraminifera (Emiliani, 1954).

Until recently, few studies of living forms have been undertaken so that our knowledge of the biology of this enormous protozoan group has been neglected. Life cycles of planktonic foraminifera are unknown, and little is known of their cytology or physiology.

The cytological studies of Rhumbler (1909) and Lee *et al*. (1965) constitute all that we know at the light microscope level. In both studies, two unusual organelles were found which were present in no other protozoan group. Large undifferentiated structures called cryptosomes (Rhumbler's *Kern*) and a fibrillar system (called *Gallertsstrange* by Rhumbler) are peculiar to planktonic foraminifera. The latter consists of 3-4μm thick tubules ending in many loops, and may aid in flotation. In addition to these organelles, Lee *et al*. found

"multinuclear inclusions," which probably arise from nuclear division but do not resemble the nucleus of either the host or its zooxanthellae.

Two recent studies of planktonic foraminiferan ultrastructure contribute to our knowledge of these organisms. Febvre-Chevalier (1971) studied the large somatic nucleus, microbodies, pore structure, and reticulopods. The fibrillar system and cryptosomes were not reported. Towe (1971) dealt mainly with pore and test construction and their taxonomic value.

There have been some cytological and ultrastructural studies of shallow water forms. However, none of the organelles peculiar to planktonic foraminifera has yet been found in the small number of littoral benthic species observed. Generally, our knowledge of foraminiferan cytology is incomplete at all levels.

In this study, the fine structure of Globigerinoides ruber will be investigated to further our understanding of these planktonics. The fibrillar system and other unique structures will be compared with those found in the recent cytological study (Lee et al., 1965).

Nothing is known about the physiology of planktonic foraminifera, primarily because they are difficult to keep alive in the laboratory for long periods of time. No species has been kept in continuously reproducing culture for over a year. One of the most important questions to answer is the problem of flotation. Spines increase the surface area but are not present in all species. Bé and Ericson (1963) found that specimens from deeper water (below 500m) have thicker tests, due to terminal deposition of a calcium crust. They suggest that crust formation occurs in response to some unknown physical stimulus at these depths.

Bradshaw (1959) states that oxygen produced by symbiotic algae during the day increases the host's buoyancy. This theory has not been experimentally substantiated, and besides, not all planktonic foraminifera have symbionts. At present, there is no substantiated theory of foraminiferan flotation.

The closely-related Radiolaria have a frothy, gelatinous extracapsular structure called the calymma, which is primarily hydrostatic in function. Brandt (1895) found that the vertical movement of _Thalassicolla_ was due to buildup and degeneration of the numerous liquid-filled calymma vacuoles. He claimed that in order to rise, the liquid (probably sea water) would have to be saturated with carbonic acid. This would insure the proper specific gravity for flotation. Rough waves or extreme temperatures cause extended reticulopodia to withdraw, and this stimulus causes the calymma to collapse. Vacuoles burst and the organism descends to calmer depths, where a newly-vacuolated calymma can be formed.

The closely-related Acantharia also have a gelatinous layer, but it is more peripheral than in the Radiolaria, and is not included in the ectoplasm. In this group there is a unique ectoplasmic structure involved in flotation. It consists of cone-like arrangements of myofrisks, which are short contractile bands linking the rest of the cytoplasm with the spicules. When contracted, they cause expansion of the extracapsular layer. This promotes buoyancy by increasing cell volume while maintaining a constant cell weight. Sinking results from myofrisk relaxation and the resulting cell volume decrease (Schewiakoff, 1926). Pseudopods and spines are also involved in flotation in these plankters, since they increase surface area.

The fibrillar system is a good starting-point for the study of flotation mechanisms of planktonic foraminifera. These heavy-shelled organisms have neither myofrisks nor calymma, and some do not have spines. The existing possibilities point to some internal mechanism, particularly the well-developed fibrillar system.

Many Radiolaria and Acantharia, as well as some foraminifera, contain algae. This unique type of symbiosis has been studied in many invertebrates, but little is known about the relationship in foraminifera. Algal symbionts are involved in coralline calcification (Goreau, 1960), and evidence for nutrient transfer between symbiont and host has been demonstrated in tridacnid and other invertebrate symbioses (Muscatine, 1967; Muscatine et al., 1967; Provasoli et al., 1968; Trench et al., 1970). Few foraminifera have been studied. They include the littoral benthics Peneroplis pertusus (Winter, 1907), Orbitolites duplex (Doyle and Doyle, 1940), and Archais angulatus (Lee and Zucker, 1969). These studies have nevertheless provided us with vital details of marine calcification. In both Orbitolites and Archais symbiotic enhancement of host calcification was demonstrated.

The Archais algae also provide an auxiliary carbon source. The zooxznthellae of Globigerinoides ruber may play similar roles in the host's nutrition and calcification processes. Some aspects of the symbiont-host relationship can only be seen at the fine structural level. These include the state of the algae within the host, condition of foraminiferan protoplasm, and structure of the host-symbiont interface. They are all vital to our understanding of endozoic algal symbioses.

Only a few endozoic algae have been identified, and little is known about the *Globigerinoides* algae. They are believed to be similar but not identical to *Symbiodinium microadriaticum* (Lee *et al.*, 1965). Observations of motile stages just released from a crushed host have confirmed this (Lee and Freudenthal, unpubl.). The symbionts have been tentatively assigned to the Dinophyta.

It is important to compare these with other dinoflagellate symbionts studied at the electron microscope level, such as *Symbiodinium microadriaticum* (Kevin *et al.*, 1969). A detailed fine structure study of the foraminiferan symbiont will also help to qualify Taylor's (1968) theory that one widespread marine species is involved in all dinoflagellate symbioses.

In summary, the study of planktonic foraminiferan ultrastructure will accomplish several things. The small endosymbionts will be more thoroughly identified and the fine structure of the cryptosomes and fibrillar system will be clarified. Symbiont-host interaction will also be studied at the electron microscope level.

MATERIALS AND METHODS

Planktonic foraminifera were collected at 32° 10' N., 64° 30' W. in the North Atlantic, on Bermuda Biological Station Research Vessel Panulirus cruise Pan 69-301, July 16, 1969. A one meter diameter 200μm mesh plankton net was used in vertical 0-200m tows. The sample from each of nine tows was allowed to stand about ten minutes, permitting most foraminifera to sink to the bottom. Larger crustacea and other organisms were then poured off. The foraminifera-rich plankton sample was added, with as little water as possible, to a cold (4 C) 6% glutaraldehyde solution. The organisms were fixed for two hours, followed by post-fixation in Palade's fluid. Both fixatives were at pH 7.2. Cold solutions were used through the 95% ethanol treatment. The samples were then rinsed in cacodylate buffer, which was also used in the fixatives and uranyl stain. This buffer was found to be successful in preliminary studies of planktonic foraminiferan fixation.

Specimens were stained with a saturated aqueous uranyl acetate solution at pH 4.9 for one hour. Some decalcification probably occurred during exposure to this acidic solution, but electron-translucent spaces in the test region were primarily due to removal of non-decalcified material during ultramicrotomy. After the uranyl acetate staining solution, they were placed in a 50% ethanol bath for ten minutes, followed by two ten-minute changes of 75% ethanol. They

were brought back to the laboratory in a third 75% ethanol solution in stoppered vials. The foraminifera were picked clean and identified in this solution, and further dehydrated in 95% and 100% ethanol. They were allowed to come to room temperature in the 95% solution, and subsequent steps were at ambient temperature. These included three absolute ethanol changes, a short (five minutes) 1:1 absolute ethanol/propylene oxide treatment, fifteen minutes in propylene oxide, and one hour in another change of propylene oxide. They were then left overnight in 1:1 propylene oxide/Epon 812 with 2, 4, 6-dimethylaminomethylphenol (DMP-30), followed by one and one half hours in Epon 812 with DMP-30, and curing for forty-eight hours at 60 C in BEEM capsules. One individual was embedded in each capsule.

The Epon A mixture consisted of 94 gm dodecenyl succinic anhydride (DDSA) and 80 gm Epon 812, while mixture B contained 78 gm nadic methyl anhydride (NMA) plus 100 gm Epon 812. Hard Epon blocks were obtained with a ratio of 1A:2B. The final mixture plus 1.4% DMP-30 was stirred for ten minutes with a mechanical stirrer. The Epon 812 used had a weight per epoxide equivalent of 159.

Blocks were sectioned with a diamond knife on a Sorvall MT-2 ultramicrotome, and picked up on collodion-coated 200 mesh copper grids having a thin carbon layer. The sections were then stained with lead citrate (Reynolds, 1963) and viewed in a Philips EM 200 electron microscope at 60 kv.

OBSERVATIONS

I. PLANKTONIC FORAMINIFERA

Several species of planktonic foraminifera were collected. These included _Globigerinoides ruber_, _Globigerina bulloides_, _Globigerinoides conglobatus_, and _Globigerinita glutinata_. Except as indicated otherwise, observations refer to specimens of _Globigerinoides ruber_, the most extensively studied species.

Test Microstructure

In _Globigerinoides ruber_ the test measured 2 - 3μm between chambers and at least 12μm between the endoplasm and the exothalamous protoplasm (Figs. 1 and 4). The interchamber shell consisted of up to four calcite layers (Fig. 2) originally deposited on membrane-like strands. Up to six layers were found between the endoplasm and exothalamous protoplasm. The most distal layers were elongated and columnar in shape (Fig. 1). Fewer layers (sometimes one) were found next to foramina (Fig. 5). Protoplasm was continuous between chambers via these passageways.

Calcite test material had originally occupied the electron-transparent areas of my specimens (Figs. 1 and 2). Decalcification was accomplished during uranyl acetate staining. The dark, membrane-like strands (e.g., in between chambers) were organic centers of

calcification (Angell, 1967a). Membranes were easiest to see during early stages of chamber formation, when minimal calcification was evident.

All calcification layers were continuous, and showed similar morphology and arrangement in both G. ruber and G. bulloides. Internally, the test was separated from the endoplasm by a deeply-staining inner calcification layer (Figs. 3, 5, and 31). Beneath this layer there occasionally was a layer-like arrangement of granular material (Fig. 31). Internal to this was the cell membrane bordering the endoplasm. The more peripheral endoplasmic organelles (i.e., those closest to the test) included fibrous bodies (Fig. 3), sea water vacuoles (Fig. 1), and mitochondria (Fig. 31). The latter were often found near pores. Externally, the test was bordered by a similar outer calcification layer (Fig. 31), followed by the exothalamous protoplasm (ectoplasm) (Fig. 6). Small portions of membrane-bound protoplasm were found near either side of this most external layer (Fig. 6). These probably originated as folds of neighboring areas of this layer.

Thin portions of exothalamous protoplasm were bounded on either side by unit membranes. The distal and proximal membranes each measured about 7 nm wide. They were often only 35 - 65nm apart, separated by granular groundplasm. The distal membrane had a fringe-like antennular glycocalyx (extracellular coat; Bennett, 1969). Its strands (Fig. 7) extended into the surrounding sea water environment and ranged in length from 25 - 50nm. The strands measured about 17nm wide and were often spaced about 20nm apart along the membrane.

The portions of membranes which bounded protoplasmic islands had few or no glycocalyx antennulae (Fig. 6). These masses of protoplasm were bordered by the same unit membranes as the rest of the

exothalamous protoplasm. Protoplasmic islands were found in all pores (Fig. 1, arrows; Figs. 4 and 31), and contained a variety of organelles. These included membrane-bound vesicles, fibrous bodies, and mitochondria, in a granular groundplasm.

Pores were conical depressions in the exterior surface of the test (Figs. 8, 9, 31, and 60). _Globigerinoides_ _ruber_ and _Globigerina bulloides_ had similar pore morphology. Toward the pore base there was a seive-like diaphragm. At the diaphragm level, pores were constricted. The pore base was shorter than the more distal conical portion, and the sides of the base gradually flared out to approach the cell membrane. The perforated diaphragm was composed of deeply-staining organic material. Between the cell membrane and the pore diaphragm were portions of membrane-bound protoplasm which contained oval bodies (Fig. 31). Oval bodies are discussed later in the reticulopod section (page 15). These and perhaps other portions of the basal protoplasm in which they were located could later be extruded through the sieve-like diaphragm (Figs. 8, 9, arrows).

Protoplasmic islands were located external to the pore diaphragm (Fig. 1, arrows; Figs. 4 and 31), and few were found in the more external portions of the narrow exothalamous protoplasm.

The pores in newly-formed chambers had fewer membrane-like layers associated with them than those in more mature chambers. In the basic pore form there were only three of these 50nm wide, granular, membrane-like layers. In both _G_. _ruber_ and _G_. _bulloides_ these coalesced to form the sieve-like diaphragm at the pore constriction (Fig. 31).

The longer, more distal conical portions of the pore were bordered by the outer calcification layer. The inner calcification layer bordered the more basal part of the pore. Both these layers then continued laterally, roughly paralleling the primary calcification layer. The primary calcification layer extended laterally from the pore constriction on all sides. Additional calcification layers were added between the primary and outer layers.

All calcification presumably takes place between these layers (Bé and Ericson, 1963). The inner lamellar unit of the shell would form between the inner and primary calcification layers, while the outer lamellar unit would form between the primary and outer calcification layers.

Fibrillar System

The most unusual organelle in all species of planktonic foraminifera studied was the fibrillar system (Lee's vesicular system). It consisted of a radially-arranged network of 2 - 4μm wide channels which were found primarily in the most recently formed chambers. These were usually found in pairs, each channel coiled around the other. The fibrillar channels were membrane-bound (Fig. 10, arrow), except in the loose apertural protoplasm, where unbound fibrils were observed (Fig. 14). The channels were bordered by mitochondria, fibrous bodies, and oval bodies (Fig. 13).

The channels contained intertwined uniformly cylindrical thread-like fibrils in a faintly granular matrix (Fig. 59). Fibrils measured 30 - 150nm in diameter and had a rough, woolly texture (Fig. 11). They were not bounded by membranes. Thicker forms sometimes had dark bands (Figs. 10 and 12).

Thinner, hair-like fibrillae were often found attached to thicker fibrils (Figs. 10 and 11). They measured up to 200nm in length.

The fibrils intertwined in an apparently helical pattern, and resembled a cylindrical woven basket. This was suggested by cross-sections of the main, untapered portions of channels (Fig. 15). Fibrils were closely-associated with the channel membrane (Fig. 13). Medial longitudinal sections showed a clear central cavity, while the more lateral longitudinal sections of a channel show many stub-like fibril cross-sections (Fig. 13). Longitudinal sections show various views due to the helical curvature of the channel.

The ends of some of the channels tapered abruptly to become protoplasmic pockets with thinner fibrils (Fig. 13). Other channels branched to end in reticuloid masses of fibrils (Fig. 16) within end-pockets. The majority, however, ended in thicker segmented fibrils (Fig. 12), which appeared to be 1.3μm crescent-shaped structures in peripheral oblique sections through the basket-like woven arrangement of fibrils.

Banded figures with a periodicity of about 0.2μm were present in the protoplasm of larger chambers (Fig. 30). They were not associated with any particular organelle or with symbionts. They were 0.8μm wide and were continuous within 1.3-2.7μm wide serpentine vesicles. Because of twisting, no more than an 8μm length of any vesicle was observed in a section.

Banded figures may be related to the segmented forms of fibrillar strands, but their channels were not observed to be connected to the fibrillar system.

Vacuoles

Many different types of vacuoles were present in the foraminiferan protoplasm. One type of vacuole measured 1μm in diameter and probably contained sea water since it was only moderately electron dense (Fig. 1). These were more prevalent in younger chambers. Deeply stained granular inclusions were sometimes found in these vacuoles (Fig. 2, arrows).

Many food vacuoles were also present. They were bounded by a single unit membrane (Fig. 17, arrow), as were the symbiont vacuoles. Unlike symbiont vacuoles, they contained deeply-stained digested food organisms. Diatom frustules were observed in some digestive vacuoles (Fig. 18).

Nuclei

The planktonic foraminfera were heterokaryotic, and a variety of nuclear types were found. As in the individuals examined by Lee *et al*. (1965), up to six small generative nuclei were found in the largest chambers. These were spherical and measured 2-4μm in diameter (Fig. 20). Some had chromatin dispersed in a faintly granular matrix. A few had condensed chromatin in the equatorial region (Fig. 21). They were not surrounded by large amounts of Golgi or endoplasmic reticulum.

Somatic nuclei were spherical or ellipsoidal and ranged in section from 10 - 50μm. These were found in the larger chambers and were much less numerous than generative nuclei. Both intact forms with tightly-coiled chromosomes (Fig. 2) and pycnotic types (Fig. 19) were observed. In the latter, the chromatin formed a shrunken, deeply-stained mass.

Large (45μm) oval somatic nuclei were also found in Globigerina bulloides (Figs. 22-24) and Globigerinoides conglobatus. In all cases the nucleus occupied almost an entire chamber. Oblique sections through some nuclei showed nuclear folds, which enclosed portions of protoplasm (Fig. 23).

There are at least 150 chromosome sections in the nucleus of Fig. 22. In the same figure, most chromosomes are segregated in one-half of the nuclear section. Mitotic spindle fibers (Fig. 24) connect the denser chromosome-containing half with the lighter part, which has fewer chromosomes and projecting nuclear folds.

The envelopes of several somatic nuclei were each surrounded by an external ribosomal border (Fig. 23). Between the bases of nuclear folds, vermiform perinuclear aggregates (Fig. 23) were observed.

Several other large somatic nuclei were observed with poly-energid chromosome aggregates (Fig. 25). Annulated honeycomb-like masses were also present (Fig. 25). These probably represented nucleolar material which would later be extruded. No mitotic spindle fibers were observed. These somatic nuclei had few dispersed chromosomes and their nucleoplasm was homogeneously granular. These large nuclei had a smooth envelope, with neither a ribosomal layer nor perinuclear aggregates nearby. They did not have nuclear folds extending into the surrounding protoplasm.

Cryptosomes were observed in Globigerinoides ruber and Globigerinoides conglobatus (Figs. 27 and 28). They had a broad size range from 6μm to greater than 50μm (Lee et al., 1965). Cryptosomes were bordered by many mitochondria. They differed from nuclei in being undifferentiated and homogeneously granular internally. In addition,

some had annulated vesicular borders (Fig. 29). These consisted of internal and external vesicles measuring 0.1 - 0.3μm wide, and separated by granular layers. The vesicles contained granular material.

Reticulopodia

Extended reticulopods were observed in only one Globigerina bulloides specimen. Most probably retracted due to fixation or handling during collection aboard ship. The extended reticulopods (Figs. 32 - 35), as well as unextended ones (Fig. 31), contained 0.8 - 1.3μm oval bodies which had very dense borders. These oval bodies often contained dense or membranous material (Fig. 35). All oval bodies had a similar size, shape and border. Microtubules were only present in extended reticulopods and had the typical parallel arrangement (Marszalek, 1969).

Smaller spherical vesicles, fibrous bodies, and tubular endoplasmic reticulum were also present in the granular protoplasm of reticulopods (Fig. 35). Figure 32 is an oblique section of a spine and its closely-associated reticulopod. The central clear space probably resulted from loss of calcite during sectioning.

Aggregated reticulopodial material, characterized by the presence of oval bodies, was found in apertures (Fig. 36). Reticulopod components were rare in the denser protoplasm.

Mitochondria

Mitochondrial christae arose as microvilli-like invaginations into a less dense matrix. Few reached the center (Fig. 37). Mitochondria were always present bordering fibrillar channels as well as symbiont and food vacuoles. Many were found beneath the cell membrane

near pores and around cryptosomes. Other areas of high concentration were within reticulopods and the exothalamous protoplasm. Mitochondria in the rest of the protoplasm were randomly distributed.

Vacuolar System

Smooth endoplasmic reticulum consisted of short (0.2μm) tubular vesicles (Figs. 35 and 38). These were found primarily in reticulopods and apertural protoplasm. Little rough endoplasmic reticulum was present in the protoplasm, but there were numerous 22.5nm ribosomes in the groundplasm of every chamber (Figs. 37 and 38).

The typical Golgi complex (Fig. 39) was observed in all specimens. It was most often found near host vacuoles, and at least one was associated with each nucleus.

Fibrous Bodies

Fibrous bodies were 0.5μm ovoid membrane-bound vesicles containing fibrous material. They were found in highest concentrations near the shell (Figs. 3 and 6), in reticulopods (Fig. 35), and sometimes bordering fibrillar channels (Fig. 13).

II. SYMBIOTIC ALGAE

General Description

The symbiotic dinoflagellates of Globigerinoides ruber were found either in host vacuoles or in the non-dense protoplasm near apertures (Fig. 40). Only non-motile forms were present. Thin-walled "Pseudoencysted" individuals were found outside the foraminiferan test (Fig. 42). The non-motile intracellular forms were unicellular oblate spheroids (Fig. 43). Some tapered antapically

(Fig. 44). The epicone was twice the hypocone length (Figs. 61 and 62). Based on fifty measured individuals, the non-motile vegetative form had a mean length of 10.2μm (s.d.=2.3) and was 6.1μm (s.d.=0.9) wide at its widest point. One extremely tapered alga measuring 9μm by 6μm was also observed (Fig. 45).

Nucleus

The symbiont nucleus was typically dinoflagellate and occupied about one-third of the total cell volume. The nuclear envelope was interrupted by pores (Fig. 46). Nuclei are ovoid, with occasional bulges resulting from the close apposition of peripheral chromosomes to the nuclear envelope (Fig. 47). Nuclei measured 3μm in cross-section. The chromosomes were condensed in all nuclei observed. Oblique sections were denser than longitudinal or transverse ones. All chromosomes in any one individual were in the same condensed state.

Their fine structure consisted of fibrils or fibril bundles arranged in a high amplutude sine curve, with 5-6nm diameter granules scattered along it. These granules may be chromosomal ribosomes or precursors of nucleoplasmic and cytoplasmic ribosomes, both of which are about twice their size (10nm). The DNA fibrils with which the granules were associated were either single (2nm diameter) or double (4-5nm diameter) (Fig. 47).

The granular nucleoplasm contained ribosomes and a single nucleolus (Fig. 43). The nucleolus was denser peripherally, and stained more deeply than the rest of the nucleoplasm.

Chloroplasts and Associated Structures

The two large chloroplasts were multi-lobed and peripheral. Each was bounded by a unit membrane (Fig. 48, arrow). The chloroplast matrix was granular and contained ribosomes, possible DNA fibrils (Fig. 49, arrows), and parallel lamellae. Lamellae consisted of three closely-apposed thylakoids. Discrete osmiophilic globules were also found within the chloroplast stroma (Fig. 45, arrows).

A pyrenoid projected from the inner surface of each of the chloroplasts and was capped by a deeply-staining starch sheath in the *Globigerinoides ruber* symbionts (Fig. 43). Pyrenoids were penetrated by chloroplast lamellae (Fig. 41). Deeply-staining and unstained starch granules were also present. In some individuals (Fig. 13) they occupied as much as 40% of the algal cell volume. The grains ranged in diameter from 0.5 - 1.5µm.

Other Reserve Bodies

Lightly-stained lipid vacuoles were found in most individuals (Fig. 43), and were oval or round in shape. They stained more homogeneously than starch grains, but were sometimes denser peripherally. Calcium oxalate crystals (Fig. 50) and accumulation bodies (Fig. 45) were rare in the planktonic symbionts. The structure in *Symbiodinium microadriaticum* which was formerly called an assimilation body (Doyle and Doyle, 1940; Freudenthal, 1962), is now known as an accumulation body (McLaughlin and Zahl, 1966). This new name is more appropriate in view of its probable waste storage function (Taylor, 1968). Accumulation bodies were round in cross-section and had a diameter of

about 1μm. They contained many dense peripheral 0.1μm concretions (Fig. 45, inset).

Mitochondria

Symbiont mitochondria were sausage-shaped. Small tubular cristae were distributed throughout the dense matrix (Fig. 51), and not as peripheral as in most other dinoflagellates. Mitochondria were located between the nucleus and the chloroplasts (Fig. 43).

Flagellar Apparatus

The organisms were biflagellate, but only flagellar rudiments were found in the non-motile forms. Each flagellar base consisted of two diaphragms and two basal discs, in addition to the 0.4μm long rootlet (Fig. 52). The two basal bodies originated at right angles to each other. The posterior girdle was displaced $>$ 2/10 of the body length. It extended about three-quarters of the way around the alga, terminating about 0.5μm closer to the antapex (Fig. 44).

A pusule was also present. It consisted of a series of ovoid vesicles surrounding the flagellar pore (Fig. 52). Several vesicles contained dense material.

Other Organelles

Other symbiont organelles included vacuoles, endoplasmic reticulum, and dictyosomes. The latter were rare. There were few examples of tubular and rough (Fig. 53) endoplasmic reticulum. They were always found medial to the chloroplast. These were not as common as free ribrosomes, which were often grouped in star-like polysome clusters (Fig. 53).

Host-Symbiont Interface

The algal symbionts of Globigerinoides ruber were bounded by a three-layered periplast. The first layer consisted of three closely-apposed unit membranes (Fig. 54). The innermost of these (closest to the chloroplast) was the plasma membrane. Vesicles were sometimes formed by infoldings of one or more of these membranes (Fig. 55).

Exterior to this layer was the fragile rudimentary thecal or pellicle layer, which was usually broken. It consisted of a single membrane, often undulated (Fig. 54).

The most external layer was the cell wall, which was made up of two membranes, which stained more deeply than the thinner thecal layer. This was often closely-apposed to a nearby host membrane in loose protoplasm (Fig. 56, arrows), or in dense protoplasm symbiont vacuoles. Symbiont vacuoles were structurally similar to host digestive vacuoles. Both were bounded by a single unit membrane interrupted by pores (Fig. 57).

The outer two periplast layers were often ruptured, probably during fixation. Thus, only portions of these layers were usually present.

Microtubules were observed beneath the periplasts of some algae (Fig. 48). A dividing form was observed within Globigerinita glutinata (Fig. 58).

The algae in other planktonics studied (Globigerina bulloides, Globigerinoides conglobatus, and Globigerinita glutinata) were the same species, based on similar structure of the following: lamellae

and pyrenoid penetration, pusule, mitochondria, girdle, as well as the number of periplast layers and nuclear size. Symbiont concentration and distribution were similar in all the species studied.

DISCUSSION

I. PLANKTONIC FORAMINIFERA

Many aspects of the fine structure of *Globigerinoides ruber* are similar to those found in *Globigerina bulloides* (Febvre-Chevalier, 1971). This is obvious in their nuclear ultrastructure. In addition, they have comparable pore, ectoplasm, and reticulopod morphology.

Nuclei

The large somatic nuclei observed in my *Globigerinoides ruber* and *Globigerina bulloides* specimens were similar to those Febvre-Chevalier (1971) found. Both had nuclear folds, and a similar glycocalyx-like layer of ribosomes. The latter was situated 70nm external to the nuclear envelope.

There were also some differences among these nuclei. In one of my *Globigerina bulloides* specimens, chromosomes were segregated in one-half of the large nucleus. There were more nuclear folds associated with this half of the nucleus. In addition to this difference, the perinuclear ribosomes in both my species had a smaller size range (10 - 30nm) than Febvre-Chevalier's *G. bulloides* specimens (10 - 60nm). She did not observe any perinuclear aggregates in this ribosome layer. Lastly, she did not observe any spindle fibers in the nucleoplasm.

Some structures associated with the nuclear envelope were found only in Febvre-Chevalier's individuals. These included both

internal and external microfibrillar layers, which are comparable to the fibrous lamina (Dahlgren, 1967 a and b). She also noted nuclear pores, annulated vesicles, and a zonula nucleum limitans (Patrizi and Poger, 1967).

Planktonic nuclear structure was simpler than in benthic foraminifera, such as Allogromia laticollaris (Arnold, 1955; Lee and McEnery, 1970) and those studied by Dahlgren (1967 a, b). In the large forms of planktonic nuclei, there were fewer projecting nuclear folds, compared with the gamontic nucleus of Ovammina opaca (Dahlgren, 1967, a). Planktonic foraminiferan nuclei showed little development of the exonuclear vacuome.

Nucleosomic aggregates about the same size (4.5μm) as in O. opaca were also found in Globigerinoides ruber. These were heterogeneous like those of Globergenia bulloides, but had a more defined structure than the benthic form (Dahlgren, 1967 a) or G. bulloides. They consisted of 1μm wide polygonal units with deeply-staining borders.

The planktonic foraminiferan nuclei had a unique extranuclear ribosomal layer with perinuclear aggregates. The inner fibrous lamina canals (if they were present in precursor nuclear forms) probably filled with ribosomal material which then budded off from the nuclear membrane to form these extranuclear structures, since no pores were evident in either my planktonic nuclei or in the gamontic form in Ovammina opaca. Dahlgren (1967 a) and Febvre-Chevalier (1971) suggested that the nucleosomic aggregates were a possible source of mRNA. These aggregates probably gave rise to the extranuclear

ribosomal layer and perinuclear aggregates, indicating increased nucleocytoplasmic interaction, which would involve mRNA.

Dahlgren suggested that these aggregates of nucleolar material would serve to maintain the high level of protein production necessary for reticulopod reformation. Ribosomal material, represented by the glycocalyx and perinuclear aggregates could direct the synethesis of the specific proteins necessary for reformation of these important structures. He felt that reticulopods would be continually worn out or disrupted by food capture and/or the effects of strong water currents.

Because of these factors, in addition to tearing or just retraction due to turbulent waters, the surface to volume ratio would be lowered. Foraminifera would then sink to lower levels. Brandt (1895) described an analogous phenomenon in the radiolarian, *Thalassicolla*. Water turbulence caused the organism to descend due to calymma collapse.

Rapid protein formation would also aid in maintenance of the active fibrillar system, which consists primarily of contractile protein. Precipitated fibrils and those passed out from loose protoplasm could then be readily replaced.

Morphological differences between the large somatic nuclei of *Globigerina bulloides* were minimal. The structure of the nuclear envelope and other aspects probably varied with physiological state and life cycle stage. Another factor which might be involved is season of collection, since Febvre-Chevalier's organisms were collected during the winter, while mine were collected in warmer summer waters. These organisms apparently undergo a great deal of large-

scale nuclear transformation, such as in the process of nuclear RNA extrusion in the form of ribosomes. The lack of the external ribosome layer in some of the *Globigerinoides ruber* nuclei (Fig. 26), and its presence in others collected at the same time indicates nuclear activity. The presence of a large concentration of chromosomes in one-half of the large nucleus of *Globigerina bulloides*, along with spindle fibers represents part of a massive nuclear transformation. It is therefore understandable that more nuclear folds would be present on this side of the nucleus. Thus there would be increased surface area, and greater nucleoprotoplasmic interaction would be facilitated in this region of high metabolic activity.

My observations reaffirm the heterokaryotic nuclear structure described by Lee *et al*. (1965). Their study suggested that the variable number of somatic nuclei indicated affinity with heterokaryotic forms such as *Glabratella sulcata* (Grell, 1958). The different sizes of nuclei may constitute a series, ending with the large somatic nucleus. This series, ranging up to the size of cryptosomes, suggests an affinity with these large structures. Cryptosomes were not observed by Febvre-Chevalier (1971), but were noted by Lee *et al*. (1965) in both *Globigerinoides ruber* and *Globigerina bulloides*. In the present study, they were observed in *Globigerinoides ruber* and the related *Globigerinoides conglobatus*. Lee claimed that cryptosomes were hypertrophied somatic nuclei. This conclusion is further supported by their similarly large size.

Rhumbler considered the cryptosome to be nuclear in origin when he called it "Kern," which means nucleus. The cryptosomes observed in my specimens were elongated ellipsoids. Elongated nuclear

forms such as the ones I observed were also noted by Lee et al. (1965) and Rhumbler (1909).

The giant somatic nucleus of Fig. 22 may be nearing transformation into a cryptosome. Evenly-distributed chromosomes would assume this eccentric position prior to extrusion. At this time, nucleoprotoplasmic interaction through the portion of nuclear envelope nearest this high concentration of chromatin would increase. This is manifested in an increased number of nuclear folds. The annulated vesicular border in cryptosomes also suggests enhanced nucleoprotoplasmic interaction, as in the case of Noctiluca scintillans (Afzelius, 1962). It may be a further morphological development of the somatic nuclear envelope and may be related to the annulated lamellae observed by Febvre-Chevalier (1971). The cryptosome may thus represent an hypertrophied somatic nucleus devoid of chromatin and nucleolar material. The annulated vesicles were probably formed shortly after extrusion of nuclear material by portions of the somatic nuclear envelope.

These conclusions are further supported by the similarity of planktonic cryptosomes to a stage in nuclear transformation occurring in benthic foraminifera.

The cryptosome was similar both ultrastructurally and cytochemically (Lee et al., 1965) to the gamontic nucleus of the saccaminid, O. opaca (Dahlgren, 1967 a). The complicated vesicular border resembled the inner fibrous lamina vesicles of the benthic saccaminid. Lee et al. (1965) found similar staining properties in the cryptosome and the nucleus of O. opaca. Its large size also suggested derivation from a somatic nucleus, which was of comparable

size and the largest structure in the planktonic foraminifera. The cryptosome is probably the result of a Zerfall-like purgation (Arnold, 1955) of nuclear material. Nuclear transformation, eventually resulting in a cryptosome, may aid in flotation by facilitating synthesis of proteins for reticulopods and the fibrillar system.

Pores and Associated Structures

The pore structure of the *Globigerina bulloides* specimens described by Febvre-Chevalier (1971) is similar to that observed in my *G. bulloides* and *Globigerinoides ruber* specimens. In all cases there was a pore diaphragm, separating membrane bound ecto- and endoplasm. The diaphragm was situated at the pore constriction. In addition, calcification layers were observed in both studies, and in both, reticulopods were associated with the ectoplasm.

The basic pore structure, which served as a framework for future calcite deposition, involved granular membrane-like layers associated with the test. I found that the simplest form of pore construction consisted of three calcification layers. Each lamellar unit of test was deposited between two calcification layers, resulting in two lamellar units of deposited calcite test.

Febvre-Chevalier's pore diaphragms were transversely striated, and some contained microtubules, but neither were found in any of Towe's (1971) or my specimens. Her pores did show a constriction at the level of the pore diaphragm, but she did not mention this. This constriction was also observed in my specimens. Her primary calcification layer did not extend straight lateral to the pore as it did in my specimens, but paralleled the pore base. No early shell forms were

observed by her, so the bead-like calcification loci of my Fig. 3 were not observed. Her specimens also lacked fibrous bodies in association with nearby endoplasm. This may have been due to the age of her specimens. The absence of fibrous bodies indicates a less active stage of test calcification (Angell, 1967 a).

The pore and test pattern in these planktonic foraminifera was basically similar to that of Rosalina leei (Angell, 1967 a). This consisted of an organic calcification layer lying just external to the cell membrane. External to this was the primary calcification layer, followed by outer calcification layers. These layers merged to form the pore diaphragms. The presence of microtubules may be accounted for by different environmental variables in the Mediterranean Sea compared to Bermuda waters. But the presence of transverse striations may have a genetic basis and represent one of the characteristics which has evolved during geographic isolation from North Atlantic forms. Other differences may also be accounted for by geographic isolation. Minor differences in pore structure were found when Febvre-Chevalier's specimens were compared with mine. The difference in number of layers in the basic pore form was probably due to a difference in specimen age. The bilamellar pattern with only two test lamellae formed is the basic pattern, as pointed out by Bé and Hemleben (1970).

The protoplasm which penetrated Globigerinoides ruber and Globigerina bulloides pore diaphragms included portions of groundplasm and not just fibrils. The pores were partially closed off by sieve-like diaphragms (one to a pore), which included organic material in addition to some calcite. The tearing observed in pores (Hemleben, 1969) may have been due to the presence of this organic diaphragm

closely-associated with calcite and perhaps some membranes. It was probably destroyed during his scanning electron microscope preparation, which included drying and formalin fixation. The pores served as strengthening structures for test layer deposition, similar to pylons on a bridge. The pattern of pore formation in planktonic foraminifera may have taxonomic value. Bé and Hemleben (1970) suggested that pore constriction and the presence of an associated bilamellar structure would distinguish these from other foraminifera. According to the theory of bilamellar structure, each lamellar unit of calcite test would be deposited between two calcification layers. The initial process involves formation of one inner and one outer lamellar unit within the limits defined by the surrounding membrane-like calcification layers. After this basic form, the next outer lamellar unit would be added between the first outer calcification layer and a second, more distal one. The next would then be deposited between the second and third, and so on. The most distal outer calcification layer would lie next to the exothalamous protoplasm. Calcification layers apparently merged to become the pore diaphragm in the pore area.

Towe (1971) doubts the value of placing all planktonic foraminifera in the same group, based on bilamellar shell structure. He claims that there is clear evidence of this characteristic in some, but not all planktonics. Towe states that widespread mass calcification is a more probable alternative to the gradual calcification scheme suggested by the bilamellar theory. This mass calcification would presumably take place within a short period of time.

There is evidence, however, in support of the bilamellar theory. It is found in tests of fossil planktonic foraminifera

(Bé, pers. comm). This theory is further supported by the addition of a calcite crust in Globorotalia truncatulinoides (Bé and Ericson, 1963), which suggests layered test formation. The bilamellar pattern was observed in both my Globigerinoides ruber and Globigerina bulloides specimens, in addition to the G. bulloides specimens of Febvre-Chevalier (1971). Towe's G. ruber specimens did not show this pattern for a number of reasons. Towe may have observed a different stage of pore development. This is plausible in view of the complicated life cycle suggested for these organisms. For instance, the replica in his Fig. 1, Plate 6 represents the obliteration of the basic bilamellar form by a large amount of deposited calcite. At an earlier stage, the calcification layers may have been visible.

Specimen treatment may also have been a factor. His were decalcified with EDTA and treated with sodium hypochlorite, which may have removed the primary calcification layer, in addition to test calcite. The calcification layers consisted of some organic (and possibly membranous) material, which probably disintegrated during the latter treatment. Angell (1967 a) used sodium hypochlorite to remove all organic material in the pores of his specimen.

I feel that if the bilamellar form is present to any extent in certain foraminifera, it has taxonomic value. Therefore, the similar pore structure in Globigerinoides ruber and Globigerina bulloides reinforces taxonomic affinity of these organisms.

Exothalamous Protoplasm (Ectoplasm)

The exothalamous protoplasm of my specimens was similar to that observed by Febvre-Chevalier (1971) in many respects. In both

studies this layer was associated with pores. Reticulopods arose from this layer in all specimens. In addition, this layer contained few organelles. Both studies showed oval bodies in oral ectoplasm.

The thin (35 - 65nm) portions of exothalamous protoplasm on which the glycocalyx was located were only found in my specimens. My specimens lacked microbodies and microtubules in this layer. However, I did observe microtubules in reticulopods. The presence of the exothalamous protoplasm demonstrates that planktonic (and perhaps all) foraminifera have an internal shell. This external protoplasm is shown in Fig. 1, Tafel 11 of Hemleben's article. A mass of fine filaments (Gorycki, 1971) was not observed in this protoplasm or in nearby calcification layers. Perhaps these filaments are only found in benthic foraminifera.

The exothalamous protoplasm of foraminifera may have a variety of functions, including calcium transport and reticulopod formation. Angell (1967 b) suggested calcium transport for shell-building as a function of the mitochrondria found in this layer. Studies of kidney mitochondria (De Luca _et al_., 1962) supported this hypothesis. In addition, the exothalamous protoplasm of the planktonic was coated with small knob-like structures in a glycocalyx arrangement. These increased the surface area and would thereby facilitate calcium ion incorporation from the surrounding sea water.

Bennett (1969) claims that glycocalyces behave like anionic polymers. Strongly acidic carbohydrate groups on most glycocalyces are immediately adjacent to the cell membrane, which has a different charge distribution. The close proximity of differently-charged layers results in electrical rectifying properties which facilitate the

binding of nutrients, as well as ions. Brandt and Pappas (1960) described the importance of binding properties of the amoeba's glycocalyx in uptake of prey and nutrients.

Glycocalyces usually contain sulfated mucopolysaccharides. Moss' (1963) cytochemical demonstration of these in the outer "membrane" of *Globigerinella aequilateralis*, *Globigerinoides ruber*, and *Globigerinoides sacculifer* provides further evidence of the classical glycocalyx. The well-developed glycocalyx, in addition to a lack of microbodies may indicate a different physiological state. My specimens were probably in a more active state of calcification than Febvre-Chevalier's. This is further supported by the greater test microstructure development in the organisms in the present study.

Reticulopodia

The reticulopods of my specimens were similar to those observed by Febvre-Chevalier (1971). In both, microtubules formed a strengthening axis (stereoplasm) for extended reticulopods. The oval bodies observed in protoplasmic islands and extended reticulopods corresponded to her osmiophilic formations. She found no central cavity. However, this may correspond to vacuoles observed by her.

Reticulopods and protoplasmic islands contained similar groups of organelles. These included mitochondria, groundplasm, fibrous bodies, oval bodies, and membrane-bound vesicles. The latter originate as infoldings of the plasma membrane (Marszalek, 1969). These vesicles probably contain sea water, which increase pseudopod volume and greatly enlarge the food-gathering surface area. As Figs. 1 and 31 indicate, exothalamous protoplasm in the distal portions of pores had an

organelle population similar to more externally located exothalamous protoplasm. The proximal portions of pores frequently contained a reservoir of oval bodies. These eventually pass through pore diaphragm holes to become incorporated into protoplasmic islands via phagocytosis. Towe (1971) suggested an excretory function for pores. Thus, the proximity of portions of exothalamous protoplasm enables waste-containing oval bodies to be extruded efficiently. Their content would eventually be released into the surrounding sea water when reticulopods are formed, as in Fig. 35.

A large number of oval bodies were found in apertural protoplasm. Some of these may also be eventually extruded via pore diaphragm holes and phagocytosis as described above. Oval bodies may also pass from endoplasm into the proximal pore chamber via the cell membrane, probably by phagocytosis. Few oval bodies were observed in dense protoplasm, so they may be bundles of waste material which are packaged as they are being passed out of the foraminiferan. Oval bodies may also contain chromatin residua resulting from a Zerfall-like nuclear "purgation" (Arnold, 1955) which eventually results in cryptosome formation. Large numbers of oval bodies would be necessary to accommodate the amounts of deteriorated nuclear material produced during nuclear transformation. It is therefore significant that these oval bodies are found in foraminifera, where such nuclear transformation occurs.

Reticulopod extension could also aid in flotation. Vacuoles of extended reticulopods were probably filled with sea water which contributed to the turgidity of these structures. Release of carbon dioxide from surrounding pseudopod protoplasm respiration into these

vacuoles would increase the organisms' buoyancy by lowering its specific gravity. A similar situation was found in planktonic Radiolaria, which had carbon dioxide-supersaturated sea water in calymmal vacuoles (Brandt, 1895). Extension of pseudopodia alone would also increase the surface area:volume ratio of the organism.

Febvre-Chevalier (1971) claims that reticulopods are formed from exothalamous protoplasm. My observations support this theory, since similar organelle populations were found in both reticulopods and their precursor protoplasmic islands. The presence of oval bodies in the proximal portions of pores, in addition to reticulopods, further supports this theory. Apparently, the membrane-bound islands of protoplasm in the ectoplasm could be suddenly extended forward. Reticulopod microtubules are probably formed at the time of extension. The sites for reticulopod microtubule formation are presumably located in the protoplasmic islands.

Planktonic and benthic pseudopods were different in several respects. Opaque particles observed in Allogromia laticollaris (Lengsfeld, 1969 b) and Iridia diaphana (Marszalek, 1969) were not found in the planktonic's pseudopods, using similar fixation regimes. Another difference between them was the presence of lacunae in the benthics Allogromia and Iridia, and their absence in the planktonic pseudopodia. Lacunae are sea water-filled vacuolar spaces which extend throughout the foraminiferan protoplasm, eventually connecting with the surrounding medium (Lengsfeld, 1969 a).

Globigerina bulloides was similar in several respects to each of the benthic species. Oval bodies are found in the planktonic pseudopods and in those of Allogromia laticollaris. They were about

the same size as those of the planktonic, but lacked extensive opaque borders. These were not present in *Iridia*.

Parallel bundles of microtubules were observed in exothalamous planktonic pseudopods, and those of *Shepheardella taeniformis* (Hedley *et al*., 1967) and *Iridia*. None was observed in *Allogromia laticollaris*.

Tilney and Porter (1965) demonstrated that different fixation regimes were necessary for good preservation of heliozoan axopod microtubules and protoplasm. It was therefore not unusual to encounter similar problems in foraminiferan fixation. However, this did not eliminate the possibility that microtubules were absent from some pseudopods. Similar fixation produced good pseudopodial and protoplasmic fine structure in *Globigerina bulloides*. Lengsfeld used various fixatives and no microtubules were present. Since the pseudopods were not withdrawn in his electron micrographs, a shock to the organism resulting from fixation could be eliminated as the cause of microtubule absence. Thus, existing microtubules were not disintegrated due to the fixative, since they are strengthening structures and the pseudopods would have then collapsed. Therefore these structures were not present in *A. laticollaris*. The lacunes were another means of support for *Allogromia* pseudopods.

Febvre-Chevalier (1971) did not observe several important structures. These include the fibrillar system, nuclear variety, and the cryptosome.

Fibrillar System

The most unusual structure in these planktonics is the fibrillar system (Rhumbler's Gallertsstrange), characterized by Lee et al. (1965) as a network of serpentine channels. On the basis of staining reactions with hematoxylin, iron hematin, acetocarmine, and azure A-Schiff, and lack of ability to stain with naphthol yellow S, they deduced that it consists of strands of protein with few available dibaso amino acid residues (Lee et al., 1965). In light microscopy, strands appeared helical in cross-section, with 1 - 2μm periodicity, and they suggested that it had contractile properties. Fine structural details give more insight into their possible function, in addition to substantiating Lee's results. Reticuloid masses in fibrillar channel end-pockets suggest a transformation due to protein precipitation. This could result from a lowered pH in the channel liquid due to lack of channel water mixing in the remote end-pockets. Fibril ultrastructure provides further evidence of protein composition. At higher magnifications, a fibrous protein is suggested by the fibrils' wooly texture, with radiating smaller strands (fibrillae).

Fibrillar bands were probably areas of maximum contraction along the fibrils. This is further supported by the greater thickness of fibrils with bands, as in Fig. 12. Alternatively, these bandings may represent centers of fibril protein synthesis, between which the woolly-textured strands are formed. These are found primarily near the ends of channels, where channel turbulence and loss of fibrils via apertural sea water exposure would be minimized. Fibrils may lengthen to eventually extend the entire length of the channel. The conidia-like structures observed by Lee et al. (1965)

in cross-sections of channel endings were these compact banded early fibril stages. The fibrillar system is only found in planktonic foraminifera, and probably functions in flotation in these pelagic organisms. Flotation is a major problem in planktonic organisms such as these, which have heavy tests. Bé (1959 b) measured a specific gravity of 1.40 for these and the related Radiolaria. Since the specific gravity of sea water is 1.03, they would be expected to sink.

Radiolaria and Acantharia, which are primarily epipelagic and have about the same specific gravity as planktonic foraminifera, have unique flotation mechanisms. Radiolaria are believed to rise in the water column due to buildup of numerous liquid-filled calymma vacuoles (Brandt, 1895). The water in these vacuoles is more saturated with CO_2 than in surrounding sea water, thus decreasing the specific gravity of the protozoan. Brandt measured a specific gravity of 1.026 for vacuole liquid. Sinking occurred when rough waves caused reticulopod withdrawal and subsequent calymma vacuole collapse. The numerous sea water vacuoles of planktonic foraminifera, particularly in the largest chamber, may aid in flotation, but these vacuoles are internal and collapse would not occur.

A more analogous flotation adaptation is found in Acantharia, where myofrisks can increase or decrease cell volume. At the same time cell weight is constant, so that the organism can sink or float depending on the degree of contraction of these structures. Febvre (1971) found that these structures were cylindrical organelles with a double microfibrillar ultrastructure consisting of longitudinal and transverse components called L zones and T bands. They are apparently

helical, as in the foraminiferan fibrillar system, but no organelles such as mitochondria are associated with them. Febvre suggests that the lack of associated organelles indicates that myofrisks may be passive structures. Thus they may be elastic structures controlled externally.

Myofrisk contraction is limited to the extracapsular portion of Acantharia. In contrast, contractile protein strands extending throughout the foraminiferan make widespread endoplasmic contractions possible. The medial channel space in some sections (e.g., Fig. 15) suggests contraction of the helically-arranged fibrils. The mass of channel fluid might thus be compressed by contraction of these interwoven contractile strands and forced out of the organism via channel openings in the loose apertural protoplasm. Water thus could propel the organism in the direction opposite that aperture. The motion produced would be similar to jet propulsion, and more distance would be travelled with repeated contractions. There may be an environmental factor involved in stimulation of contraction, such as increased hydrostatic pressure or gravity. This would make oriented movement toward the surface possible during day or night. Nearby mitochondria would provide ATP for fibril contraction. There would also have to be some source of oxygen for production of the large numbers of ATP molecules needed. Symbiotic algae would supply this as a product of photosynthesis. Photosynthetic oxygen production would increase as the symbiont-containing foraminiferan got nearer the ocean surface, especially during periods of intense insolation. More ATP's would be produced as the sun reached its highest intensity, and the rate of pulsating contractions would increase. Thus, a positive phototaxis,

which continually increased as the organism rose in the water column, would result.

Harmful rays would be shielded out by the symbiont pigments. Both would benefit. The foraminiferan would be able to stay afloat longer, while its symbiotic algae would be exposed to high levels of solar radiation, thus aiding photosynthesis. Symbiont-containing planktonics are usually found near the surface, while foraminifera without symbionts predominate in deeper waters (Bé, 1960).

Reticulopodia can be extended out in all directions. Then interconnections or anastomoses, which are characteristic of this type of pseudopod, would further increase surface area. The resulting snowshoe-like effect would be enhanced by the many spines present. It is significant that Bé (1966) found spinose species of foraminifera to be generally epipelagic.

Morphological adaptations also aided flotation in these organisms. Sinking rate is determined by the ratio of surplus weight to friction (Sverdrup et al., 1942), while friction is determined to a great extent by the surface area. The elaborate foraminiferan test ornamentation, including spines, increases the surface area to volume ratio. These factors alone would result in a slower sinking rate. Bradshaw (1959) states that the presence of empty tests in upper water tows proves that they do not sink immediately.

Protoplasmic oil droplets and sea water vacuoles also increased buoyancy. The lipid reduces specific weight, and may also serve as a food source. The foraminifera probably reached their level in the water column via fibrillar system activity. The "snowshoe"

effect of reticulopods would then be the primary factor in maintaining this optimum position.

II. SYMBIOTIC ALGAE

The algal symbionts of *Globigerinoides ruber*, as well as *Globigerinoides conglobatus*, *Globigerinita glutinata* and *Globigerina bulloides*, were dinoflagellates. Nuclear and chloroplast structure, in addition to the presence of a girdle, support this conclusion. The foraminiferan algae were, however, different from the mutuals of *Anemonia sulcata*, *Condylactis sp.*, and other benthic invertebrate hosts.

They were distinguished from other endozoic dinoflagellates by lamellar penetration of the pyrenoid. Similar flask-shaped pyrenoids were observed in some chrysophytes and euglenids (Taylor, 1968). They were also found in some symbiotic and free-living dinoflagellates (Taylor, 1968), such as the free-living *Aureodinium pigmentosum* (Dodge, 1967). The latter may be symbiotic. Two chloroplasts were present in the foraminiferan symbionts, while only one was found in *Symbiodinium microadriaticum* (Kevin *et al.*, 1969). They were further distinguished from the other endozoic dinoflagellates by the absence of girdle lamellae.

Few of the foraminiferan algae had accumulation bodies. When they were present, the cell usually had a high starch content and few calcium oxalate crystals. Taylor (1968) reported a similar situation in the *Anemonia sulcata* symbiont. However, the accumulation bodies in all foraminiferan symbionts were larger. Lee *et al.* (1965) did not find accumulation bodies in their *G. ruber* algae. This may have been

due to differences in physiological state. PAS-positive halos were observed in association with the zooxanthella chloroplasts. These were sections of the dome-shaped starch sheaths capping each pyrenoid (See Figs. 43 and 45).

Unlike the other endozoic dinoflagellates, the foraminiferan algae did not contain fibrous bodies or striated patches (Taylor, 1968).

The foraminiferan symbiont nucleus was also different than those of other endozoic dinoflagellates. It was up to twice as large as the one found in Symbiodinium microadriaticum (Freudenthal, 1962). The planktonic symbiont's nucleus was centrally located, while the Symbiodinium nucleus was found in the hypocone. The nucleolus was not found next to the nuclear envelope as in the other symbionts, and no fibrillar bridges connected the nucleoplasm matrix with chromosome fibrils. As reported by Lee et al. (1965), the planktonic foraminiferan symbiont nuclei were always observed in prophase. The algae had a six-membraned periplast, as in the other endozoic forms. They also formed vesicles by periplast membrane-infolding, as in the Anemonia sulcata symbionts. The outer two periplast layers were often ruptured. This may have been due to fixation, as in Woloszynskia micra (Leadbeater and Dodge, 1966). No thecal vesicles were found, so the thecal layer was rudimentary. The periplast was thick enough to provide an effective barrier to host enzyme penetration, even with disruption of the two outer layers. Those with disrupted outer layers showed good internal organelle integrity. The rarity of digested symbionts also bore this out. The host foraminiferan may use some of the algae for food when external food organisms are scarce. This

would always be possible since the algae were found in host vacuoles (presumably digestive).

Other adaptations to symbiotic life included loss of flagella (only bases left), and loss of the cell wall and rudimentary thecal layer. The latter may have been due to fixation, as pointed out before. In addition, no trichocysts were present in the *in situ* foraminiferan algae. These factors would necessarily be involved in mutualism as well as parasitism.

Since the planktonic foraminiferan alga was endozoic, it should be placed in the Blastodiniales, along with such mutuals as *Symbiodinium microadriaticum*. This was further supported by the occurrence of binary fission. They both had flask-shaped pyrenoids and similar periplast structure, in addition to lacking chloroplast starch grains. These ultrastructural characteristics may be common to all Blastodiniales. They were also evident in the free-living *Aureodinium pigmentosum*, which Dodge (1967) suggested as a possible endozoic form. Lee *et al.* (1965) suggested taxonomic affinity of the planktonic foraminiferan symbionts with parasitic rather than mutualistic dinoflagellates. The foraminiferan symbiont was quite different from the mutuals in many respects, including the presence of many dense starch granules, which is considered diagnostic of the parasitic Syndiniaceae (Chatton, 1952).

The living zooxanthellae just released from a crushed *Globigerinoides ruber* observed by Lee (McLaughlin and Zahl, 1966) were morphologically similar to many of the forms in my electron micrographs. The algae were described as follows:

> They have the typical golden-brown color and a dinoflagellate-type nucleus which appears to remain in a 'continuous prophase' instead of going to a resting interphase. The zooxanthellae of the pelagic foraminifera Globigerinoides ruber are generally spherical to oval, 6.1 - 9.9μ in diameter. The nucleus appears to be discoidal and is generally regular in shape, although it may be stellate when the cell is highly vacuolated. The mean diameter of the nucleus is 3.5μ. Because of their size, counting the chromosomes is difficult, but there would appear to be between 20 and 30 (roughly twice the number as in Symbiodinium microadriaticum), either condensed or intertwined, or relatively spread apart, oriented so that one end is in the direction of the disc's center.

Based on the information from Lee's recent study, as well as unpublished light micrographs, a precise taxonomic assessment is possible.

One of the most important criteria for dinoflagellate classification is the external morphology of motile forms. Many of the non-motile forms in my electron micrographs were morphologically similar to motile zoospores just released from a crushed host (Lee, unpubl. obs.). They could have probably developed flagella quickly since they were not encysted and already had some motile characteristics, such as flagellar bases and a girdle. Pseudoencysted forms were rare and did not resemble dinospores. The majority of the forms in my specimens would be better described as "pre-motile" rather than non-motile vegetative forms. Oblate spheroidal forms were non-motile vegetative stages and lacked flagellar bases.

The motile and "pre-motile" forms observed by Lee and myself were clearly different from Symbiodinium, based on external morphology. The small zoospores of Symbiodinium microadriaticum and those from the foraminiferan were about the same size (10μm), but differed in shape. The epicone and hypocone were not subequal as in Symbiodinium, and the hypocone was not domed. The domed epicone was about twice the length

of the antapically-tapered hypocone. The girdle was not equatorial, but arose posteriorly. It had a torsion of < 1.0 turn and was displaced $\leq 2/10$ of body length. The sulcus was very shallow and not as well-developed as in *Symbiodinium*.

These major differences in zoospore morphology reinforced conclusions based on protoplasmic fine structure. The planktonic foraminiferan symbionts belonged in the genus *Merodinium* of the parasitic Syndiniaceae. Chatton proposed this genus in 1923. The type species, *M. brandti*, was originally called *Syndinium brandti* (Hovasse, 1923), and is an endoparasite of *Collozoum inerme*.

Chatton identified biflagellate tapered unicells within the same host as radiolarian isospores. Their nuclear structure was probably hard to observe with the light microscope because of their small size. They are probably dinoflagellates. The nucleus is typically dinoflagellate, and the rest of the organism's internal fine structure closely resembles the other symbiotic algae present in these planktonic foraminifera. Starch grains, which are characteristic of the Syndiniaceae, may have been indicated in Chatton's (1923) Fig. A. He may have hesitated to place them in the Dinophyta because this stage lacked a girdle and sulcus. They were also observed in motile stages just released from *G. ruber* into sea water (Lee, unpubl. obs.). They probably were transitional stages, which would explain the lack of a girdle.

The epicone and hypocone of *M. brandti* zoospores were approximately equal in length. The foraminiferan dinoflagellate had a more posterior girdle and a slight girdle notch. Therefore a new species is suggested.

Diagnosis

Merodinium rhumbleri.

Symbiotic Stage. Single cells, oblately spheroid. 7 - 15μm long (mean = 10.2μm), 5 - 8μm wide (mean = 6.1μm). Surrounded by periplast consisting of 6 layers. Two golden-brown chloroplasts, lobulate, peripheral, lamellae arranged in parallel rows composed of 3 closely-apposed thylakoids, girdle lamellae absent. Stalked pyrenoid, projecting from inner chloroplast surface, penetrated by lamellae, enclosed by chloroplast envelope and surrounded by starch sheath. Nucleus centrally located, 3 - 4μm in diameter, with typical dinoflagellate chromosomes. Accumulation bodies and calcium oxalate crystals rare, lipid globules and many starch grains distributed in protoplasm. "Pseudoencysted" forms rare, dividing forms rare. Found primarily as zooxanthella in the planktonic foraminiferan, Globigerionides ruber. Also found in the following species of planktonic foraminifera: Globigerina bulloides, Globigerinoides conglobatus, and Globigerinita glutinata.

Motile Stage. Body stout, oblately spheroid, its length 1.7 transdiameter, widest at hemispherical epicone, hypocone sometimes tapered. Epicone and hypocone subequal. Girdle posterior, shallow, rarely notched, displaced $\leq$ 2/10 body length, extending 3/4 around terminating 0.5μm closer to antapex. Biflagellate.

In all these symbioses, sufficient light intensity is necessary for photosynthesis to take place. The planktonic foraminiferan host's pelagic habitat is ideally suited to this need, since it is found in the upper 200m of the North Atlantic (Bé, 1960), where insolation is intense. Most endozoic symbioses involve a host organism which lives near a high intensity light source, which is the case with corals and tridacnids, as well as *Anemonia sulcata* and the *Symbiodinium* hosts. Corraline larvae actively seek prominent reef positions. Planktonic foraminifera remain within the upper 100m during the day (Rhumbler, 1909; Bradshaw, 1959; Bé, 1960). The endozoic algae of planktonic foraminifera may provide an explanation of this phenomenon. Planktonic foraminifera are limited to the euphotic zone (0 - 200m), implicating a possible alga-host interaction. Food alga pigments may also be involved, since digestive vacuoles did contain other algae.

The presence of the same algal species in planktonic foraminifera other than *G. ruber* indicates that they may be more widespread than previously thought. However, their occurrence in these other foraminifera is rare. It is therefore understandable that Febvre-Chevalier did not find any in her Mediterranean *Globigerina bulloides* specimens.

The protoplasm or test of some species of planktonics without symbionts is colored with inorganic compounds such as Fe_2O_3 (Lipps and Ribbe, 1967), which could serve as protective filters from the high light intensities. Non-pigmented and zooxanthella-deficient organisms were more prevalent in deeper waters (Bé, 1960).

In the absence of other protection from insolation, this symbiont-host relationship may have evolved. Perhaps originally all planktonic foraminifera lacked pigmentation. Those with a diet including algae with the essential pigment survived the high light intensities of the euphotic zone.

BIBLIOGRAPHY

Afzelius, B. A. 1962. The nucleus of *Noctiluca scintillans*. J. Cell Biol. 19:229-238.

Angell, R. W. 1967a. The test structure and composition of the foraminifer *Rosalina floridana*. J. Protozool. 14:299-307.

________. 1967b. The process of chamber formation in the foraminifer *Rosalina floridana* (Cushman). J. Protozool. 14:566-574.

Arnold, Z. M. 1955. Life history and cytology of the foraminiferan *Allogromia laticollaris*. Univ. Calif. Publ. Zool. 61:167-252.

Bé, A. W. H. 1959a. Ecology of recent planktonic foraminifera. I. Areal distribution in the western North Atlantic. Micropaleontology 5:77-100.

________. 1959b. A method for rapid sorting of foraminifera from marine plankton samples. J. Paleont. 33(5):846-848.

________. 1960. Ecology of recent planktonic foraminifera. II. Bathymetric and seasonal distributions in the Sargasso Sea off Bermuda. Micropaleontology 6:373-392.

________. 1966. Distribution of planktonic foraminifera in the world oceans. Proc. 2nd Intern. Oceanogr. Congr. 26.

________ and Ericson, D. B. 1963. Aspects of calcification in planktonic foraminifera (Sarcodina). Ann. N.Y. Acad. Sci. 109:81-99.

________ and Hemleben, C. 1970. Calcification in a living planktonic foraminifer, *Globigerinoides sacculifer* (Brady). N. Jb. Geol. Paläont. Abh. 134(3):221-234.

Bennett, H. S. 1969. The cell surface: components and configurations. Chapt. 47. Handbook of Molecular Cytology. A. Lima-de-Faria, Ed. North-Holland, Amsterdam. 1261-1293.

Bradshaw, J. S. 1959. Ecology of living planktonic foraminifera in the North and equatorial Pacific Ocean. Contr. Cush. Found. Foram. Res. 10:25-64.

Brandt, K. 1895. Biologische und faunistische Untersuchungen an Radiolarien und anderen pelagischen Thieren. I. Untersuchungen über den hydrostatischen Apparat von Thalassicolla und colonie-bildenden Radiolarien. Zool. Jahrb. 9:27-74.

Brandt, P. W. and Pappas, G. D. 1960. An electron microscopic study of pinocytosis in ameba. I. The surface attachment phase. J. Biophys. Biochem. Cytol. 8:675-687.

Buchner, P. 1965. Algal symbiosis. Chapt. 1. Endosymbiosis of Animals with Plant Microorganisms. Interscience, New York. 3-22.

Chatton, E. 1923. Les péridiniens parasites des radiolaires. C. R. Acad. Sci. Paris. 177:1246.

________. 1952. Class des dinoflagellés ou péridiniens. Traité de Zoologie. P. P. Grassé, Ed. T. 1. f. 1. Masson et Cie, Paris. 309-390.

Christiansen, B. O. 1965. A bottom form of the planktonic foraminifer Globigerinoides rubra (D'Orbigny, 1839). Publ. Staz. Zool. Napoli 34:197-202.

Dahlgren, L. 1967a. On the ultrastructure of the gamontic nucleus and the adjacent cytoplasm of the monothalamous foraminifer Ovammina opaca. Zool. Bidr. Uppsala 37:77-112.

________. 1967b. On the nuclear distribution of RNA and DNA and on the ultrastructure of adjacent cytoplasm of the foraminifers Hippocrepinella alba Heron-Allen and Earland and Globobulimina turgida (Bailey). Zool. Bidr. Uppsala 37:113-138.

De Luca, H. R., Engstrom, G. W., and Rasmussen, H. 1962. The action of vitamin D and parathyroid hormone in vitro in calcium uptake and release by kidney mitochondria. Proc. Nat. Acad. Sci. 48:1604-1609.

Dodge, J. D. 1967. Fine structure of the dinoflagellate Aureodinium pigmentosum gen. et sp. nov. Br. Phycol. Bull. 3:327-336.

Doyle, W. L. and Doyle, M. M. 1940. The structure of the zooxanthellae. Pap. Tortugas Lab. 32:127-142.

Emiliani, C. 1954. Depth habitats of some species of pelagic foraminifera as indicated by oxygen isotope ratios. Amer. J. Sci. 252:149-158.

Ericson, D. B. 1959. Coiling direction of Globigerina pachyderma as a climatic index. Science 130:219-220.

Febvre, J. 1971. Le myonème d'Acanthaire: essai d'interpretation ultrastructurale et cinétique. Protistologica 7(3):379-391.

Febvre-Chevalier, C. 1971. Constitution ultrastructurale de *Globigerina bulloides* d'Orbigny, 1826 (Rhizopoda-Foraminifera). Protistologica 7(3):311-324.

Freudenthal, H. D. 1962. *Symbiodinium* gen. nov. and *Symbiodinium microadriaticum* sp. nov., a zooxanthella: taxonomy, life cycle, and morphology. J. Protozool. 9:45-52.

Goreau, T. and Goreau, N. I. 1960. Distribution of labelled carbon in reef building corals with and without zooxanthellae. Science 131:668-669.

Gorycki, M. A. 1971. A compound embedding technique for the microscopical study of living and fossil foraminifera. Micropaleontology 17(4):492-500.

Grell, K. G. 1958. Untersuchungen über die Fortpflanzung und Sexualitat der Foraminiferen. III. *Glabratella sulcata*. Arch. Protistenk. 102:449-472.

Hedley, R. H., Parry, D. M., and Wakefield, J. St. J. 1967. Fine structure of *Shepheardella taeniformis* (Foraminifera: Protozoa). J. Roy. Microscop. Soc. 87:445-456.

Hemleben, C. 1969. Zur Morphogenese planktonischer Foraminiferen. Zitteliana 1:91-133.

Hovasse, R. 1923. Les péridiniens intracellularies--zooxanthelles et *Syndiniums*--chez les radiolaires coloniaux. Remarques sur la reproduction des radiolaires. Bull. Soc. Zool. France 48:247-254.

Jones, J. I. 1968. The relationship of planktonic foraminiferal populations to water masses in the western Caribbean and lower Gulf of Mexico. Bull. Mar. Sci. 18:946-982.

Kevin, M. J., Hall, W. T., McLaughlin, J. J. A., and Zahl, P. A. 1969. *Symbiodinium microadriaticum* Freudenthal, a revised taxonomic description, ultrastructure. J. Phycol. 5:341-350.

Leadbeater, B. and Dodge, J. D. 1966. The fine structure of *Woloszynskia micra* sp. nov., a new marine dinoflagellate. Br. Phycol. Bull. 3:1-17.

Lee, J. J., Freudenthal, H. D., Muller, W. A., Kossoy, V., Pierce, S., and Grossman, R. 1963. Growth and physiology of foraminifera in the laboratory. Part 3--Initial studies of *Rosalina floridana* Cushman. Micropaleontology 9:449-466.

________, Freudenthal, H. D. Kossoy, V., and Bé, A. 1965. Cytological observations on two planktonic foraminifera, *Globigerina bulloides* d'Orbigny, 1826, and *Globigerinoides ruber* (d'Orbigny, 1839) Cushman 1927. J. Protozool. 12:531-542.

________, and McEnery, M. E. 1970. Autogamy in Allogromia laticollaris (Foraminifera). J. Protozool. 17:184-195.

________, and Zucker, W. 1969. Algal flagellate symbiosis in the foraminifer Archais. J. Protozool. 16:71-81.

Lengsfeld, A. M. 1969a. Zum Feinbau der Foraminifere Allogromia laticollaris. I. Mitteilung: Zellen mit ausgestrecten und eingezogenen Rhizopodien. Helgoländer Wiss. Meeresunters. 19:230-261.

________. 1969b. Zum Feinbau der Foraminifere Allogromia laticollaris. II. Mitteilung: Ausgestreckte und durch Abreissen isolierte Rhizopodien. Helgoländer Wiss. Meeresunters. 19:262-283.

________. 1969c. Nahrungsaufnahme und Yerdauung bei der Foraminifere Allogromia laticollaris. Helgoländer Wiss. Meeresunters. 19:385-400.

Lipps, J. H. and Ribbe, P. H. 1967. Electron-probe microanalysis of planktonic foraminifera. J. Paleontol. 41:492-496.

Marszalek, D. S. 1969. Observations on Iridia diaphana, a marine foraminifer. J. Protozool. 16:599-611.

Moss, M. L. as addendum in Be, A. W. H. and Ericson, D. B. 1963. Aspects of calcification in planktonic foraminifera (Sarcodina). Ann. N.Y. Acad. Sci. 109:65-81.

Muscatine, L. 1967. Glycerol excretion by symbiotic algae from corals and Tridacna and its control by the host. Science 156:516-519.

________, Karakashian, S. J., and Karakashian, M. W. 1967. Soluble extracellular products of algae symbiotic with a ciliate, a sponge, and a mutant hydra. Comp. Biochem. Physiol. 20:1-12.

Patrizi, G. and Poger, M. 1967. The ultrastructure of the nuclear periphery. The zonula nucleum limitans. J. Ultrastruct. Res. 17:127-136.

Phleger, F. B. 1960. Ecology and distribution of recent foraminifera. Johns Hopkins Press, Baltimore. 297p.

Provasoli, L., Yamasu, T., and Manton, I. 1968. Experiments on the resynthesis or symbiosis in Convoluta roscoffensis with different flagellate cultures. J. Mar. Biol. Ass. U. K. 48:465-479.

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

Rhumbler, L. 1909. Die Foraminiferen (Thalamophoren) der Plankton-Expedition. Humbolt-Stiftung Plankton-Expedition, Ergebn. 3:1-476.

Schewiakoff, W. T. 1926. Die Acantharia des Golfes von Neapel. Faune u. Flora Golfes Neapel 37:1-755.

Sverdrup, H. U., Johnson, M. W., and Fleming, R. H. 1942. The Oceans. Prentice-Hall, Englewood Cliffs. 764; 821-823.

Taylor, D. L. 1968. In situ studies on the cytochemistry and ultrastructure of a symbiotic marine dinoflagellate, J. Mar. Biol. Ass. U. K. 48:349-366.

Towe, K. M. 1971. Lamellar wall construction in planktonic foraminifera. Proc. II Plankt. Conf., Roma, 1970. A. Farinacci, Ed. 1213-1224.

Trench, M. E., Trench, R. K., and Muscatine, L. 1970. Utilization of photosynthetic products of symbiotic chloroplasts in mucus synthesis by Placobranchus ianthobapsus (Gould), Opistobranchia, Sacoglossa. Comp. Biochem. Physiol. 37:113-117.

Winter, F. W. 1907. Zur Kenntnis der Thalamophoren. I. Untersuchung über Peneroplis pertusus (Fårskal). Arch. Protistenk. 10:1-113.

Yonge, C. M. 1944. Experimental analysis of the association between invertebrates and unicellular algae. Biol. Rev. 19:68-80.

LIST OF FIGURES

All figures are of Globigerinoides ruber, except for Figures 22 - 24, 31 - 37, 51, and 56, which are of Globigerina bulloides; Figures 17, 28, and 29 are of Globigerinoides conglobatus and Figures 48 and 58 are of Globigerinita glutinata. Magnification data represents original magnification and enlargement.

Figure

1. Overall view of chambers, test layers (tl), and exothalamous protoplasm (ep). Arrows indicate portions of exothalamous protoplasm extending into pores. Many sea water vacuoles (v) are present. Test layers were originally calcified but are now electron-transparent due to decalcification. X2,350.

2. Internal boundary between three chambers. Note test layers (tl), somatic nucleus (sn), symbionts (s), and fibrillar channels (f). Note deeply-stained granular inclusions in vacuoles (arrows). X2,600.

3. Test. Note fibrous body (fb), cell membrane (cm), calcification loci (cl) associated with membranes (arrows), and inner calcification layer (icl). X42,700.

4. Detail of portion of protoplasmic island at distal end of pore in Fig. 1. X4,525.

5. Foramen, with inner calcification layer (icl). X8,700.

6. Protoplasmic island in exothalamous protoplasm. Note fibrous body, vesicles (ve), mitochondria (m), and small length of outer calcification layer (ocl). Glycocalyx (arrows) is only present on non-island portions of outer membrane layer. X27,200.

7. Detail of external membrane of protoplasmic island in Fig. 6. Note glycocalyx. X88,500.

8. Longitudinal section of pore showing protoplasmic extrusion (arrow). X34,000.

Figure

9. Oblique section of pore showing three protoplasmic extrusions. X19,850.

10. Membranes of fibrillar system channel and neighboring symbiont vacuole (arrow). The thin symbiont periplast layer is a fragile rudimentary theca (th). X25,000.

11. Fibrillar system strands. Note woolly texture and fibrillae (arrows). X73,250.

12. Peripheral oblique section through end-pocket of fibrillar channel. Note thicker banded fibrils. X4,575.

13. Fibrillar channel with end-pocket. Channel is surrounded by mitochondria (m), fibrous bodies, and an oval body (ob). Symbiont at upper right has large number of starch granules. X6,600. Inset. Detail of thinner end-pocket fibrils. X28,600.

14. Fibrils in loose apertural protoplasm. They are bounded by symbionts and islands of host protoplasm (h). X6,100.

15. Cross-section of fibrillar channel, showing helical fibrils. X1,550.

16. Branched fibrillar channel endings. Note reticuloid mass of fibrils (rm). X8,575.

17. Food vacuole, containing deeply-stained digested material. Note membrane (arrow). X42,000.

18. Deeply-stained diatom frustules in food vacuole. Note host mitochondria to right of vacuole. X10,300.

19. Pycnotic somatic nucleus. Compare with Fig. 2. X6,100.

20. Generative nucleus. X22,600.

21. Generative nucleus with condensed chromatin in equatorial region. X27,600.

22. Somatic nucleus, occupying most of the volume of a chamber. Note chromosomes (arrows). X1,900.

23. Closeup of somatic nucleus showing glycocalyx-like border (arrows), perinuclear aggregates (pa), and chromosomes (ch). X13,500.

24. Spindle fibers in large somatic nucleus. Deeply-stained bodies are chromosomes. X12,600.

Figure

25. Polyenergid chromosome aggregates (pc) and annulated honeycomb-like structures in large somatic nucleus. X4,575.

26. Honeycomb-like nuclear structure. X10,550.

27. Cryptosome. X4,800.

28. Cryptosome. X3,500.

29. Annulated vesicular border of cryptosome in Fig. 28. Note view of homogeneously granular internal portion of cryptosome in upper part of photomicrograph. X25,000.

30. Banded figure (bf) in vesicle next to fibrillar channel. Note peripheral arrangement of fibrils in channel. X6,700.

31. Unextended reticulopod in pore. Note pore diaphragm (pd), protoplasmic island (pi) and its continuation as exothalamous protoplasm (ep), inner calcification layer (icl), primary calcification layer (pcl), outer calcification layer (ocl), oval bodies. Note many mitochondria near cell membrane (cm). X73,700.

32. Reticulopod closely-associated with spine. Note central clear space (cc), formerly occupied by spine. Oblique section. X2,530.

33. Base of extended reticulopod with ruptured outer calcification layer. X12,600.

34. Tip of reticulopod. Microtubules (mt) are arranged in parallel bundles. X22,100.

35. Reticulopod base with oval bodies, tubular endoplasmic reticulum (ter), fibrous body, mitochondria, vesicle, and central clear space (cc). X14,200.

36. Anastomosed apertural reticulopod material. Note test (t) and oval bodies. X6,500.

37. Mitochondrion with villi-like cristae (arrow). X57,500.

38. Fibrous body, ribosomes (r), and tubular endoplasmic reticulum. X43,750.

39. Golgi apparatus. X6,455.

40. Symbionts in loose and dense protoplasm. X3,750.

41. Lamellae (1) penetrating pyrenoid. Note starch sheath (ss). X17,000.

42. Pseudoencysted symbiont. X7,850.

Figure

43. Symbiont in loose apertural protoplasm. Note chloroplast lamellae penetrating pyrenoid (py) which is surrounded by dense starch sheath. Nucleolus (nu) is denser peripherally. Note also starch granules (sg), lipid vacuole (lv), and mitochondria (m) located between nucleus and chloroplasts. X9,500.

44. "Pre-motile" symbiont with girdle. X8,675.

45. Symbiont with one end sharply-tapered. Note accumulation body (ab) and osmiophilic globules (arrows). X10,000. Inset. Detail of accumulation body. X18,250.

46. Pore in nuclear envelope of symbiont (arrow). X69,600.

47. Symbiont chromosomes. Note large granules and fibrils arranged in sigmoid fashion. X69,300.

48. Microtubules in cell wall. Note chloroplast membrane (arrow). X34,000.

49. Chloroplast with possible DNA helices (arrows), ribisomes (r), two- and three-thylakoid lamellae. X94,000.

50. Calcium oxalate crystals (Ca). X86,600.

51. Symbiont mitochondrion. X25,800.

52. Closeup of Fig. 44. Two flagellar bases with rootlet (rt) and pusule (pu). One is emerging into the flagellar pore (arrow). X40,700.

53. Nuclear membrane (nm), rough endoplasmic reticulum (rer), and polysomes. X21,700.

54. Periplast membranes making up inner layer (il), rudimentary theca (th), and cell wall (cw). X86,600.

55. Vesicles formed by periplast infolding (arrows). X2[illegible],500.

56. Cell wall adhering to host protoplasmic island (h). X25,800.

57. Symbiont vacuole membrane with pores (arrows). X25,000.

58. Dividing symbiont. Note newly-formed cell wall. X88,700.

59. Protoplasm of *Globigerinoides ruber*. Note fibrillar channels (fc), containing banded and unbanded fibrils. Banded fibrils, which are most numerous in channel end-pocket, have numerous fibrillae (fi). Note also cryptosome with its granular matrix and vesicular border. Also shown is the large somatic nucleus, with nuclear folds, perinuclear

Figure

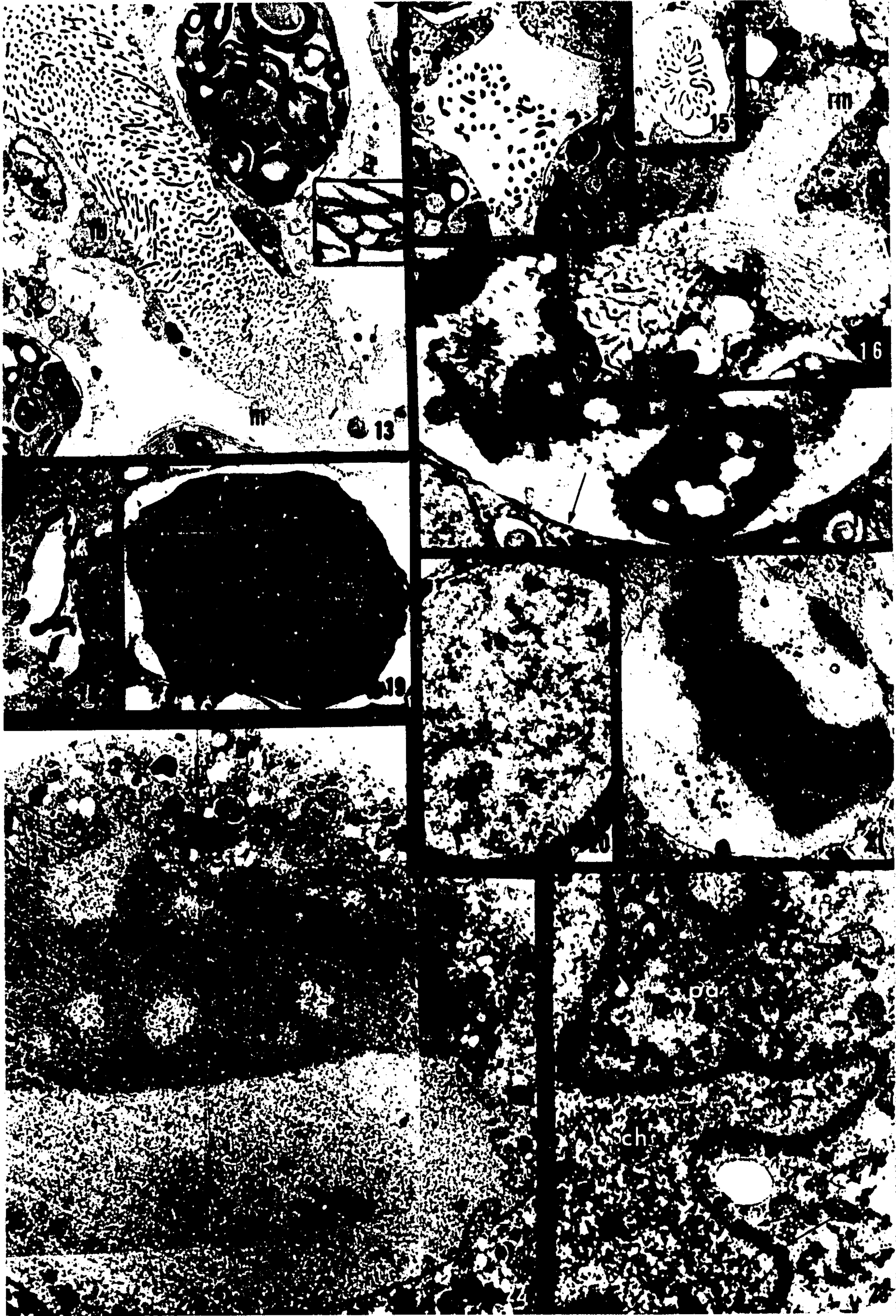
aggregates (pa), ribosomes (r), chromosomes (ch), and nuclear envelope (ne). This section also includes portions of protoplasm (pr). Other structures present in the foraminiferan protoplasm include small somatic nuclei (sn), symbionts (s), food vacuoles (fv), Golgi apparatus (G), and sea water vacuoles (v).

60. Mature pore. Longitudinal section showing pore diaphragm (pd) with two protoplasmic extrusions. Inner calcification layer (icl), primary calcification layer (pcl), and outer calcification layer (ocl) merge to form pore diaphragm (pd) at constricted point along length of pore. Between the inner and primary calcification layers is the inner lamellar unit (ilu) of the test. The outer lamellar units (olu) are more distally located. Note exothalamous protoplasm (ep) with glycocalyx (gly), and the continuation of this layer as a protoplasmic island (pi) in the distal portion of the pore.

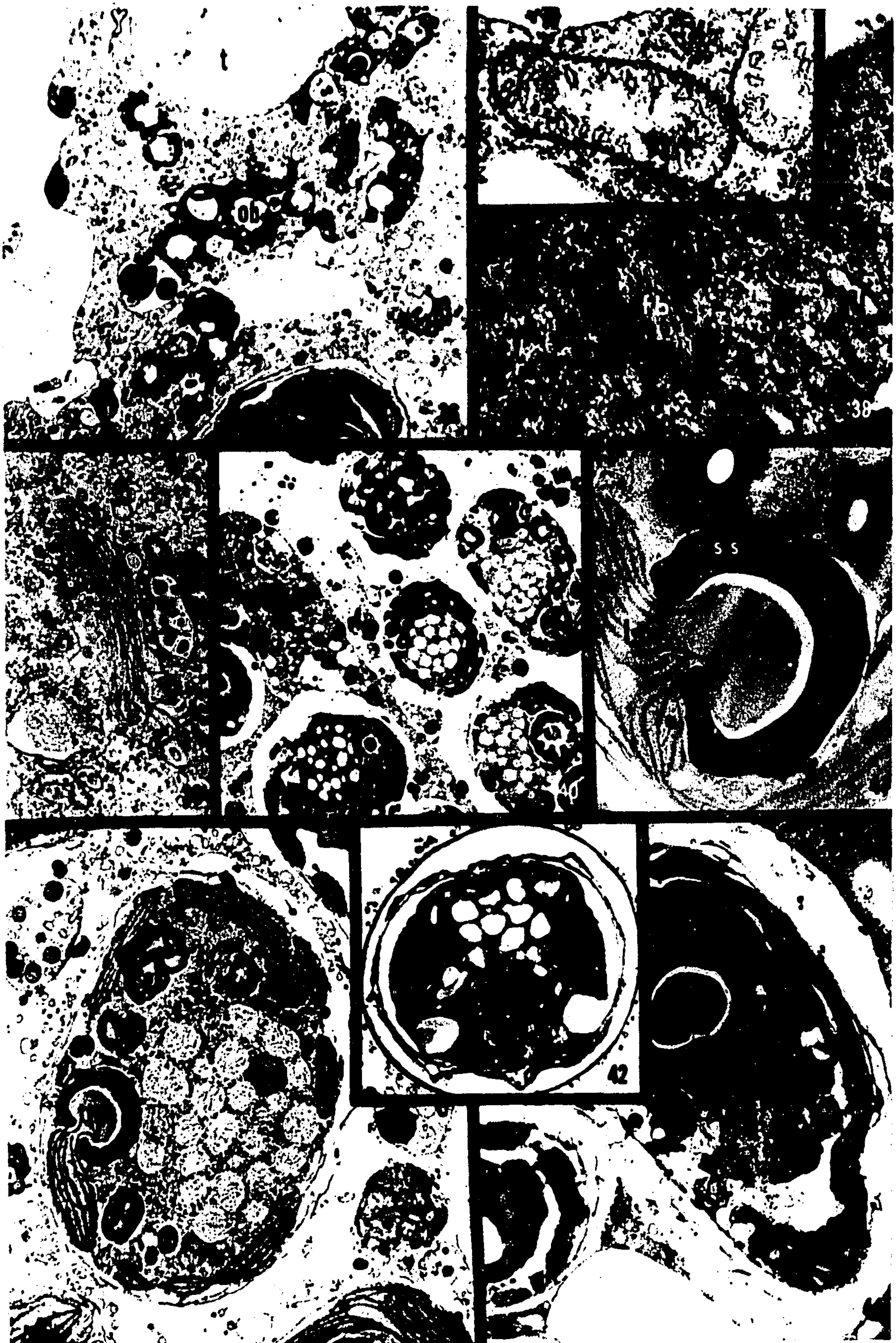
61. Symbiont. Ventral view of "pre-motile" form. Note typical dinoflagellate chromosomes (ch) and girdle (g). Chloroplast lamellae (l) penetrate pyrenoid (pyr), which is surrounded by a deeply-staining starch sheath (ss). Chloroplasts (chl) are peripheral. Mitochondria, starch granules (sg), the nucleolus (nu) and lipid vacuoles (lv) are also shown.

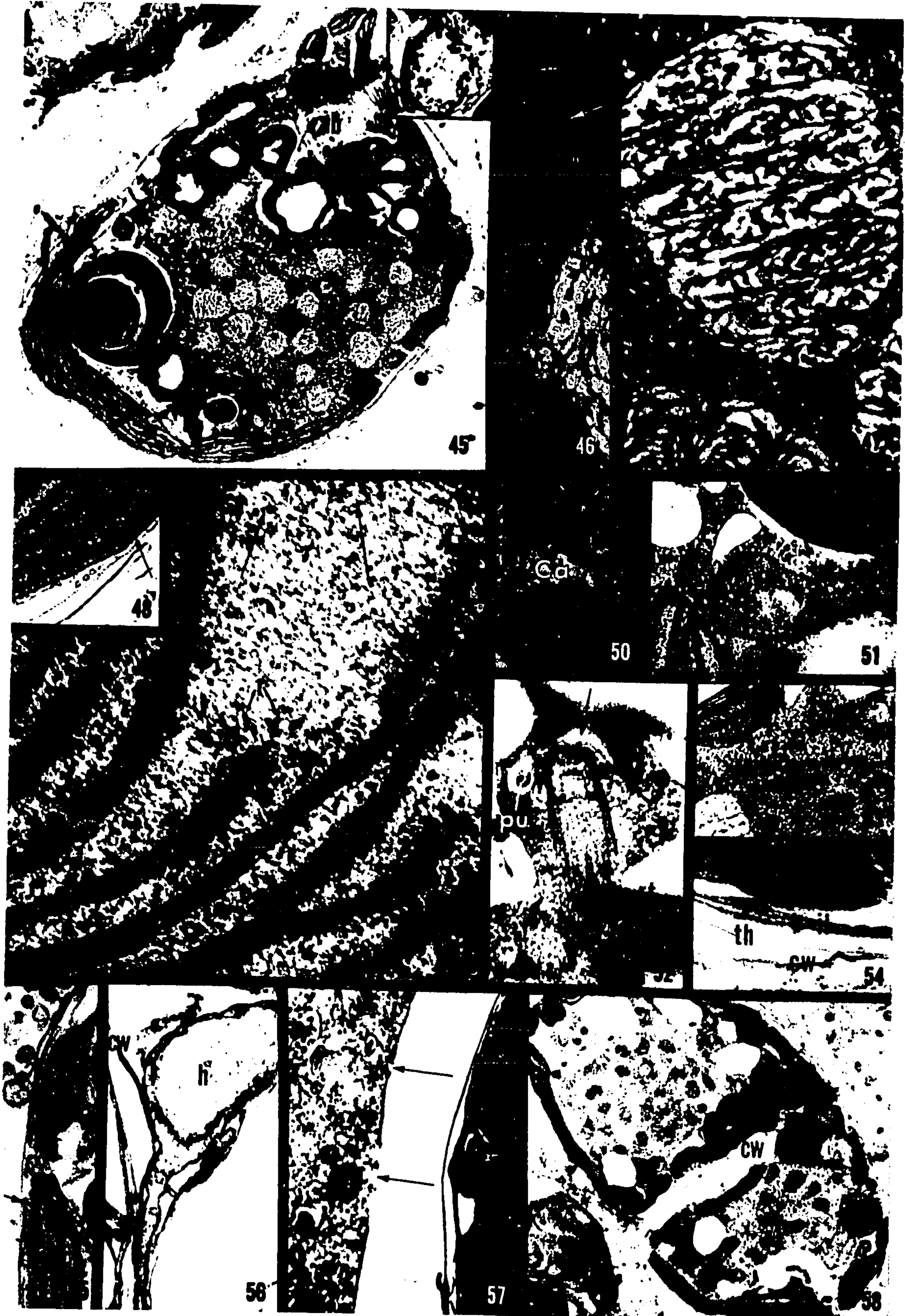
62. Symbiont. Dorsal view of "pre-motile" form.

ep
tl
icl
ocl
6
7
9
10
8
11
12


rm
15
16
13
19
20
21
pa
Sch
23

pc
24
25
26
28
29
bf
ocl
pi
ob
ep
pd
ob
pcl
icl
cm
m
cc
ocl
33
m
34
m
ter


t
ob
38
s s
42


45
46
48
Ca
50
51
pu
th
cw
54
cw
h
56
57
cw
58

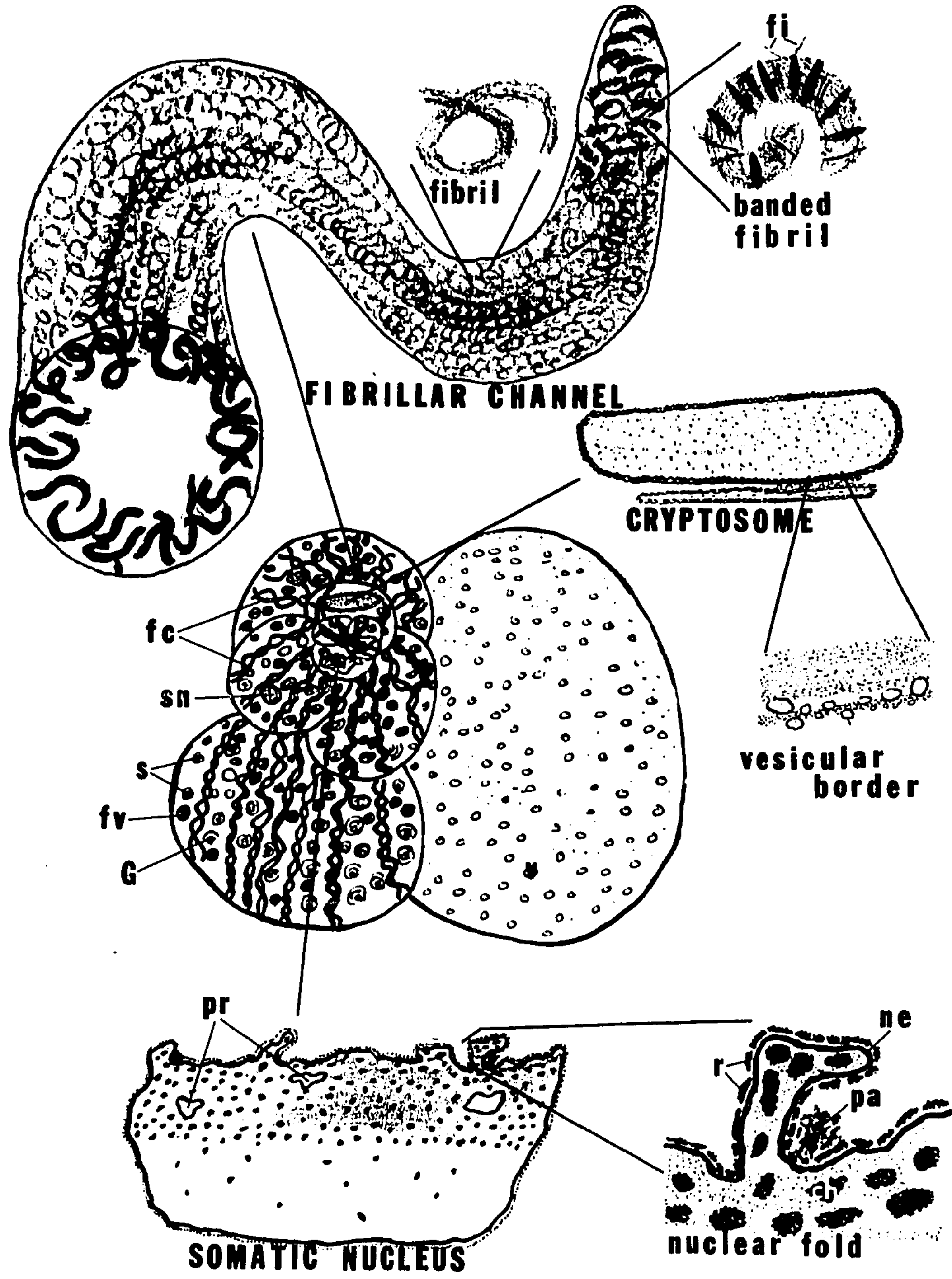


FIGURE 59. Globigerinoides ruber

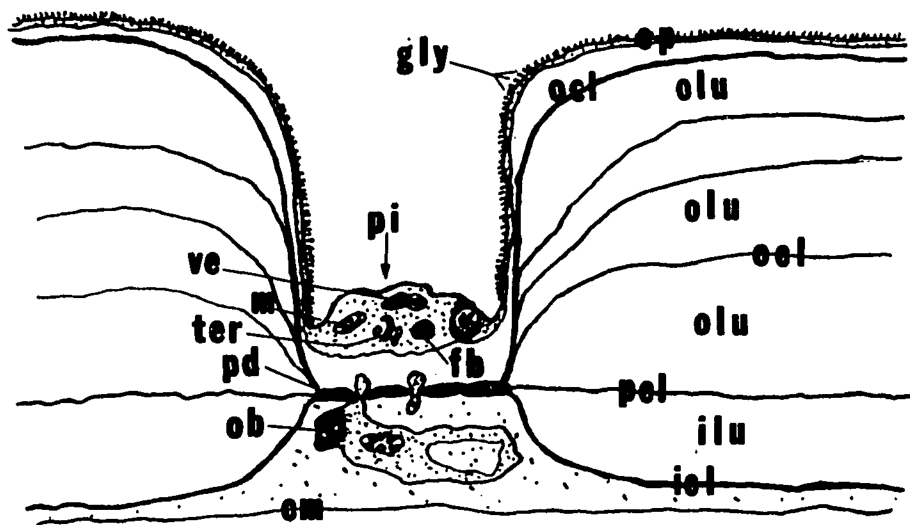


FIGURE 60. MATURE PORE

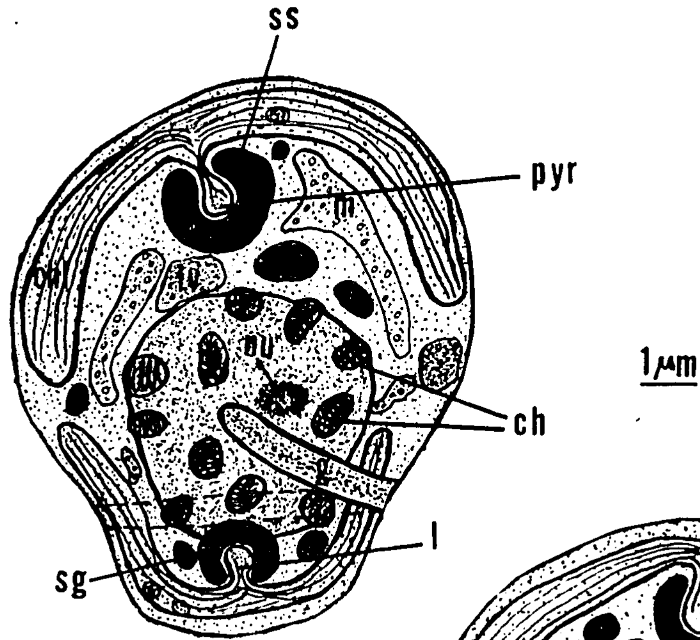


FIGURE 61. SYMBIONT.
VENTRAL VIEW.

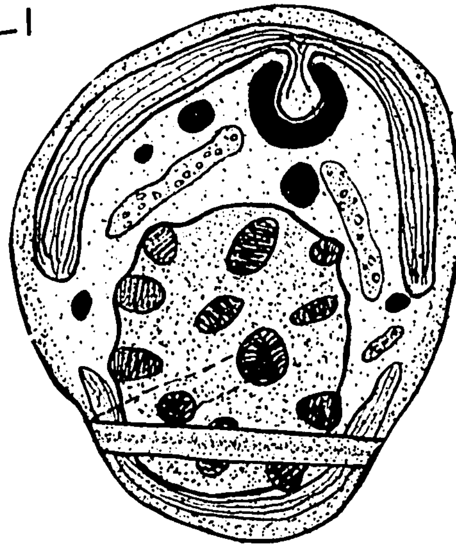


FIGURE 62. SYMBIONT.
DORSAL VIEW.