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## CONSTRUCTIONAL ASPECTS IN TEST FORMATION OF SOME AGGLUTINATED FORAMINIFERA

by

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With 2 plates

### ZUSAMMENFASSUNG

REM- und TEM-Untersuchungen an mehreren agglutinierten Foraminiferen, die in Laborkulturen gehalten wurden, ergaben morphologisch verschiedenartige Ausbildungen des organischen und kalzitischen Zements. Der organische Zement kann durch drei Morphotypen charakterisiert werden: 1) Isolierte Stränge, die graduell in ein 2) fibröses Netzwerk von Strängen übergehen können; 3) schaumähnlicher Zement.

Die agglutinierten Partikel sind mit einer organischen Lage umhüllt, die anzeigt, daß die Partikel innerhalb des Zytoplasmasystems inkorporiert waren. Entsprechende Hüllen wurden bei agglutinierten Foraminiferen mit kalzitischem Zement beobachtet.

### ABSTRACT

High resolution SEM and TEM studies of some species of agglutinated foraminifera raised in laboratory cultures show various morphologies of organic and calcareous cement. The organic cement is organized in three ways: (1) either in single strands, which may gradually pass over to (2) a fibrous meshwork of strands, or (3) in a foam-like mass. The agglutinated grains are coated with an organic envelope suggesting a former incorporation into the cytoplasmic system. Similar envelopes were found in agglutinated foraminifera with calcareous cement, also cultured under laboratory conditions.

### INTRODUCTION

The capability to secrete a protective calcareous test seems to be an advanced feature among the foraminifera as these types occur rather late (Carboniferous) in the geological record. The arenaceous foraminifera, one of the earliest foraminiferal groups (? Late Precambrian: Riding and Brasier 1975; Hansen 1979), form their test by agglutinating foreign particles. These particles are held together by biogeneous cements which usually contain organic compounds. However, various authors described calcareous cements in early agglutinated foraminifera, inferring a carbonate secreting system of the foraminiferal cell. This would imply an early development of a calcifying

system, although the capability to form an entirely calcareous test did not "evolve" before the Carboniferous.

In order to evaluate the differences between a "normal" calcifying system based on matrices and a system which secretes organic and calcareous cements, we started a program to investigate cements in living agglutinated foraminifera. Since the early 1960s, the nature of organic cements has attracted considerable attention in view of its taxonomic and ecological significance. Although various descriptions have been published, only a few presented detailed investigations on the cement itself (Hedley 1963; Murray 1973; Hansen and Hanzliková 1974; Jørgensen 1977).

In this study, we will discuss the morphology of the organic cement in some agglutinated species from different environments.

## MATERIAL AND METHODS

Living agglutinated foraminifera were collected from the intertidal zone of the North Sea coast (north of Nordenham, Weser estuary), and the Kenya coast (30 km south of Mombasa and 120 km north of Mombasa (Watamu)), from near shore sediments of the Gulf of Aqaba (10 km south of Aqaba), from Bermuda (Ferry Reach) and from New York Bight (Lawrence Bay, Nassau County; New York). Several methods were used to prepare both living and dead specimens for scanning electron microscopy (SEM). Before being mounted on SEM stubs, specimens were either: 1) washed in distilled water and air-dried; 2) critical-point-dried in liquid CO<sub>2</sub> after being fixed in 3% glutaraldehyde containing 0.1 M cacodylate buffer prepared in seawater, postfixed in 2% osmium tetroxide prepared in the same buffer and dehydrated in graded ethanol series; or 3) SEM freeze-etched (plate 1, figure 1).

Living, as well as fixed specimens, were put in a pair of Balzers double replica rivets and frozen in nitrogen slush. They were fractured manually under liquid nitrogen in a Balzers double fracture device. Inside a specimen holder, the probe was transferred to the freezing chamber of an Emscope Cryo sputter in order to attach the holder to the transfer unit. The chamber was under atmospheric pressure and fluted with argon while the nitrogen evaporated. The specimen holder was retracted into the transfer unit, kept under argon, and then docked to the pre-cooled working chamber. Here the specimen was etched in vacuum and sputter-coated with Au/Pd. Inside the transfer unit, the probe was introduced onto a stage of the scanning electron microscope and kept under liquid nitrogen during observation (Bardele, Huttenlauch and Schoppmann, in press).

Initially, transmission electron microscopy (TEM) was precluded in several species because it appeared almost impossible to dissolve the siliceous agglutinated particles of the test without destroying the organic material. An alternative solution was found by culturing specimens on a calcareous substrate.

Foraminifera with newly formed calcareous chambers were then fixed as described above, decalcified in EDTA, dehydrated in graded acetone series and embedded in Epon 812 medium. Oriented serial sections were made with an ultramicrotome, collected on copper grids and stained with uranyl acetate followed by lead citrate.

All methods applied produced the same results, inferring that no formation of artifacts occurred. Fixation was carried out to prevent post-mortem alterations, critical-point-drying was applied to preclude surface tension which may occur during air-drying, and freeze-etching SEM to determine possible effects of chemical fixation. An additional check was made by studying specimens in the SEM as well as in the TEM.

The following organically agglutinating species were studied:

*Jadammina macrescens* (Brady, 1870); New York.  
*Miliammina fusca* (Brady, 1870); Nordenham.  
*Miliammina oblonga* var. *sabulosa* Rhumbler, 1936; New York.  
*Trochammina inflata* (Montagu, 1808); New York.  
*Trochamminopsis* sp.; Mombasa.  
*Paratrochammina* sp.; Bermuda.  
*Spiroplectammina earlandi* Parker, 1952; Aqaba.

Calcitic cementing species:

*Clavulina nodosaria* d'Orbigny, 1839; Watamu.  
*Clavulina angularis* d'Orbigny, 1826; Watamu, Bermuda.  
*Clavulina difformis* Brady, 1884; Watamu.  
*Clavulina tricarinata* d'Orbigny, 1839; Bermuda.  
*Textularia aperturalis* Cushman, 1911; Watamu.  
*Textularia candeiana* d'Orbigny *kenyaensis* Banner and Pereira, 1981; Watamu.  
*Valvulina oviedoiana* d'Orbigny, 1839; Bermuda.

Species identified only on the generic level are to be designated in a later paper as these are probably new species.

## RESULTS

Our current observations indicate that chamber formation is completed in 24 hours in laboratory cultures. Prior to the chamber formation the foraminifer forms a protective cyst of detrital particles in which the chamber formation takes place. The constituents for the new chamber are collected from the cyst material by pseudopods, then organically enveloped and finally cemented together with a calcareous or an organic cement.

### Organic cement

Three different morphological types of organic agglutinating material can be differentiated in sectioned specimens of the following groups:

Group 1: *Miliammina fusca* (plate 1, figures 1-5)  
 The test is composed of a very fine granular "matrix" (less than 3 µm grain size), in which a few, much larger grains (up to 20 µm), are embedded. Sectioned specimens show that the "matrix" consists

of grains joined by numerous organic strands filling the interstitial space (plate 1, figure 2). The strands are closely packed, covering the entire surface of each grain. The ends are often branched (plate 1, figure 3). Even particles of less than 1 µm in size are fixed in the same way. Strand length measures approximately 0.1 to 0.5 µm, depending on the texture of the joined grains (plate 1, figure 4). The diameter is in the order of 30 µm and seems to be rather constant. In the TEM the strands may exhibit electron-dense nodes half-way between the grains (plate 1, figure 5). Sometimes the nodes may occur pairwise, but their nature remains unknown. In addition, the surface of each grain is coated by an organic sheath hiding the crystal faces (plate 1, figures 4-5). The inner surface of the wall is separated from the cytoplasm by an inner organic lining (IOL) (plate 1, figure 2). An outer organic lining (OOL) is missing.

Group 2: *Trochammina inflata* (plate 1, figures 6,8; plate 2, figure 1)  
*Spiroplectammina earlandi* (plate 1, figure 7)

Two grain size classes are found in the test wall of this group. One measures less than 3 µm (about  $\frac{2}{3}$  of all grains), the other is considerably larger, up to 10 µm. The grains are cemented by a fibrous meshwork with interspaces of variable size (plate 1, figures 6-7). Depending on the distance between two adjacent grains, the agglutinating material shows a different morphology. Strands with branched ends are found between grains that are less than 0.5 µm apart (plate 1, figure 8). The morphology seems to be identical to the strands of group 1. Larger intergrain distances, ranging up to 2 µm, show grains joined by a three-dimensional network of strands. The grains are organically enveloped (plate 1, figure 6). In *T. inflata* and *T. earlandi* the test wall is covered on the outside with an OOL as well as exhibiting an IOL (plate 2, figure 1).

Group 3: *Jadammina macrescens* (plate 2, figure 5)  
*Trochamminopsis* sp. (plate 2, figures 2-4)  
*Paratrochammina* sp.  
*Miliammina oblonga* var. *sabulosa*

*M. oblonga* var. *sabulosa* and *Trochamminopsis* sp. possess a test wall composed of grains ranging from less than 1 µm to more than 50 µm in size. In this group, intergrain spaces of up to 4 µm are filled by an organic foam-like mass (plate 2, figures 2-3).

*J. macrescens* and *Paratrochammina* sp. agglutinate particles that are less than 20 µm in size, showing an intergrain space up to 2 µm filled with a foam-like mass (plate 2, figure 5). In all species, the densely packed bubbles themselves adhere to the grain surface by a fibrous meshwork of similar

morphology to that observed in group 2 (plate 2, figure 4). All species develop organic envelopes around the particles, such that cleavage planes and edges are not visible (plate 2, figure 3). Also, all species form an IOL while *Paratrochammina* sp. and *J. macrescens* show an additional OOL (plate 2, figure 5).

The agglutinating organic material which comprises single strands (group 1), fibrous meshwork (group 2) and foam-like masses (group 3), shows considerable interspecific variation, but remarkable consistency within each species. Transitions, however, between the types seen in groups 1 and 2 are observable (plate 1, figure 8).

Dissolution experiments, carried out by using concentrated hydrochloric acid and hydrogen peroxide, as well as low temperature ashing, demonstrate the stable condition of the organic material. Groups 1 and 2 appeared less sensitive to the treatment than did group 3. Tests of juvenile specimens as well as newly formed chambers became brittle, but adult ones showed no significant reaction. This points towards alteration processes during ontogeny.

To conclude, all species exhibit wall structures composed of grains coated with an organic envelope. The space between these grains is filled by either strands, a network, or a foam-like mass. None of these types show completely filled interspaces with a massive organic cement.

#### Calcitic cement

Specimens were cultured on a mono-substrate of chromite or carborundum grains. Hence, it was possible to differentiate between the secreted material and the agglutinated particles in newly formed chambers (plate 2, figure 6). Although no calcareous substrate was available, the mineralogical composition of the cement could be identified as calcite (plate 2, figure 7). Thus, we conclude that the cement has been biomineralized by the foraminifer itself. The same cement structure has been found in specimens from the natural environment (plate 2, figure 8). The agglutinated grains are coated by an organic envelope, therefore being incorporated into the cytoplasmic system (Bender and Hemleben, in press).

## DISCUSSION

The morphology of the organic cement has largely escaped the attention of previous authors because of the low magnifications used. Most organically agglutinating species have been described in the literature as having agglutinated grains embedded in extremely fine granular particles (Hansen and

Hanzliková 1974; Mendelson 1982), perhaps even consisting of colloidal-sized particles (Sliter 1968). Others were reported to construct their test by loosely cemented grains leaving a large intergranular space (Loeblich and Tappan 1964; Murray 1971, 1973). Delaca, Lipps and Hessler (1980) described an Antarctic species showing grains firmly bound by a "fibrous organic sheath", and Jørgensen (1977) examined organic material forming a "spongy matrix" as well as a "rather solid matrix of organic matter" in two Recent species. Organic material forms a "framework of membranes" which partially fills the intergrain space (Jørgensen 1977, plate 2, figure 9) in *Heterostomella foveolata*, a Maastrichtian species. The figured meshwork of this fossil species corresponds very closely to the organic meshwork (group 2) distinguished here. In their review of *Trochammina inflata*, Brönnimann and Whittaker (1984) illustrated a dissected test showing the same meshwork as described in group 2. Hedley (1963) found the organic interstitial substance of alcohol-preserved specimens to be present in the form of strands joining the grains. These were interpreted as artifact, as living species showed interstices to be completely occupied by a relatively strong gel. However, our observations demonstrate that artifact formation can be excluded. Air-drying, critical-point-drying, and freeze-etch SEM as well as TEM produced the same results.

Very little is known about the biochemical composition of the organic material. Hedley (1958, 1962, 1963) and Buchanan and Hedley (1960) considered the organic cement to be a glycoprotein. Chromatographic analysis, obtained in four species representing four genera, indicated that the amino acid content of the organic material showed no significant differences (Hedley 1963).

The stability of the organic material of groups 1 and 2 against chemical treatment, together with its morphology, suggests that it may be of a mucopolysaccharide nature. Additional chemical investigations are necessary to firmly establish this or other possibilities. Our dissolution experiments support Towe's (1967) and Lipps (1973) assumption that the biochemical structure of the organic substance changes during ontogeny, resulting in the final stabilized condition and group specific morphology of the organic matter in adult individuals. These observations are essential to assess the preservation potential of organically agglutinating foraminifera. With reference to Jørgensen's (1977) observation of intact cement in Maastrichtian species, the organic meshwork must be extremely resistant or diagenetically stabilized to persist over the past 70 million years.

The morphology of the organic cement seems to be of systematic and, hence, of phylogenetic importance as identical cement structures are found in species belonging to different genera. Furthermore, the nature of the cement appears to be important from an ecological point of view and may be applied to reconstruct paleoenvironments. Agglutinated foraminifera with organic binding material generally occupy hyposaline and normal saline environments, while those with calcareous cements are restricted to normal and hypersaline environments. This is claimed to be due to the amount of free  $\text{Ca}^{2+}$  in the sea water (Pokorny 1958; Lipps 1971; Murray 1973). The results of the present study do not support this view with respect to species with organic cement. The latter were found in hyposaline (Nordenham, New York), normal saline (Mombasa, Bermuda) as well as hypersaline (Aqaba) environments. On the other hand, calcareously cementing species were not found in hyposaline environments. SEM investigations on deep sea species from the Antarctic realm showed only organic cements. All three morphological cement groups were detected from sites below the Calcite Compensation Depth (CCD) (Hemleben, unpublished data). Weston (1984) suggested that species which normally secrete a calcareous cement reduce the cement when they live below the CCD. Thus, it seems that the calcifying system in agglutinated foraminifera is somehow dynamically controlled by environmental effects on genetically controlled processes. We do not know at present whether there is suppression of calcite deposition or if the organism deposits calcite in the organic matter, but it is dissolved owing to the pressure effects below the CCD.

Further experimental work should clarify the mode of test formation, essential to assess the preservation potential of agglutinated foraminifera and establish their value in group systematics and phylogeny. Several experiments and further investigations, especially on the calcitic cement (Bender and Hemleben, in press), are under way and will be published elsewhere.

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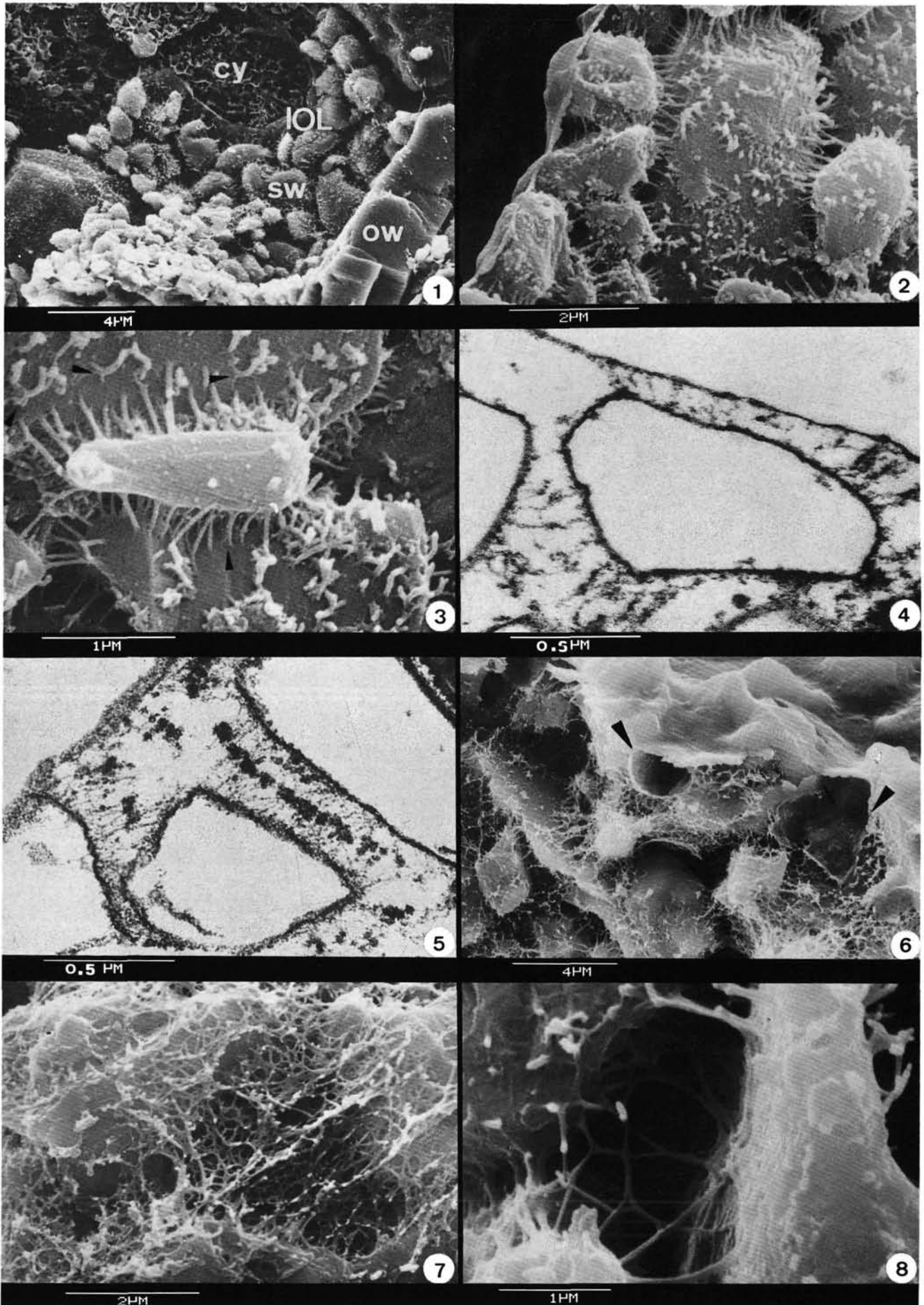
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PLATE 1

- Figure 1 *Miliammina fusca*; freeze-etch scanned specimen cultured on natural substrate. The cytoplasm (cy) is separated from the wall by an inner organic lining (IOL). Within the sectioned wall (sw), all particles are covered by densely packed strands joining the grains. On the outer test surface (ow), one can hardly observe the organic structures.
- Figure 2 Same species. Part of the sectioned wall showing the IOL and particles agglutinated by organic strands. Specimen cultured on natural substrate.
- Figure 3 Same specimen as shown in figure 2. High magnification of a particle, surrounded by numerous single strands. Due to sectioning strand-stumps with branched ends remain on the grain surface (arrows).
- Figure 4 Same species. TEM-micrograph; although the test was completely decalcified, the originally calcareous particles can be distinguished by their organic envelopes. Particles are agglutinated by strands. Specimen cultured on calcareous substrate.
- Figure 5 Same species. TEM-micrograph; the strands may exhibit electron-dense nodes half-way between the grains. These nodes have never been observed in the SEM. Specimen cultured on calcareous substrate.
- Figure 6 *Trochammina inflata*; a fibrous meshwork fills the intergrain space. Particles which were broken out leave organic envelopes (arrows). Specimen cultured on natural substrate.
- Figure 7 Same specimen as shown in figure 6. Higher magnification of the dense meshwork in which the grains are agglutinated.
- Figure 8 *Trochammina inflata*; single strands join grains within intergrain space of less than 0.5  $\mu\text{m}$ . Within larger distances they intertwine among themselves to form a three-dimensional network. Specimen cultured on natural substrate.

All specimens, except that in figure 1 were air-dried and sectioned.

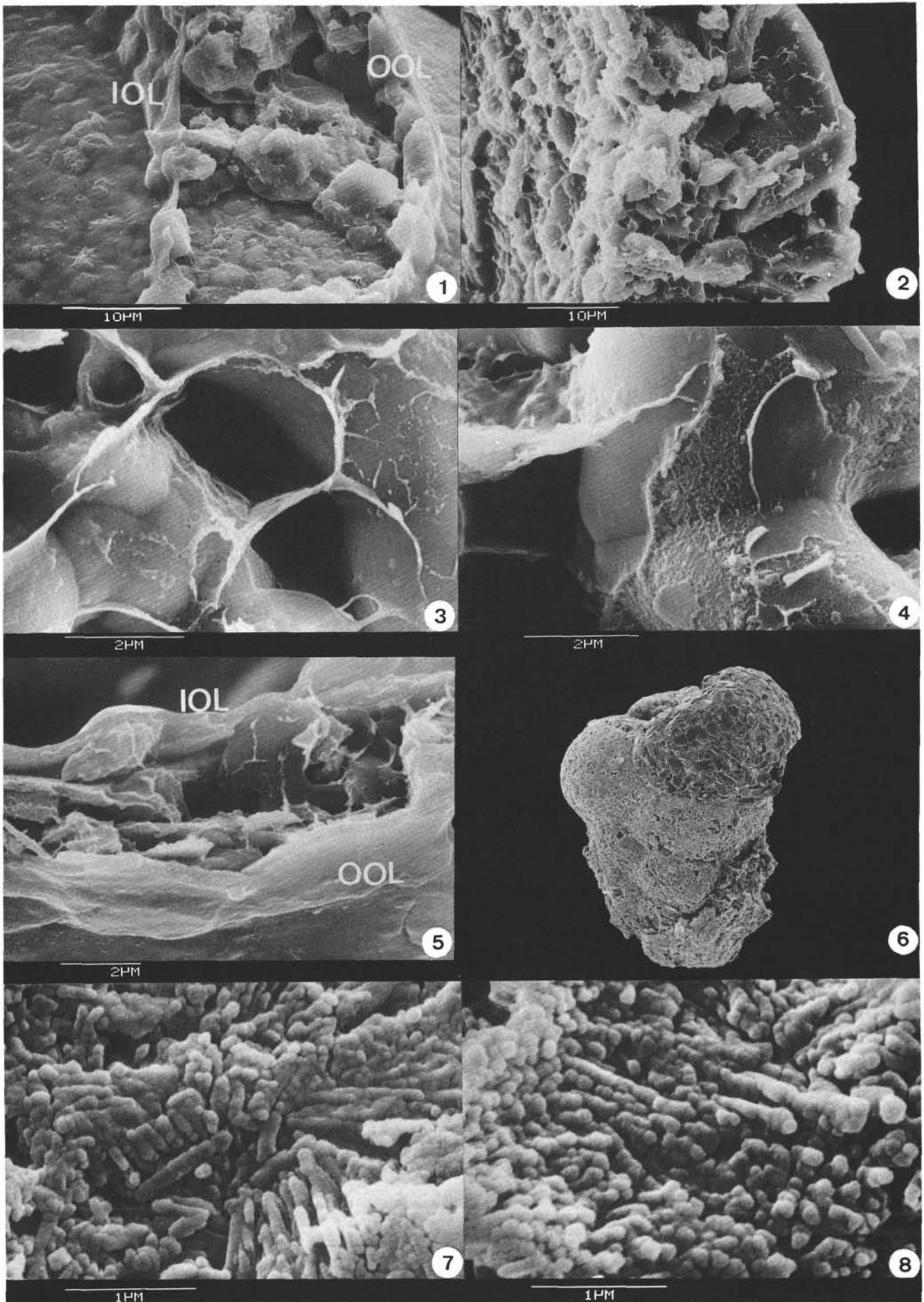


## PLATE 2

- Figure 1 *Trochammina inflata*; the test is covered by an IOL (inner organic lining) as well as an OOL (outer organic lining). Therefore, the meshwork is not visible on the outer test surface. Specimen cultured on natural substrate.
- Figure 2 *Trochamminopsis* sp.; a foam-like mass agglutinates the particles. Specimen cultured on natural substrate.
- Figure 3 Same species. Between two adjacent chromite grains the densely packed bubbles can be distinguished; particles are organically enveloped. Specimen cultured on a mono-substrate of chromite.
- Figure 4 Same specimen as shown in figure 3. Due to sectioning, the organic material is peeled off. It adheres to the grain surface by a meshwork.
- Figure 5 *Jadammina macrescens*; the sectioned chamber wall is covered by an IOL as well as an OOL. Specimen cultured on natural substrate.
- Figure 6 *Valvulina oviedoiana*; a newly formed chamber consists exclusively of carborundum particles and secreted calcite cement, x65.
- Figure 7 Same specimen as shown in figure 6. Detail of the calcareous cement of newly formed chamber built on a non-calcareous substrate in laboratory culture.
- Figure 8 Same species. Detail of the cement's ultrastructure of a chamber being formed in the natural environment of Bermuda.

All specimens air-dried and sectioned.





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