THE EXPERIMENTAL SILICIFICATION OF MICROORGANISMS

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ABSTRACT. A number of samples containing microorganisms was silicified at atmospheric and deep-sea pressures with the aim of studying the process of fossilization using SEM and TEM techniques. The samples included a bacteria-fungi-diatom culture, a bacteria-diatom culture, a microorganism-rich water sample from the interface of south-eastern Atlantic deep-sea sediments, and a microbial mat from the surface of other south-eastern Atlantic deep-sea sediments. Silicification commenced with the impregnation of organic material (e.g. cell walls, cytoplasm) by subelectron-microscope-sized crystallites, and the nucleation of spheres of porous hydrated silica within the mucus (extracellular polymeric substances, EPS) of the groundmass. With increasing silicification time the silica precipitates became more electron dense (due to hydrolysis and polymerization) and larger, forming thicker deposits which resulted in an encrusted mammillated surface on the microbial fossils. Eventually, the microorganisms were completely engulfed by the siliceous deposits. The formation of artefacts during fossilization, such as collapsed, silicified cytoplasm, looking like false nuclei in the bacteria, was common. These experiments were undertaken in order to understand the process of silicification in nature. In particular, with respect to the exceptionally well-preserved, silicified microbial mat communities in uppermost Oligocene to middle Miocene sediments from the south-eastern Atlantic, they demonstrated that both the microorganisms and the polymeric slime in which they lived were mineralized. The degree of mineral impregnation and encrustation is related to the availability of the mineralizing ions. The heavily encrusted microorganisms in each of the upper Oligocene to middle Miocene samples from the south-eastern Atlantic Ocean were thus probably subjected to mineralization for a longer period than weakly impregnated/encrusted microorganisms from the same sample.

REPORTS of silicified microbial remains, consisting of coccoid and filamentous carbonaceous structures, are relatively common in Precambrian cherts (e.g. Schopf 1974; Awramik *et al.* 1983; Hofmann and Schopf 1983; Walsh 1992). Palaeoecological interpretations suggest that they represent the remains of microbial communities in shallow marine waters which became embedded in a colloidal silica gel and were thus entombed. Observations of silicified microorganisms in Phanerozoic rocks are, however, less common. Fungi in silicified peat of Devonian and Miocene to Pliocene ages were described by Knoll (1985), and possible cyanophytes in silica nodules of middle Cretaceous age by Carson (1988, as reported in Carson 1991). Rare examples of exceptionally well preserved, silicified deep-sea microbial mats in uppermost Oligocene to middle Miocene diatomaceous sediments from the south-eastern Atlantic Ocean (Text-fig. 1) were described by Monty *et al.* (1991) and Westall (1994). These are, to date, the only report of silicified deep-sea bacteria.

It was the discovery of this interesting biota which stimulated the experiments described in this paper. The biota includes isolated microbial filaments, colonies of microbial filaments and a siliceous coating on almost all substrates. The isolated filaments are of various types, ranging from thin filaments 1–10 μm in length and 0·1–0·6 μm in width (Pl. 1) to short, stubby structures 0·3–2·0 μm in length and 0·1–0·5 μm in width. Morphologically, the filaments are straight, bent or wavy and are attached vertically or horizontally to the substrate. The surface texture of the filaments ranges from smooth to highly mammillated. The filaments are sometimes segmented, represented by hollow crusts (Pl. 1, fig. 2), or completely impregnated (Pl. 2, fig. 2). Other clay-coated oval structures of bacterial size containing a 'core' were observed in section (Pl. 2, fig. 4),

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but, at the time the microstructures were first described (Westall 1994), could not be interpreted. Their origin is further discussed below and the results of the research described in this paper are used to aid their interpretation.

A large colony of radiating, silicified filaments measuring 10 by 13 μm was found in the uppermost Oligocene sediments. The individual filaments were up to 3 μm in length and between 0·15–0·20 μm in diameter. They were generally smooth-surfaced although part of the colony presented an encrusted appearance.

Associated with the silicified microbial filaments was a siliceous coating on almost all surfaces, in which the microbial filaments were clearly embedded (Pl. 1). The coating was characterized by a smooth to mammillated surface, the latter consisting of microhemispheroidal structures ranging from 0·2–0·7 μm in diameter. Within this coating were some tiny filamentous structures generally $< 0{\cdot}1$ μm in length which, sometimes, formed an interlocking network (Pl. 2, figs 1, 3). It was identified as fossilized biofilm by Westall (1994).

The occurrence of the silicified microbial filaments and biofilm in the south-eastern Atlantic sediments was restricted to the almost pure diatomaceous horizons of the pre-middle Miocene interval of the ODP drillsite Hole 699A (water depth 3617 m). More clayey, intervening horizons did not contain such structures. Furthermore, extensive observation of numerous sediment samples of different ages, lithologies and subsurface depths from the southern Atlantic did not reveal such a characteristic association of fossilized microbial filaments and biofilm.

Westall (1994) interpreted the rare silicification of a deep-sea microbial mat community as being due to unusual environmental conditions prevailing in the Southern Ocean in the early–middle Miocene. At this period a prolonged hiatus of 3·5 My was caused by strong current activity related to the development of the Circum Antarctic Current after the complete separation of Antarctica from South America. The strong currents inhibited sediment deposition which led to the formation of a very well developed manganese nodule pavement at the surface of the sediment (some nodules are still visible in the sediment cores; Ciesielski *et al.* 1988). With the diagenetic dissolution of the biogenic siliceous deposits in the sediment column, there was a build-up of dissolved silica in the pore waters. Flux to the water column above would have been strongly inhibited by the presence of the manganese nodule pavement. The high silica concentrations in the pore waters led to the nucleation of silica on any suitable organic surfaces, such as a bacterium wall or the biofilm coating of a sediment particle (N.B. an SEM study of south-eastern Atlantic surface sediment shows that biofilms and microbial mats are common in the deep-sea environment; Westall 1993).

PREVIOUS EXPERIMENTAL WORK

Stimulated by the Precambrian finds, many researchers undertook microbial silicification experiments in order to understand the processes involved (e.g. Oehler and Schopf 1971; Oehler 1976; Walters *et al.* 1977; Francis *et al.* 1978*a*, 1978*b*; Ferris *et al.*, 1988). Leo and Barghoorn's (1976) experimental silicification of wood was a major step in this research. These latter authors, as well as Walters *et al.* (1977), Francis *et al.* (1978*a*, 1978*b*) and Ferris *et al.* (1988) based their experiments on the impregnation of organic matter by silica. Leo and Barghoorn's (1976) study

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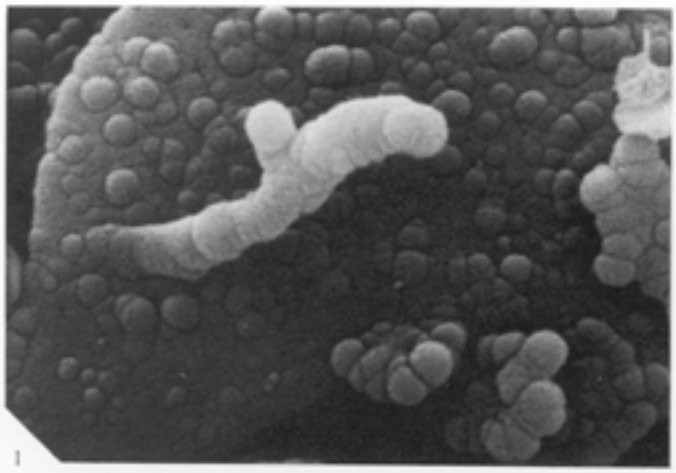
EXPLANATION OF PLATE 1

Figs 1–2, 4, 6. Sample 114-10H-1, 92–94 m (upper Oligocene, SE Atlantic Ocean). 1, silicified filamentous bacterium embedded in silicified mammillated biofilm; note large silica sphere attached to surface of the bacterium; × 15000. 2, encrusted hollow filamentous bacterium; × 16500. 4, silicified filamentous bacterium; note minute filament heads protruding from silicified biofilm in the background (arrows) and compare with TEM section in Plate 2, figure 1; × 22000. 6, small bacterium filament heavily encrusted at one end; × 24000.

Figs 3, 5. Sample 114-8H-2, 108–110 m (early-middle Miocene, SE Atlantic Ocean). 3, diatom frustule coated with silicified mammillated biofilm; × 15000. 5, small segmented silicified filamentous bacterium; × 22000.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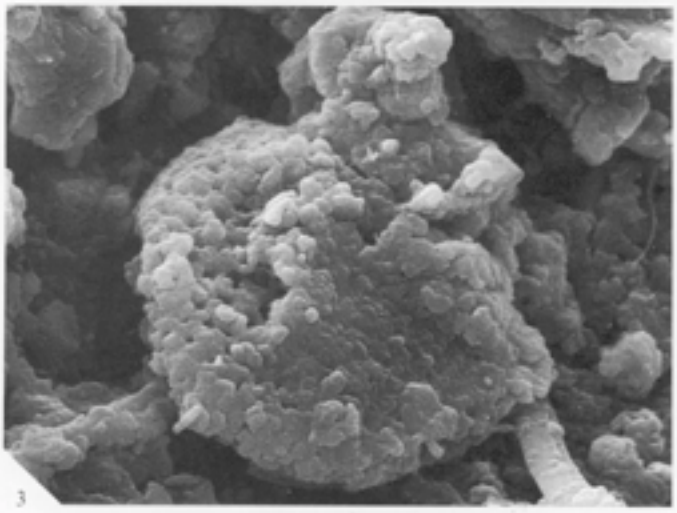
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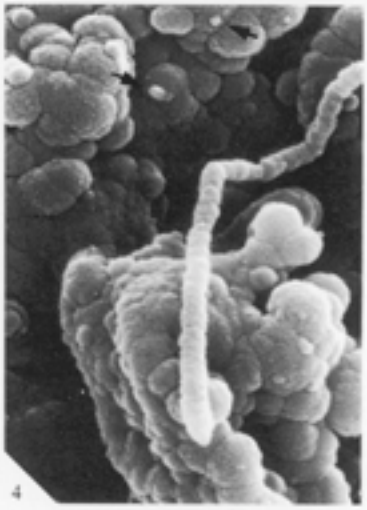
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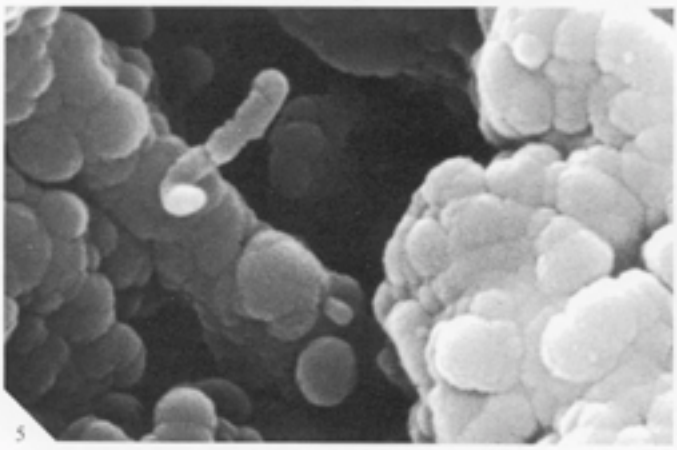
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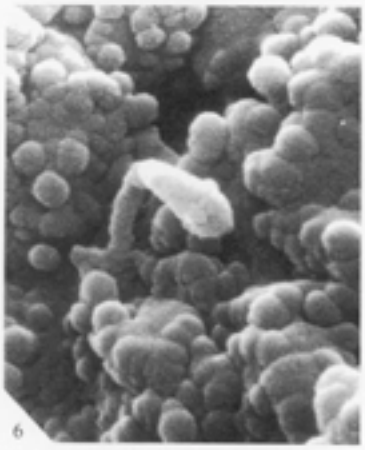
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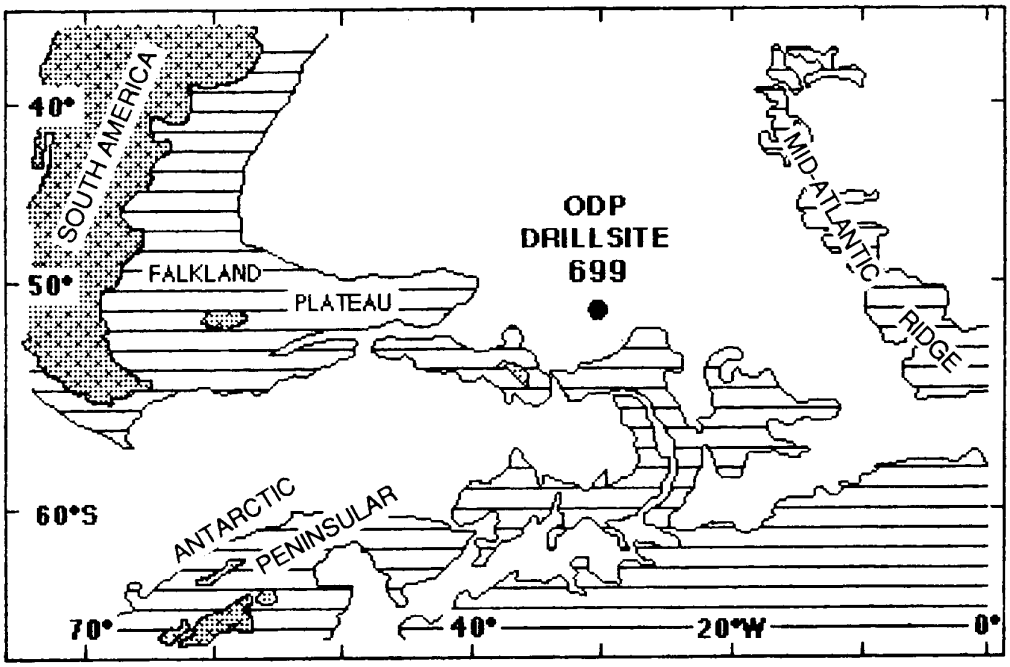
WESTALL *et al.*, ODP samples



TEXT-FIG. 1. Location of the ODP drillsite, Hole 699A, in the south-eastern Atlantic. Depths < 3000 m shaded.

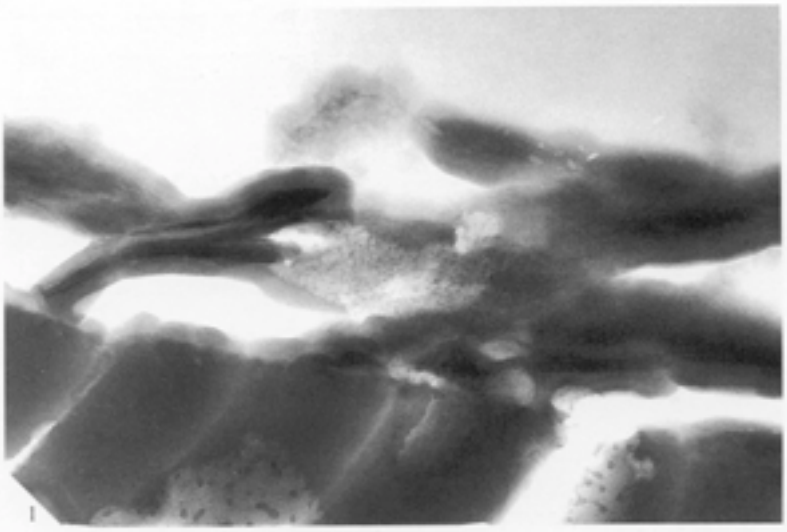
provided a clue as to the processes of silicification in nature: the vehicle for silica impregnation in nature is probably silicic acid in weak solution. The $Si(OH)_4$ molecule is small enough to penetrate into organic structures. It becomes attached to functional organic groups, such as the hydroxyl or carboxyl groups of the organic template and, with time and hydrolysis, the hydroxyl or carboxyl bonds are transformed into siloxane bonds. With further hydrolysis and polymerization the material becomes increasingly crystalline. Although bacteria can take up Si, possibly as a replacement for S or P (Heinen 1960; Heinen and Oehler 1979), the experiments of Walters *et al.* (1977) indicate that silicification is a passive process; bacteria simply provide suitable surfaces and surface area for precipitation and mineral nucleation. A summary of the present understanding of the silicification of fossils can be found in Carson (1991).

The earliest silicification experiments were undertaken by Oehler and Schopf (1971) and Oehler (1976), who impregnated microorganisms with silica, and then subjected them to high temperatures (up to 160 °C) and pressures (3000 bars) to simulate the conditions which the Precambrian rocks

EXPLANATION OF PLATE 2

Figs 1, 3–4. Sample 114-8H-2, 108–110 m (early–middle Miocene, SE Atlantic Ocean). 1, TEM section through the silicified biofilm coating a diatom frustule (lower part of the photograph); the complex layered construction of the biofilm, penetrated by a minute filament with a clearly tubular internal structure, is clearly observable; × 74000. 3, general view of the silicified biofilm coating demonstrating its structured, fibrous, network-like aspect; × 30000. 4, two oval bacterium moulds (arrowed), outlined by clay minerals and containing artificial 'nuclei'; × 52000.

Fig. 2. Sample 114-8H-1, 50–52 (early–middle Miocene, SE Atlantic Ocean); section of a bacterium completely permeated by silica attached to a diatom frustule; × 52000.



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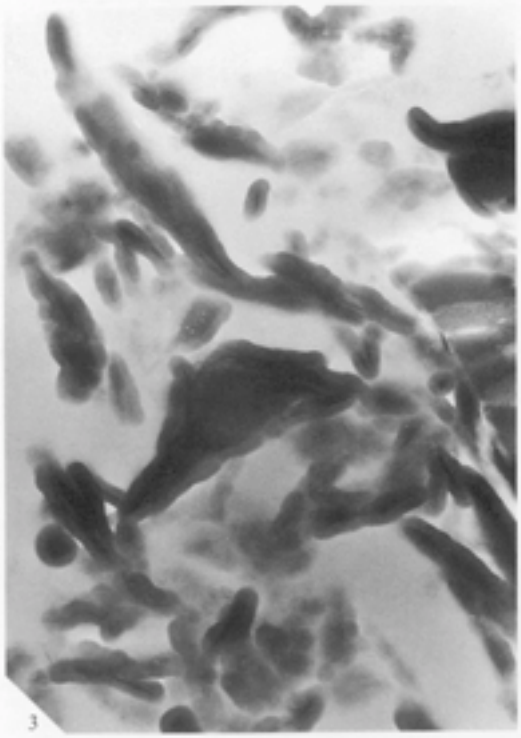

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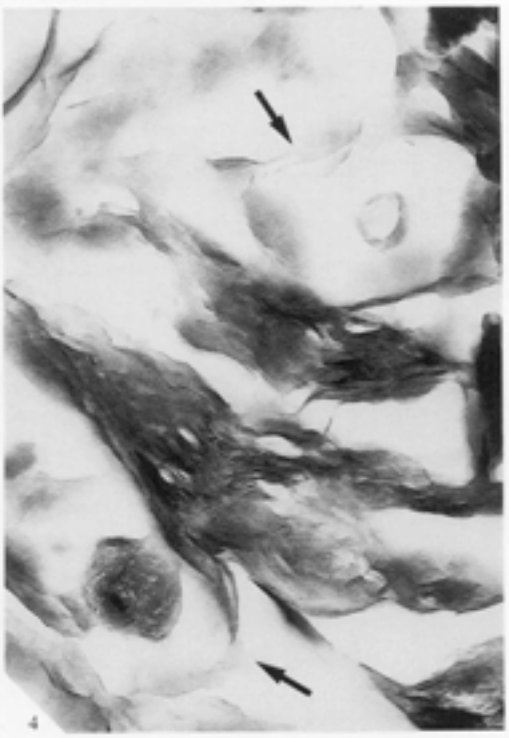

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WESTALL *et al.*, ODP samples

TABLE 1. Samples and methods used in the silicification experiments.

Sample				Conditions			
	Origin	Composition	Experiment	Pressure (atmospheres)	Temperature (°C)	Light conditions	Time
1	Culture	Diatoms (*C. fusiformis*) bacteria	2	1	30	Light	2,4 mo
2	Culture	Diatoms (*C. fusiformis*) bacteria, fungi	1	1	4	Dark	1,2,3,4 w
			3	500	4	Dark	1,2,3,4 w
3	SE Atlantic sediment water interface 5695 m	Bacteria, fungi, yeast diatoms, other	4	500	4	Dark	1,2,3,4 w
4	SE Atlantic microbial mat 3018 m	Bacteria, diatoms, minerals	5	500	4	Dark	1,3 mo

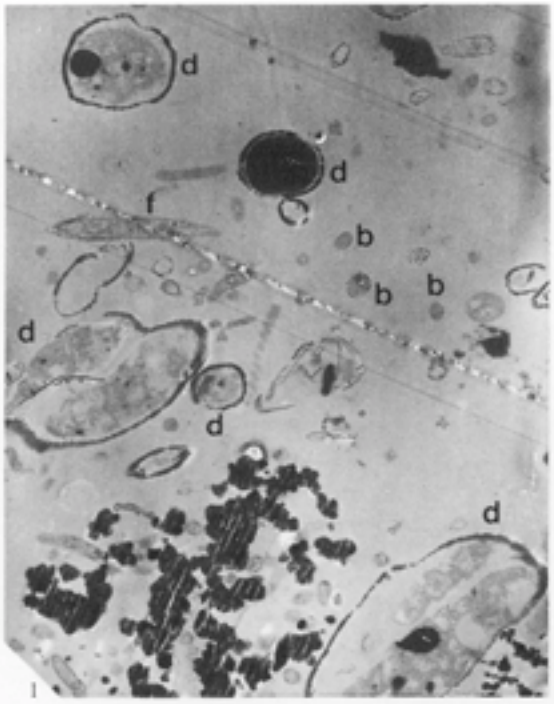
containing the microfossils underwent. Walters *et al.* (1977) and Francis *et al.* (1978*a*, 1978*b*) used an organosilicon solvent and temperatures $\leqslant 50$ °C in their experiments, whereas Ferris *et al.* (1988) used a colloidal silica solution at a temperature of 70 °C in order to imitate hydrothermal conditions.

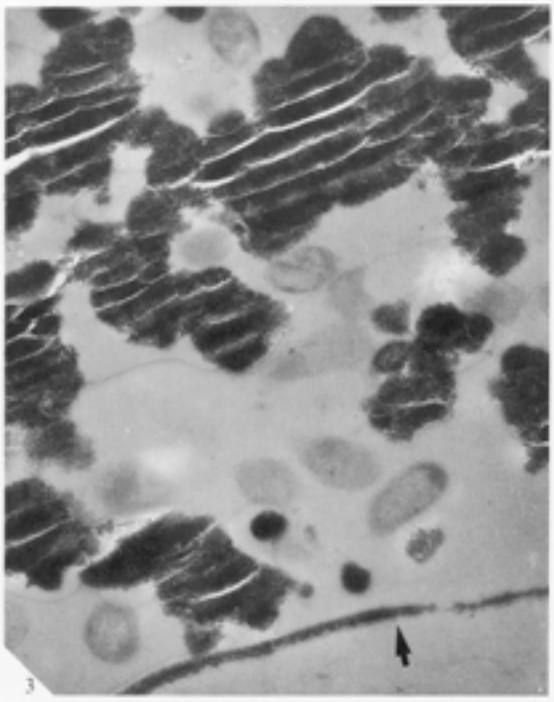
All previous bacteria silicification experiments involved the fossilization of large microorganisms such as cyanobacteria and one spirochete (Oehler and Schopf 1971; Oehler 1976; Walters *et al.* 1977; Francis *et al.* 1978*a*, 1978*b*), although the study of Ferris *et al.* (1988) involved the silicification of the Gram positive *Bacillus subtilis* (Cohn). The rationale for the use of larger bacteria was that these were the kind of organisms identified in petrological thin sections of the Precambrian rocks. Smaller bacteria have, to date, not been identified in these rocks either because of lack of preservation and/or the fact that micrometre and submicrometre-sized structures are almost impossible to observe in thin section.

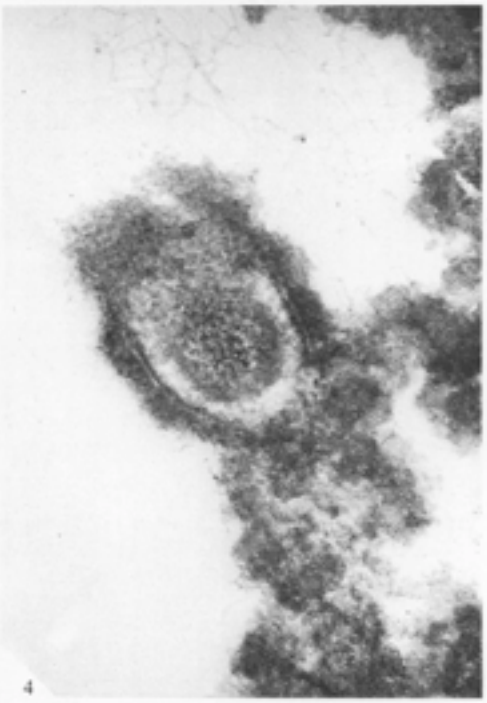
EXPLANATION OF PLATE 3

Figs 1–3. Experiment 1, Sample 2 (1 atmosphere, 1 week). 1, uncontrasted TEM section showing that all diatoms (d), fungi (f) and many bacteria (b) are mineralized; the diatom frustules are more robust and parts of the cytoplasm are replaced by very fine silica (grey areas) or by denser silica (black area or spheres); in the lower part of the photograph there is an aggregate of small porous silica spheres formed in the EPS groundmass; ×4100. 2, contrasted TEM section showing a silicified fungal hyphus adjacent to the edge of a diatom frustule; note the finely impregnated cell wall with a thicker accumulation of porous silica at one end; even the cytoplasm has been impregnated with silica; unsilicified bacterium visible, bottom left; ×41000. 3, uncontrasted TEM section of a detail of Fig. 1, showing the porous silica spheres engulfing unsilicified bacteria; note silica precipitation on the (unidentified) organic interface at the bottom of the photograph (arrow); ×16500.

Fig. 4. Experiment 1, Sample 2 (1 atmosphere, 3 weeks); contrasted TEM section showing silicified Gram negative bacterium, exhibiting the layered wall structure and an artificial 'nucleus', in the process of being engulfed by silica precipitated in the EPS groundmass; ×69000.







WESTALL *et al.*, Experiment 1

The research presented in this paper concerns a series of experiments to impregnate and fossilize microorganisms including bacteria, fungi and diatoms. Most of the bacteria silicified were small, in contrast to the majority of the above-mentioned experiments. The process of fossilization was followed in detail using TEM and SEM studies. The first part of this paper concerns the fossilization process at ambient pressures. The second part of the paper deals with fossilization under deep-sea temperature and pressure conditions, the results of which are compared with the fossilized microbial mats of latest Oligocene to middle Miocene age from the south-eastern Atlantic Ocean.

MATERIALS AND METHODS

This study is based on experimental fossilizations of a number of different microbial samples.

Sample 1. A preliminary experiment to test the chemicals and methods made use of a degrading culture of the marine diatom *Cylindrotheca fusiformis* (Rabenhorst) containing numerous bacteria (cocci, bacilli, very common spirochetes and, rarely, cyanophytes). A degrading diatom culture was chosen because the silicified bacteria from Oligocene to Miocene sediments in the south-eastern Atlantic occurred in almost pure diatomaceous sediments.

Sample 2. Another experiment used a similar degrading culture of the same diatom, *Cylindrotheca fusiformis*, which also contained fungi as well as bacteria.

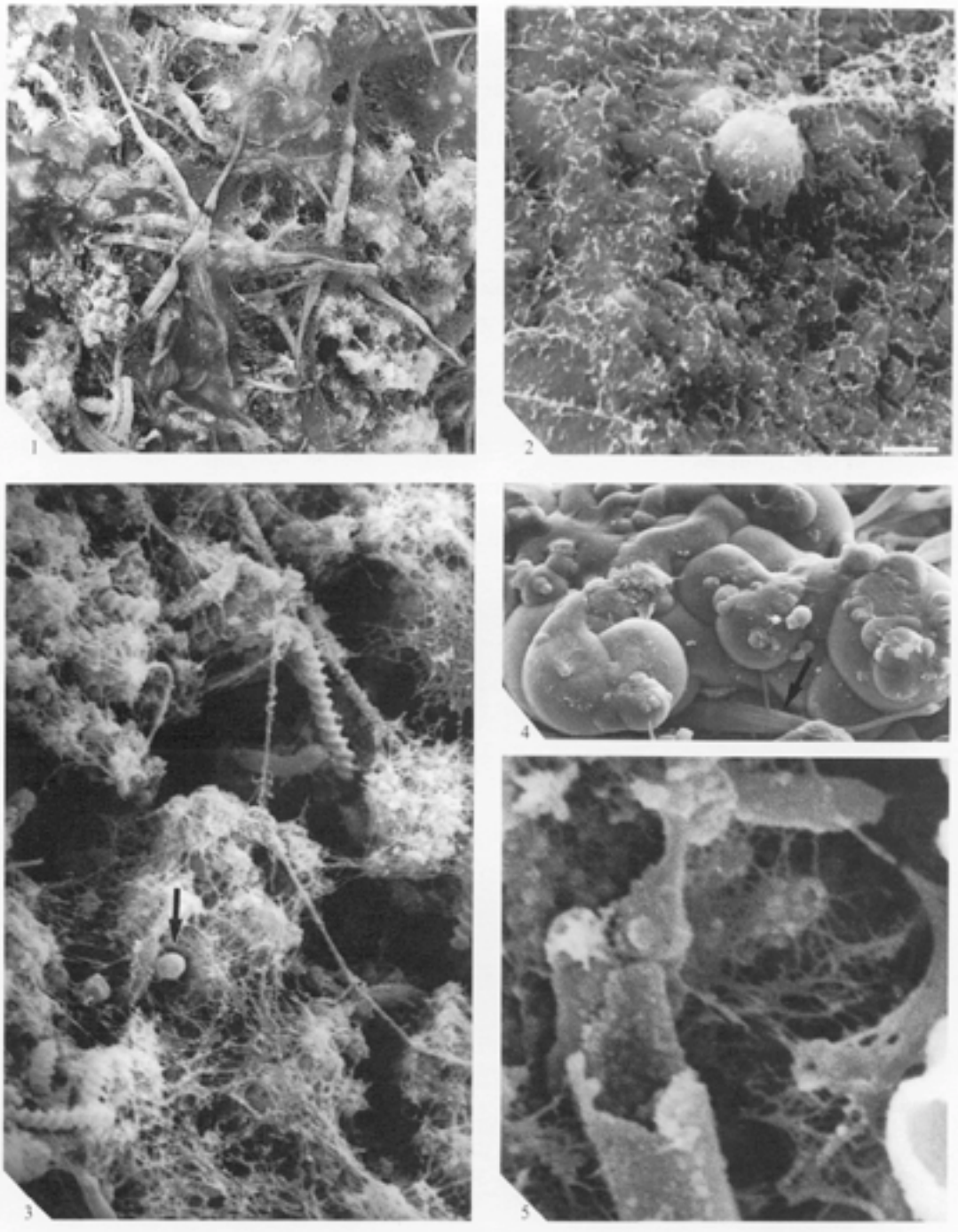
Sample 3. A third sample consisted of deep-sea microorganisms from water immediately above the sediment interface at 5695 m water depth from the south-eastern Atlantic (very close to ODP Hole 699A in which the Oligocene–Miocene silicified bacteria were found). The microorganisms at the sediment surface beneath this sample, identified in the laboratory, included Gram positive and Gram negative bacilli, Gram positive cocci, cyanobacteria, fungi (*Phialophora malorun* (Medlar) was identified from a nearby sample), yeast (*Cryptococcus albidus* (Saito) Skinner) and degraded diatom frustules (Westall 1993). Some mineral particles, such as clays attached to the surfaces of bacteria, also occurred in the sample. Bacteria cell counts in the water sample were 46×10^6 cells/cc^3 (Westall 1993).

Sample 4. The fourth sample was a microbial mat from the surface of deep-sea diatomaceous sediments in the south-eastern Atlantic at 3018 m water depth. Bacteria within the well-developed mat were abundant (24×10^6 cells/g sediment) and included the genera *Acinetobacter* (Brison and Prévot), *Neisseria* (Trevisan), *Streptococcus* (Rosenbach) and *Micrococcus* (Cohn) (Westall 1993). Interestingly, the bacteria in this sample were rarely visible with the SEM because they tended to be hidden by a coat of adsorbed clay minerals or within the aureolae of the diatom frustules (Pl. 12, figs 3–4).

EXPLANATION OF PLATE 4

Figs 1–2. Experiment 1, Sample 2 (1 atmosphere, 1 week). 1, fungal hyphae coated by a biofilm of EPS which also contains fine granules of precipitated silica (granular white areas); × 1400. 2, mammillated silica deposit coated by fibrous EPS, with larger silica sphere attached; × 9000. 3, detail showing unsilicified spirochetes and seemingly unsilicified bacilli within a fibrous EPS groundmass containing finely granular silica and some small isolated silica spheres (arrow); × 9000.

Figs 4–5. Experiment 1, Sample 2 (1 atmosphere, 3 weeks). 4, mammillated silica deposit engulfing a seemingly unsilicified fungal hyphus (arrow); × 1800. 5, encrusted hyphae showing hollow internal structure; bacterium in the upper part of the photograph has a slightly rugged, mineralized aspect; × 20000.



WESTALL *et al.*, Experiment 1

The nomenclature of the samples from the ODP drillsite Hole 699A in the south-eastern Atlantic, illustrated in Plates 1 and 2, follows from the ODP standard as noted in Ciesielski *et al.* (1988). As an example, Sample 144-699A-10H-1, 92–94: 114 – ODP leg; 699 – drillsite; A – hole; 10H – core (each core is 9·7 m long); 1 – core section (each section is 1 m long); 92–94 – sample depth in cm within each section.

For the silicification of these samples the method of Francis *et al.* (1978*a*) was used in a modified form. Two series of parallel experiments were undertaken; one at atmospheric pressures, either in the dark at 4 °C (Sample 2) or in the light at 25 °C (Sample 1). The second run involved fossilization at pressures of 500 atmospheres (corresponding with a water depth of 5000 m) and temperatures of 4 °C to imitate average conditions in the deep-sea region of the south-eastern Atlantic. This experiment was undertaken in order to fossilize samples of deep-sea microorganisms, taken from depths of 3700–5600 m, to try to obtain structures similar to those found in ODP Hole 699A (Samples 2, 3 and 4). The samples and experimental procedures used are listed in Table 1.

For the experiments, the excess water in the cultures was removed by centrifugation and the samples were then mixed with TEOS (Francis *et al.* (1978*a*) noted that a certain amount of water in the sample aided silicification). Each sample, after its respective fossilization time, was centrifuged to remove the TEOS, rinsed with a phosphate buffer and fixed with 2·5 per cent. glutaraldehyde solution in a phosphate buffer for a few days. For electron microscope studies, the samples were rinsed three times with phosphate buffer and post-fixed with 1 per cent. osmium tetroxide overnight and again rinsed three times with phosphate buffer. For the transmission microsope the samples were included in agar and cut into 1 mm cubes for ease of handling. Dehydration using first distilled water and alcohol, then alcohol and propylene oxide was made in percentage steps of 10, 30, 50, 70, 90, 100, 100, 100. The dehydrated samples were impregnated with and, finally, included in Epon resin. Ultrathin sections were made with a diamond blade mounted on a microtome and were contrasted with uranyl acetate and lead citrate. Observations were made with a Zeiss 109 transmission electron microscope. For SEM studies the samples were dehydrated with alcohol and critical-point dried. They were then mounted on brass stubs using silver paint, coated with Au/Pd and observed with a JSM JEOL 5400. Details of sample storage are to be found in the Appendix.

RESULTS

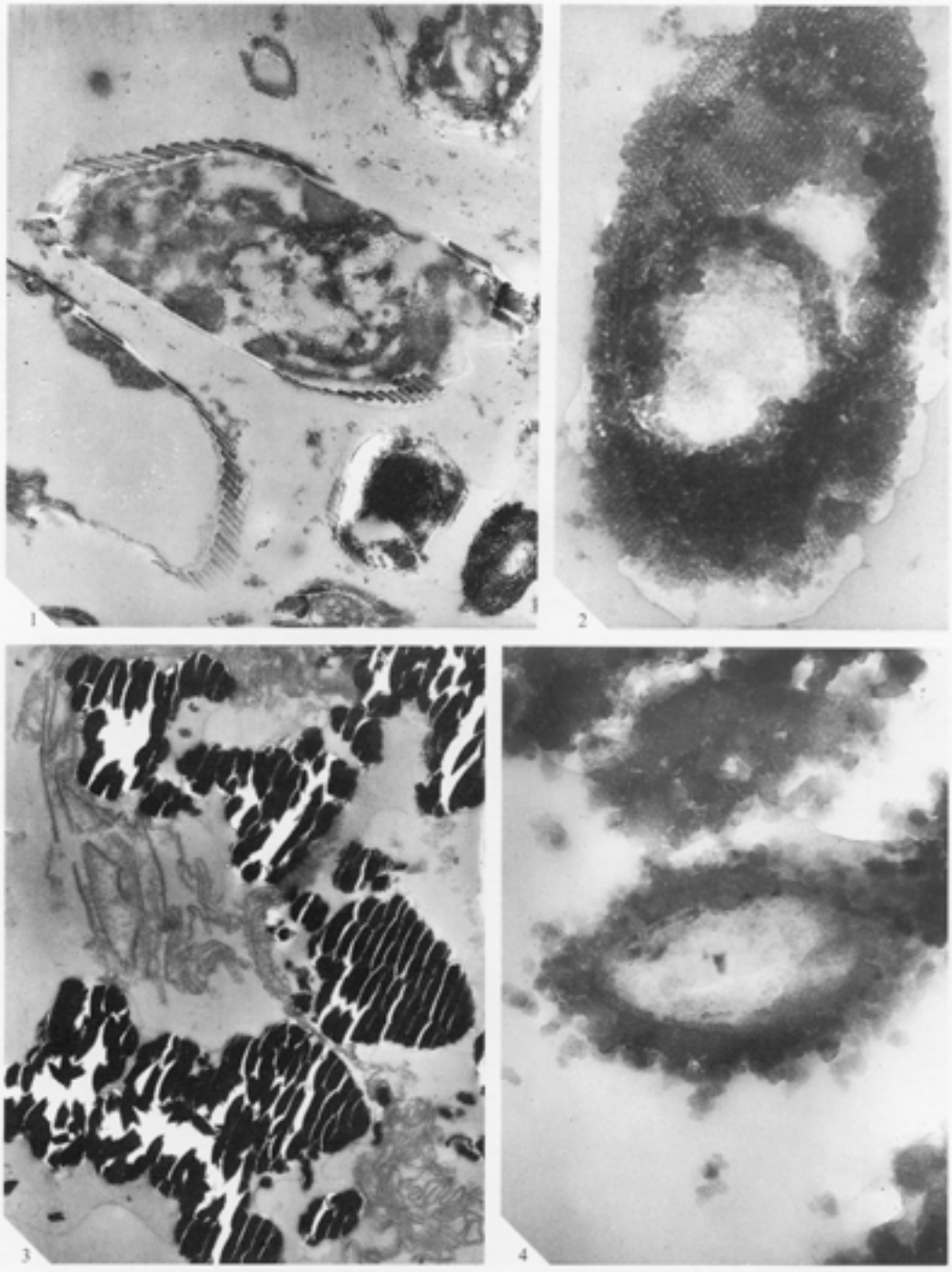
Experiment 1. Sample 2, one to four weeks at 1 atmosphere

After only one week in TEOS (at 4 °C in the dark) silica precipitation in the sample was quite pronounced (Pl. 3, fig. 1). However, many bacteria seemed to remain unsilicified (Pl. 3, fig. 3), as did much mucus or biofilm (extracellular polymeric substances, EPS, including the degradation products of the microorganisms). The EPS, when observed with the SEM, presented a relatively 'normal' fibrous-web appearance (Pl. 4, figs 1–3) (the term 'normal' is used because EPS in natural sediments prepared for SEM observation often has a fibrous morphology; Westall and Rincé 1994). Some unmineralized bacteria were engulfed by neoformed siliceous masses (Pl. 3, fig. 3).

EXPLANATION OF PLATE 5

Figs 1–3. Experiment 1, Sample 2 (1 atmosphere, 2 weeks). 1, uncontrasted TEM section showing details of the silicification of the cytoplasm within a diatom and the thickened frustule wall; × 16500. 2, uncontrasted TEM section showing details of a silicified bacterium; note finely reticulate pattern of the silica and the outline of an artificial 'nucleus'; × 69000. 3, uncontrasted TEM section showing silicified diatom chloroplast in the EPS groundmass; siliceous deposits are larger than in the 1-week old sample and more electron-dense; × 10000.

Fig. 4. Experiment 1, Sample 2 (1 atmosphere, 4 weeks); uncontrasted TEM section showing the transformation of silica around a bacterium into small but well-defined spherical structures which form stringers of spheres projecting around the crust; × 69000.



WESTALL *et al.*, Experiment 1

TABLE 2. Results of Experiment 1.

Sample	Pressure (atmospheres)	Time (weeks)	Organic matter preservation	Organic matter mineralized				Mineralization of	
				Bact.	Fungi	Diatoms	EPS	Cell wall	Cytoplasm
2	1	1	Generally very degraded. Many unmineralized cells and EPS. Much unidentified organic matter in the groundmass	Y	Y	Y	Y	Bacteria and fungi partly and completely encrusted. Diatoms encrusted	In diatoms, fungi and bacteria
2	1	2	Most organic matter mineralized. Unidentified organic matter in the groundmass	Y	Y	Y	Y (n.b. much still unmineralized)	Bacteria Fungi Diatoms	Bacteria Fungi Diatoms
2	1	3	Some unmineralized spirochetes. Less unmineralized EPS	Y	Y	Y	Y (including degraded remains of microorgs)	As above	As above
2	1	4	Some degraded, unmineralized bacteria	Y	Y	Y	Y	As above	As above

TABLE 3. Results of Experiment 2.

Sample	Pressure (atmospheres)	Time (months)	Organic matter preservation	Organic matter mineralized			Mineralization of	
				Bacteria	Diatoms	EPS	Cell wall	Cytoplasm
1	1	2	Some unmineralized bacteria and EPS	Y	Y	Y	Fused crust Small silica spheres	Bacteria Diatoms
1	1	4	Rare unmineralized Gram negative bacteria All EPS mineralized	Y	Y	Y	Thick crusts	Not all bact. cytoplasm mineralized Diatoms

Silicification was manifested by the presence of spherical deposits of silica in the groundmass as well as the presence of silcified microorganisms (Pl. 3, fig. 1). The latter were distinguishable from the unsilicified microorganisms in uncontrasted TEM sections because they presented electron dense walls and/or were filled by casts of silica (Pl. 3, figs 1–2); in uncontrasted TEM sections microorganisms without a mineral skeleton, such as bacteria and fungi, are normally poorly defined (Pl. 3, fig. 3). The walls and cytoplasm of the silicified miroorganisms seemed to be impregnated by finely crystalline amorphous silica having a porous granular texture (Pl. 3, figs 1–2). Further porous silica, sometimes in the form of $< 0{\cdot}2\ \mu m$ spheres, nucleated onto the already permineralized surfaces producing an irregular surface 20–150 nm in thickness (Pl. 3, fig. 2). Silica nucleation on the diatom frustules resulted in a more robust appearance (Pl. 3, fig. 1) (it should be noted that the diatom species *C. fusiformis* is naturally lightly mineralized). In some instances, silica precipitated

TABLE 2 (*cont.*)

Silica precipitation						
Dissem.	Small spheres	Large spheres	Irregular mass	Other	Artefacts	Comments
Y	Y			Unmineralized bacteria trapped in silica deposit	N	Two forms of silica: more dense and less dense (TEM)
Y	Slightly larger		Y	Unmineralized bacteria and fungi trapped in silica	Silica cores from collapsed cytoplasm–look like 'nuclei'	Two forms of silica as above Silica around bacteria exhibits a finely reticulate pattern
Y	Y	Y			False 'nuclei'	Silica mineralizing the cytoplasm is mostly granular: some denser patches in diatoms and fungi
Less	Forming denser silica	Y	Formed of small, coalesced spheres		False 'nuclei' Stringers	

TABLE 3 (*cont.*)

Silica precipitation					
Dissem.	Small spheres	Large spheres	Irregular mass	Artefacts	Comments
Y	Y	Rare		False nuclei, some with organic coat. Stringers	Silica spheres coated with a monolayer of organic molecules. Reticulate pattern of silica in crust around bacteria
N	Rare	Y		False nuclei	Thicker crusts 'Clean' aspect of mineralized culture

within an organism, either as small spheres 0·5–2 μm in diameter (perhaps replacing vacuoles?) or completely filling the cell as an internal cast (Pl. 3, fig. 1). Silica also precipitated on unidentified organic surfaces or interfaces in the groundmass (Pl. 3, fig. 3).

The silica precipitates within the abundant EPS of the groundmass formed loose aggregates of porous spheres ranging from 0·15–0·36 μm in diameter. Some aggregates, however, were formed of larger, fused, electron-dense silica spheres (0·5 μm diameter) with a botryoidal surface (Pl. 3, figs 1, 3; Pl. 4, fig. 2).

Continued degradation of the microorganisms led to an initial increase of organic matter making up the EPS (Pl. 5, fig. 3). However, the organic matter was generally rapidly silicified; already by the second week in TEOS most of the microorganism cells were mineralized but, in the eukaryotes especially, they often exhibited patchy mineralization of the cytoplasm, as if certain organelles

(perhaps the nuclear material) were more easily mineralized than others (Pl. 5, fig. 1). Some silicified diatom organelles, such as chloroplast, were readily recognizable in the groundmass (Pl. 5, fig. 3). The siliceous crust replacing degraded bacteria in some instances exhibited a finely reticulate pattern which obliterated the organic cell wall (Pl. 5, fig. 2). In other cases, the mineralization of the triple-layer wall structure of a Gram negative bacterium was still preserved after three weeks in TEOS (Pl. 3, fig. 4). A few, rare, unsilicified bacteria (generally Gram negative forms) were still observed in the four-week old sample. The TEM sections of the latter sample gave the impression that there was very little EPS but the SEM studies showed that it had simply migrated out of the silicified masses to form a smooth film on the surfaces of the botryoidal deposits (Pl. 8, fig. 1), as opposed to the fibrous web observed at the start of the experiment (Pl. 4, fig. 1).

General indications of increasing silicification with time include the thickening of the siliceous crust around the microorganisms (up to 0·6 μm) and increasing crystallization of the silica manifested by: (a) its less porous and more electron dense aspect; (b) an increase in the size of the denser silica spheres (up to 50 μm); (c) a decrease in the amount of loosely aggregated siliceous spheres and a corresponding increase in the denser spherical aggregates; and (d) an increase in the size of the silica crystallites nucleating on to the surface of the fossilized microorganisms.

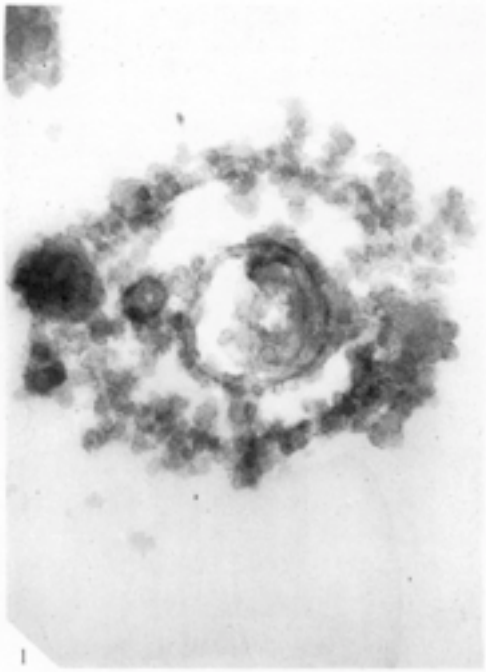
Generally, in the SEM micrographs of the early stages of fossilization the surfaces of the microorganisms, especially the bacteria, looked quite smooth (Pl. 4, figs 1, 3), except for some 10–20 nm sized silica crystallites attached to them, and appeared to be, at least superficially, 'unfossilized'. However, where, for instance, a fungus was broken, it was possible to see that the outer wall was simply a crust (Pl. 4, fig. 5). This underlines the importance of parallel TEM studies which document in more detail the state of fossilization of the microorganisms. The increase in size with time of the crystallites attached to the surface of the fossilized cell walls gave the walls of the microorganisms in the older samples a more rugged, crusty 'fossilized' appearance (Pl. 4, fig. 5). In the TEM sections, the silica forming the crusts around the microorganisms in the older samples consisted of coalesced spheres of silica (60–100 nm) rather than amorphous porous silica (compare Pl. 5, fig. 4 with Pl. 3, fig. 2). In the four-week old sample, stringers of these spheres attached to the outside of the silica crust were common (Pl. 5, fig. 4). They seem to represent artefacts. Another artefact of the silicification process, already apparent in the two-week old sample, was the appearance of cores in the silicified bacteria, formed by the silicification of the collapsed cytoplasm (Pl. 3, fig. 4; Pl. 5, fig. 2). These cores in a fossilized cell could be mistaken for a 'nucleus', which does not exist in prokaryotes.

Lastly, the engulfing of the microorganisms (generally silicified) by the botryoidal aggregates of silica in the groundmass (Pl. 3, fig. 4; Pl. 4, fig. 4) becomes more common with the duration of the silicification process.

The results of Experiment 1 are summarized in Table 2.

EXPLANATION OF PLATE 6

Figs 1–4. Experiment 2, Sample 1 (1 atmosphere, 2 months). 1, contrasted TEM section showing a bacterium wall outlined by silica spheres and stringers of spheres radiating from both sides of the wall; faint residues of the reticulate amorphous silica may still be seen (bottom right); a larger silica sphere has started to grow on the crust, left (compare with Pl. 1, figs. 1–3 and Pl. 10, fig. 2); silicified membrane structure around artificial 'nucleus' is clearly visible; remains of organic matter can be seen in the very thin dark outlines of the individual silica spheres (compare with the uncontrasted section in Fig. 2) and as a dark line around the artificial 'nucleus'; × 67000. 2, uncontrasted TEM section of two encrusted bacteria; the silica around the cell walls has fused but the outer edges are lined with small silica spheres and stringers of silica; the internal part of the cells has been completely replaced by partly fused porous silica spheres; × 67000. 3, contrasted TEM section of a bacterium cast retaining the structure of its inclusions; the diatom frustule adjacent is lined with small silica spheres; × 67000. 4, contrasted TEM section of an encrusted bacterium wall in which remnants of the organic wall are still visible; note the mass of silica spheres in the background, outlined by fine web-like EPS fibrils; × 67000.



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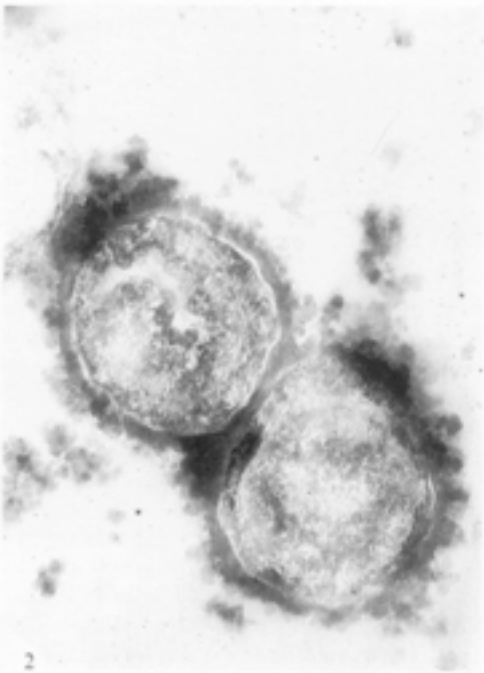

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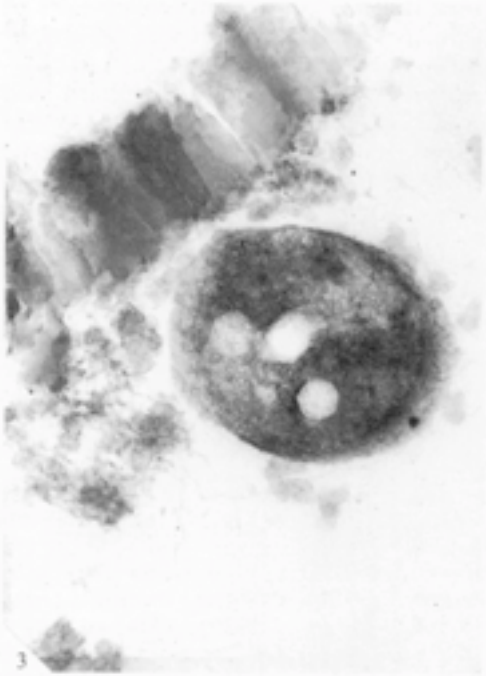

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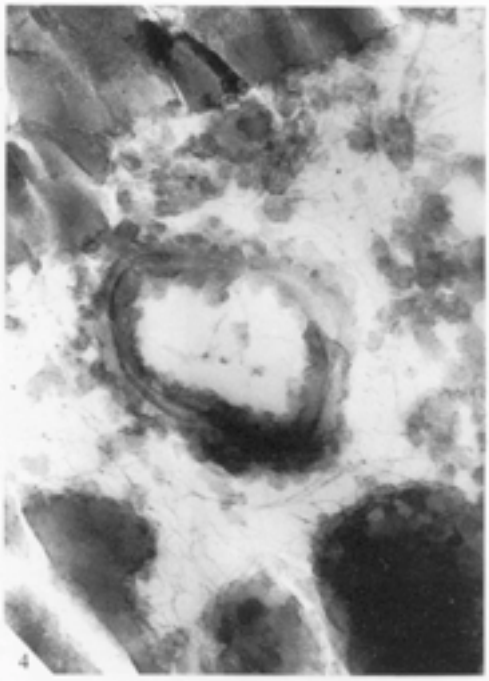


4

WESTALL *et al.*, Experiment 2

TABLE 4. Bacteria cell size changes in Experiment 2 (vol. μm^3).

	Small bacteria	Large bacteria	Average
Control	0·04	0·37	0·11
2-month	0·04	0·12	0·05
4-months	0·06	0·39	0·25

Experiment 2. Sample 1, two to four months at 1 atmosphere

Details of the results of this experiment are listed in Table 3. The silicification phenomena already noted in the previous experiment were further developed after two and four months in TEOS. At the end of the experiment very few bacteria remained unmineralized; only Gram negative cocci and spirochetes continued to resist mineralization (Pl. 7, figs 1–2) and some bacteria were only partly mineralized, even after four months, by spheres or half-moon-shaped silica deposits around the walls (Pl. 7, fig. 2).

The walls of the microorganisms were mineralized by small silica spheres, 20–300 nm in diameter, or fused crusts or casts in the two month sample (Pls 6–7), in strong contrast with the porous granular silica mineralizing the microorganisms in the early weeks of fossilization. Inclusions in the original bacteria (formerly gas or liquid?) were also preserved in casts (Pl. 6, fig. 3). Cores of silicified, collapsed cytoplasm were still common in the bacteria of the two month sample and, in some instances, were coated with an organic layer, thus increasing the (false) nuclear appearance (Pl. 6, fig. 1). Stringers of silica spheres radiating out from the silicified cell wall were also common (Pl. 6, fig. 4).

Organic-coated silica spheres continued to nucleate in the web-like EPS groundmass (Pl. 6, fig. 3), but, after four months, these disappeared and the many tiny spheres coalesced to form fewer, larger organic-coated spheres (Pl. 7, fig. 1). After four months the silica spheres mineralizing the walls of the bacteria coalesced into thicker, electron-dense crusts with a mammillated external surface (Pl. 7). However, electron diffraction of the initial sample and the two and four month old samples did not demonstrate any increase in crystallinity; the silica was, crystallographically speaking, as amorphous after four months as at the start of the experiment.

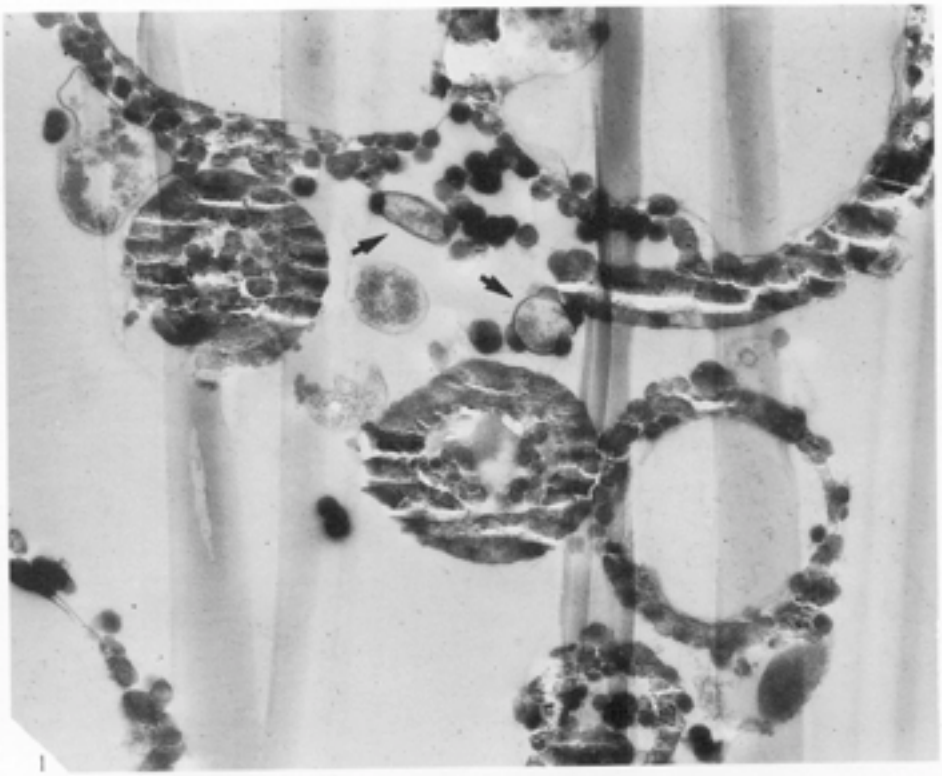
A general increase in the cell sizes of the mineralized bacteria with time was noted (Table 4), although the average cell volume of the larger bacteria actually seemed to decrease in the two month sample. Francis *et al.* (1978*a*, 1978*b*) found that fossilized spirochetes increased in volume whereas cyanobacteria decreased in size upon silicification.

Experiment 3. Sample 2, one to four weeks, 500 atmospheres

The results of this experiment are presented in Table 5. At 500 atmospheres and 4 °C (corresponding to general deep-sea conditions at a water depth of 5000 m in the south-eastern Atlantic) the rate of

EXPLANATION OF PLATE 7

Figs 1–3. Experiment 2, Sample 1 (1 atmosphere, 4 months). 1, contrasted TEM section showing heavy mineralization of the diatoms with silica deposition inside the cell walls, forming an almost solid structure; silica spheres around the microorganism fossils are larger and more electron-dense; some bacteria are still unfossilized but, on a few, silica spheres are starting to grow (arrows); note the generally clean appearance of the sample due to the disappearance of the many, small, isolated silica spheres in the groundmass; × 22000. 2, contrasted TEM section showing half-moon-shaped silica spheres which have grown around a bacterium such that the bases of the spheres are contiguous with the curvature of the cell; unfossilized spirochete upper right; × 60000. 3, uncontrasted TEM section showing a crust formed around a bacterium; × 93000.



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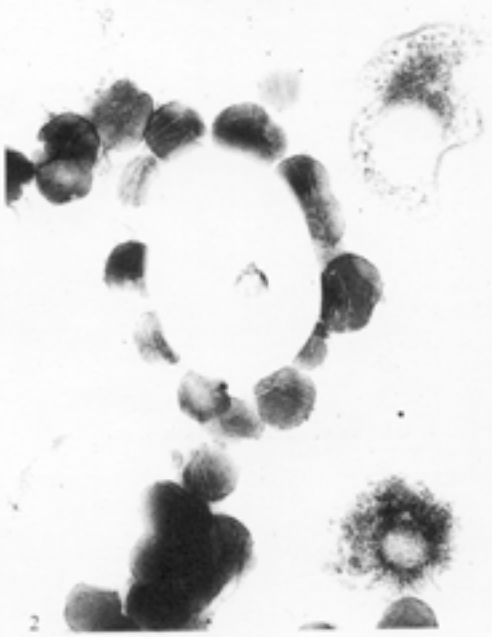

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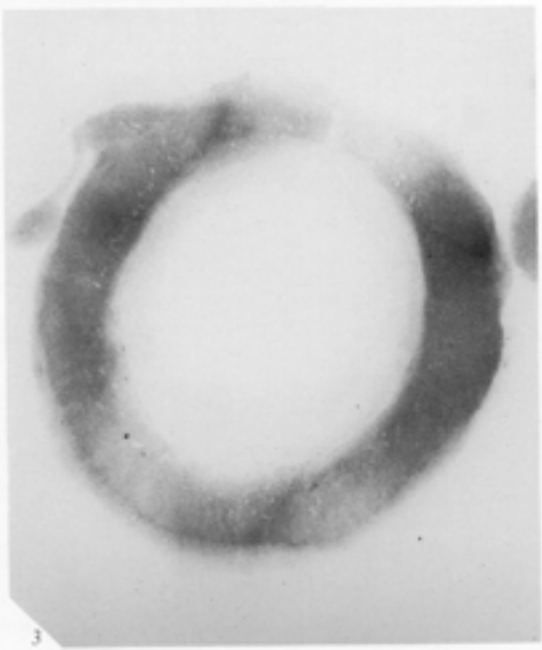

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WESTALL *et al.*, Experiment 2

TABLE 5. Results of Experiment 3.

Sample	Pressure (atmospheres)	Time (weeks)	Organic matter preservation	Organic matter mineralized				Mineralization of	
				Bact.	Fungi	Diatoms	EPS	Cell wall	Cytoplasm
2	500	1	Little loose EPS Some unmineralized bacteria.	Y	Y	Y	Y	Not all bacteria or fungi walls mineralized	Not all diatom cytoplasm mineralized
2	500	2	As above	Y	Y	Y	Y	Crusts around bacteria and fungi	Bacteria Fungi Diatoms
2	500	3	Still some unmineralized bacteria	Y	Y	Y	Y	All organisms	All organisms
2	500	4	Everything mineralized	Y	Y	Y	Y	All	All

TABLE 6. Results of Experiment 4.

Sample	Pressure (atmospheres)	Time (weeks)	Organic matter preservation	Organic matter mineralized					Mineralization of	
				Bact.	Fungi	Diatoms	EPS	Other	Cell wall	Cytoplasm
3	500	1	Little unmineralized EPS. Few unmineralized bacteria.	Y	Y	Y	Y	Cyano-bacteria	Almost all bacteria occur as crusts. Thick mineralized glycocalyxes	Mineralized where still present
3	500	2	Still some cell walls unmineralized	Y				Cyano-bacteria	Crusts, clay moulds, glycocalyx	As above
3	500	3	As above	Y					Moulds of bacteria	As above
3	500	4	Large number of unmineralized but degraded bacteria. Some degraded remnants of EPS?	Y	Y	Y	?	?	Eukaryotes	Eukaryotes

silicification of the organic structures was greatly increased, the one week-old sample exhibiting many of the same silicification manifestations as the four week-old, 1 atmosphere sample. There were few unmineralized bacteria and little unmineralized EPS in the groundmass of the TEM sections (Pl. 9, fig. 1), although in the SEM preparations EPS was visible and still had a fibrous

TABLE 5 (*cont.*)

Silica precipitation						
Dissem.	Small spheres	Large spheres	Irregular mass	Other	Artefacts	Comments
Y	Y	Y	Mineralized organisms engulfed by silica sphere mass	Silica crystal?	False nucleus	Bacteria mostly have a granular crust. Diatom cytoplasm already mineralized with dense silica as well as finely granular material
Less		Y	Containing spheres and more dense material		False nucleus Stringers	No small spheres seen
Not much		Y	Bacteria included in irregular mass		Organelles in diatoms. False nuclei Stringers	Not much disseminated silica. Mineralized bacteria starting to lose their identity in the irregular mass. Unmineralized bacteria enclosed in silica mass
Little		Y	Y		Organelles in diatoms and fungi. Stringers on diatoms. Oval, semi-rectangular in groundmass	Bacteria almost disappeared in crystalline silica. Silica mineralizing some fungi has a distinct lattice structure

TABLE 6 (*cont.*)

Silica precipitation					
Dissem.	Small spheres	Large spheres	Irregular mass	Artefacts	Comments
Y	Y	Y	Y	False nuclei	Mineralized thick glycocalyxes around voids (i.e. moulds)
Y					Microorganisms, including bacteria in clay nests are enclosed in large expanses of finely granular to dense silica
			Y	False nuclei	As above
Y	Y		Y		Many bacteria walls remaining in degraded form but other bacteria engulfed by silica have lost their form

aspect (Pl. 8, fig. 2). Over the four week period of the experiment some bacteria were still unmineralized after three weeks but, by four weeks, all microorganisms were engulfed by botryoidal silica deposits and individual silicified bacteria could no longer be distinguished from the engulfing mass (Pl. 9, fig. 2; Pl. 10, fig. 4).

TABLE 7. Results of Experiment 5.

Sample	Pressure (atmospheres)	Time (months)	Organic matter preservation	Organic matter mineralized			Mineralization of	
				Bact.	Diatoms	EPS	Cell wall	Cytoplasm
4	500	0	Many bacteria either well preserved or already partially degraded. Occurs in diatom aureolae and clay nests. Much EPS: fibrous or partly reticulate. Some EPS on mineral surfaces.					
4	500	1	None observed	Y		Y	Bacteria almost totally disappeared	
4	500	3	None observed	Y		Y		

Initially, most of the bacteria had a 'fresh', smooth appearance when viewed with the SEM, testifying to the fineness of the permeating silica. However, by three weeks many individuals showed visible evidence of fossilization, appearing as broken crusts (Pl. 8, fig. 3) or with large silica spheres attached to their encrusted surfaces (similar to the upper Oligocene to middle Miocene bacteria from the south-eastern Atlantic: compare Pl. 10, fig. 2 with Monty *et al.*, 1991, pl. 4, fig. 4). An increase in the electron density of the mineralizing silica with time was evident (compare Pl. 9, fig. 1 with Pl. 9, fig. 2).

As in the atmospheric fossilization run, with increasing time during the experiment, there was a gradual expulsion of organic matter from within the mineralizing microorganisms and the EPS changed in morphology from being finely fibrous to becoming smooth and stringy (compare Pl. 8, fig. 2 with Pl. 10, fig. 4).

Artefacts appearing within the first week of this experiment included the frequent mineralization of collapsed cytoplasm in bacteria, giving the impression of a false nucleus; stringers of silica spheres appeared around the mineralized cells by the second week. Other types of artefacts occurred in the three and four week samples, for example, regularly-shaped 'tear-drop' structures in the diatoms (Pl. 9, fig. 3) and other more irregular structures within the diatom frustules and in the groundmass (Pl. 11, fig. 1).

Experiment 4. Sample 3, one to four weeks, 500 atmospheres

This experiment concerned the fossilization of natural deep-sea microorganisms under deep-sea pressure and temperature conditions (Table 6). The organic material was rapidly mineralized although remnants of some unmineralized bacterial walls could still be observed in the three week sample. Bacteria with a thick glycocalyx were particularly susceptible to mineralization with the fossilized glycocalyx reaching 0·2–0·8 μm thickness (Pl. 11, fig. 3). Sometimes only the mineralized glycocalyx or cell wall remained, leaving the inner part of the cell hollow or containing false 'nuclei' (Pl. 11, fig. 4). Where the cytoplasm had not collapsed and had been permineralized, the original inclusions were still visible (Pl. 11, fig. 2). Mineralized Gram negative bacteria, enclosed within the fine grained siliceous deposit, were invariably surrounded by an empty halo, thus creating a cast. However, Gram positive forms were enclosed by an outer crust of silica which was more finely textured than the silica replacing the cytoplasm or that engulfing the microorganisms (Pl. 11, fig. 2).

TABLE 7 (*cont.*)

Silica precipitation					
Dissem.	Small spheres	Large spheres	Irregular mass	Artefacts	Comments
Y		Y	Y	Spheres with crusts False nuclei	Irregular mass is granular and dense
Y			Y	Full spheres	

In sedimentary environments containing clay minerals, bacteria or colonies of bacteria are invariably coated by clays (Westall and Rincé 1994). Upon degradation of the microorganisms the clay coatings retain their shape but are empty. A number of these empty clay housings were observed embedded within the siliceous precipitate (Pl. 11, fig. 4).

An important observation in the one, two and three week samples was the vast amount of silica precipitated in the groundmass, as disseminated porous granules or as a granular porous mass (Pl. 11, figs 2, 4), or as botryoidal-surfaced electron denser masses, all enclosing the abundant microorganisms. In many cases the engulfed, degraded bacteria were barely recognizable and the SEM study showed that the samples consisted of aggregates of silica spheres coated with fibrous, stringy EPS.

The four week sample seemed to have reacted differently to the TEOS, compared with the other samples. Instead of a few moulds and casts enclosed within a fine-grained porous silica deposit, the sample consisted of large numbers of degraded but apparently unmineralized Gram negative cell walls, as well as unidentifiable, unmineralized organic matter in the groundmass (Pl. 12, fig. 2). Silica spheres formed both on cell walls and within the cells in some of the microorganisms. Botryoidal-surfaced masses as well as irregular masses of disseminated silica granules occurred in the groundmass. On the other hand, eukaryote microorganisms in the sample, such as fungi, did present mineralized cell walls and cytoplasm.

Experiment 5. Sample 4, one to three months, 500 atmospheres

The deep-sea microbial mat sample (Sample 2285) consisted of a well-developed layer of EPS coating the surface of the diatomaceous sediment and embedding particles within it. Beneath the thick cohesive EPS surface coating, the particles were interlinked and bound together by a fibrillar EPS network. The EPS film was surficial and at a depth of 5 mm in the sediment only a few rare fibrils remained. Although bacteria cells were not observed with the SEM, the epifluorescence counts and TEM studies showed that they were abundant (24×10^6 cells/gm sediment; Westall 1993). They generally occurred coated with clay or within the aureolae of corroded diatom frustules (Pl. 12, figs 3–4).

This particular sample was subjected to long-term fossilization in TEOS under 500 atmosphere pressure. After one month, no organic matter could be distinguished with the TEM; the SEM studies showed that the degraded and denatured organic matter coated the exterior of granular-

surfaced aggregates of silica spheres. The silica precipitate formed large expanses of porous granular material which engulfed mineral particles and diatom frustules. The diatom frustules were distinguished in the siliceous precipitate only with difficulty (Pl. 12, fig. 5). Mineralized bacteria were rare and occurred as bacteria-sized moulds (possibly of Gram negative forms, based on a comparison with Sample 3) or as clay moulds. Large, non-bacterial, artificial spheres of silica were common (3–> 13 μm diameter) as well as spherical or crescent-shaped artefacts, either filled or as crusts of 0·3–3·3 μm diameter (Pl. 12, fig. 6). After three months fossilization at 500 atmospheres, microorganism remains were no longer visible in the granular mass and only minerals such as clays could be distinguished, although artificial spheres, either filled or as crusts were still observed. The results of this experiment are summarized in Table 7.

DISCUSSION

The process of fossilization

In nature, an important factor in the process of microorganism fossilization is the preservation of the dead cell for a period of time long enough to allow its mineralization. Microorganisms with thick sheaths such as cyanobacteria or the cysts of protists, or with thick glycocalyxes, are more resistant to decay and, therefore, are more readily fossilized (Golubic and Barghoorn 1977; Francis *et al.* 1978*a*; Knoll 1985; Gerdes and Krumbein 1987). However, the studies of Ferris *et al.* (1988) suggest that the chemical environment of the dead microorganisms may also play an important role, with the binding of Fe ions to cell surfaces effectively retarding the rate of degradation. These authors also put forward the opinion that the preservation of the microorganisms in the experiments of Oehler and Schopf (1971), Oehler (1976) and Francis *et al.* (1978*a*, 1978*b*) was due to the denaturization of the autolytic enzymes which naturally degrade a bacterial cell wall upon the death of the organisms. However, they cite no evidence to support their statement. Other factors influencing cell wall fossilization are discussed below.

In the experiments described in our paper, the supply of dissolved silica was made readily available in the form of the orthosilicate TEOS. The time delay between cell death and mineralization was, therefore, probably not a limiting factor.

Silicification takes place by the attachment of the silica molecule to a functional group, such as hydroxyl or carboxyl groups of the organic material, i.e. the organic matter serves as a template for the nucleation of silica. Once the initial nucleation has taken place, silica is precipitated by silicic acid polymerization (Leo and Barghoorn 1976). A certain degree of cell degradation may, in fact, increase the number of hydroxyl groups available for silica nucleation (Leo and Barghoorn 1976; Ferris *et al.* 1988); indeed, most of the Proterozoic microfossils present a slightly degraded aspect according to Knoll (1985). Schultz-Lam *et al.* (1993) note that Gram positive bacteria have a higher metal-binding capacity than Gram negative bacteria. This is a result of the former containing more peptidoglycan in their cell walls than the latter. It seems that metals bind primarily to the carboxyl functional groups of the peptidoglycan and teichuronic acids and the phosphoryl groups of the teichoic acids. Could Gram positive bacteria, therefore, be more susceptible to silicification than

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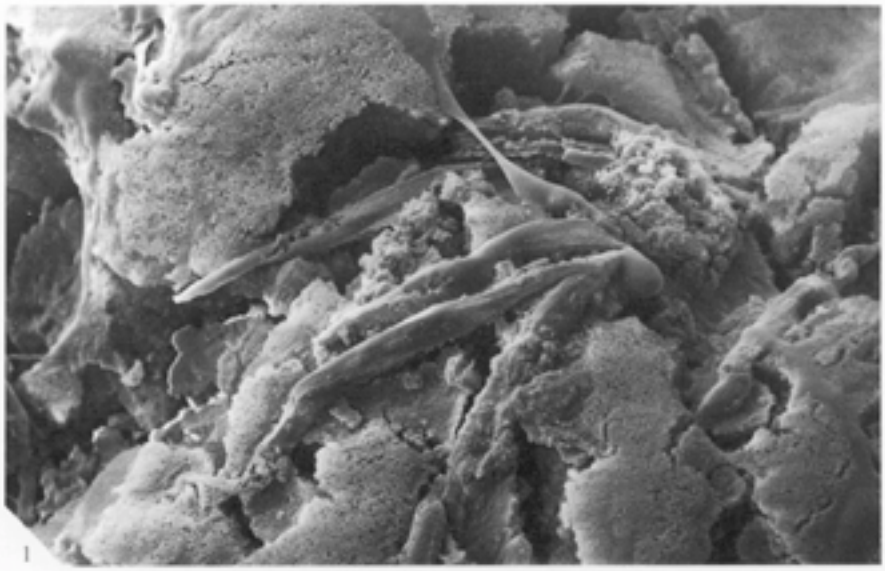
EXPLANATION OF PLATE 8

Fig. 1. Experiment 1, Sample 2 (1 atmosphere, 4 weeks); the silica precipitates are volumetrically important enough to almost engulf all organic structures in the sample; the EPS has changed morphology from fibrous to smooth glue-like masses which coat the silica deposits; × 1900.

Fig. 2. Experiment 3, Sample 2 (500 atmospheres, 1 week); fibrous EPS web with mineralized fungal hyphae (note cracked crust in centre) and seemingly unmineralized spirochete; × 15000.

Fig. 3. Experiment 3, Sample 2 (500 atmospheres, 2 weeks); thickly encrusted fungal hyphus with part of a smooth-surfaced, seemingly unfossilized, bacterium, bottom left (arrow); note the still fibrous aspect of the EPS; × 7000.



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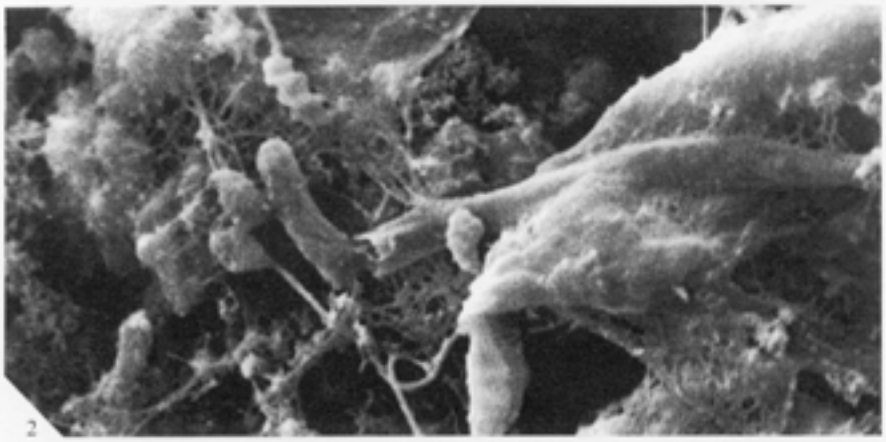

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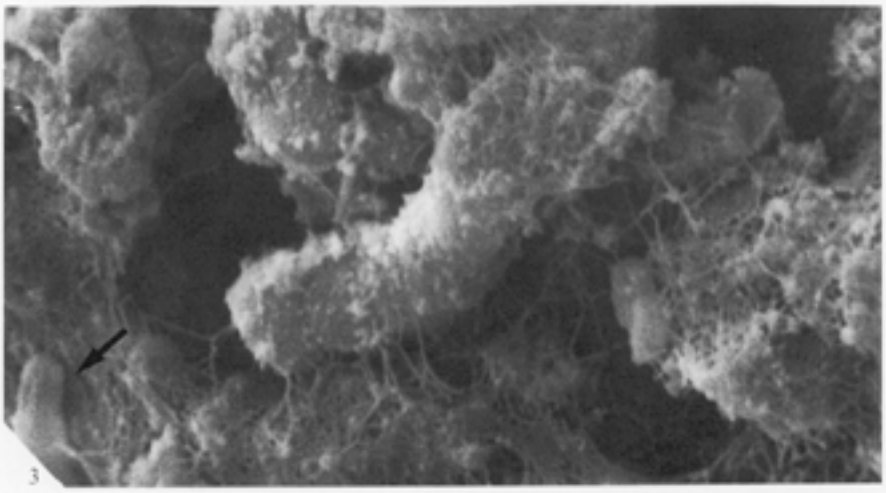


3

WESTALL *et al.*, Experiments 1 and 3

other types of bacteria? Other very important factors to take into account, however, are the physico-chemical conditions of the environment, as there is a very close interaction between microorganisms and their physico-chemical environment (Guerzoni *et al.* 1995). Microbial respiration and metabolic products change conditions, such as pH, within the immediate environment of the bacteria. On the other hand, the physico-chemical properties of the environment and the interactions between cells and the surrounding water are influenced by the nature and properties of the ions present, which affect the hydrophobic balance of the environment (Guerzoni *et al.* 1995).

In the experiments described in this paper, it was noticed that although all the eukaryotes and many of the bacteria were rapidly silicified (within one week at atmospheric pressures), a number of the latter, especially Gram negative forms (mostly spirochetes) in Experiments 1 and 2, resisted complete degradation for long periods and became only partially mineralized after four months' fossilization (at atmospheric pressures). The fact that, when they did silicify, only certain parts of the cell, such as the apices of some bacilli and spirochetes, or certain locations on the walls of some cocci, became mineralized may be related to differences in the composition and hydrophobicity of the heterogenous cell wall. Of the microorganisms which were rapidly mineralized, it appears that the cell wall and, often, all or much of the cytoplasm and internal organs are initially permeated by silica. This would explain the fact that the fossilized microorganisms in the early phases of fossilization often had a smooth rather than rough, encrusted surface when viewed with the SEM, and that individual organelles in the eukaryotes could sometimes still be identified.

Interestingly, not only did the microorganism cells become rapidly silicified but also the EPS in the groundmass. In the early stages of fossilization the volume of EPS in the groundmass actually seemed to increase, probably due to the addition of the degradation products of the microorganisms which, in turn, became rapidly mineralized. In some cases specific organelles, such as chloroplasts from diatoms, could still be distinguished. The importance of macromolecule mineralization rather than simply cellular mineralization in carbonate reefs was noted by Reitner (1993). He studied the formation of modern cryptic microbialites and concluded that 'Calcifying organic macromolecules are mainly responsible for microbialite formation by cementing detritical material' (Reitner 1993, p. 3). In this reef situation, Ca^{2+} is bound to the carboxyl groups of the macromolecules which make up the microbial biofilm.

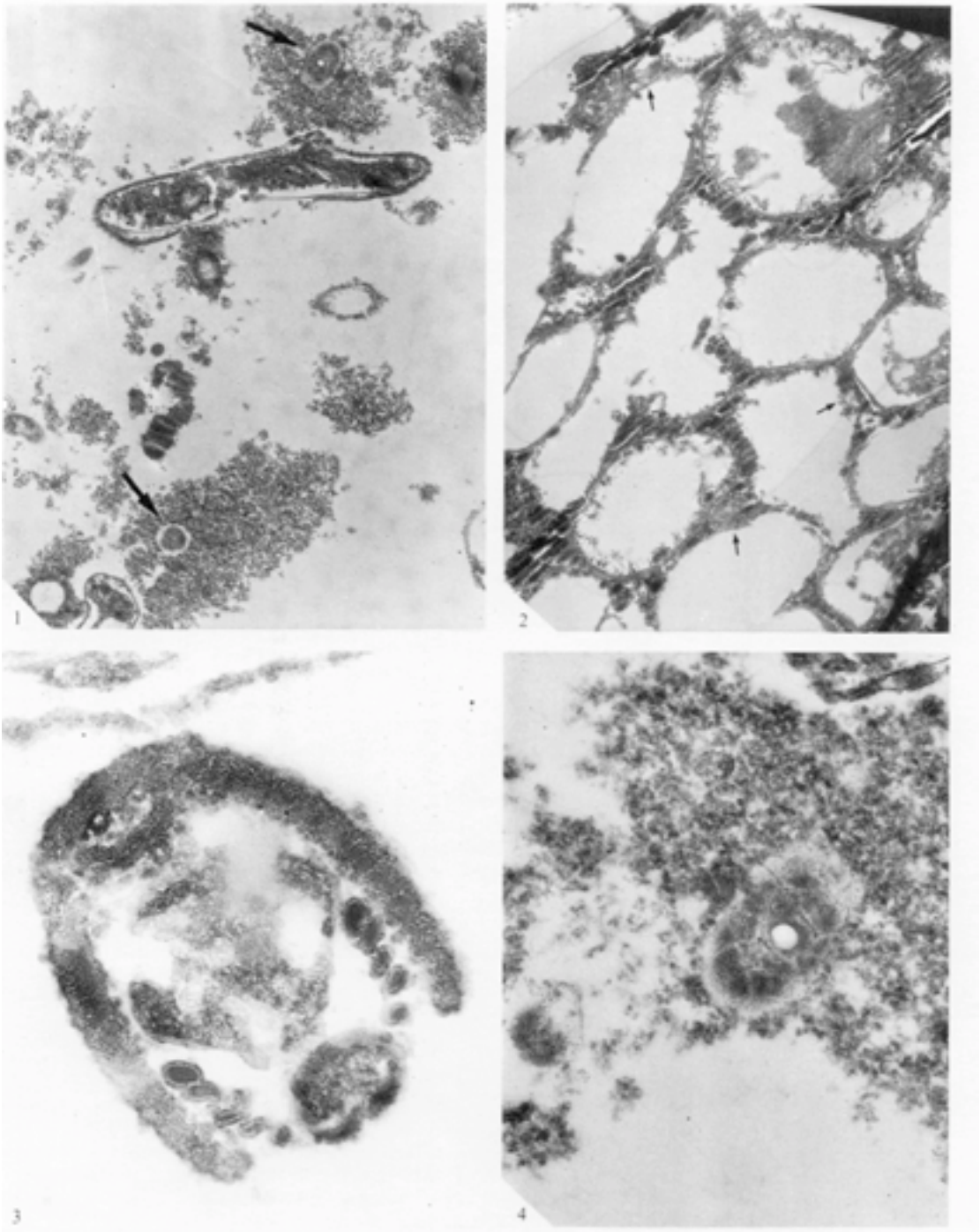
We have seen that organic matter acts as a template for initial silica nucleation but what happens to it during the fossilization process? The experiments demonstrated that most of the degraded cellular material ends up in the groundmass as EPS which, in its turn, becomes fossilized. However, in situations where the microorganisms are rapidly entombed in precipitated silica, often some unmineralized parts of the organic cell wall remain, as a comparison of contrasted and uncontrasted TEM sections shows. This is especially apparent in the high pressure fossilization experiment of Sample 3, which contained microorganisms in water from above the deep-sea sediment surface.

EXPLANATION OF PLATE 9

Figs 1, 4. Experiment 3, Sample 2 (500 atmospheres, 2 weeks). 1, contrasted TEM section showing well encrusted fungus filled with silica and a number of silicified bacteria (moulds and casts, arrows) engulfed in a granular silica deposit; × 10000. 4, contrasted TEM section showing detail of a bacterium cast (with inclusion) engulfed in the granular silica deposit; × 41000.

Fig. 2. Experiment 3, Sample 2 (500 atmospheres, 4 weeks); contrasted TEM section showing that the silica deposit has completely engulfed all the organic structures; there is also distortion of some diatom frustules, probably as a result of the pressure; bacteria are hardly recognizable – some small oval structures which may be bacteria are arrowed; × 28000.

Fig. 3. Experiment 3, Sample 2 (500 atmospheres, 3 weeks); uncontrasted TEM section showing a heavily mineralized diatom with parts of the cytoplasm also mineralized; the symmetrical structures may represent remnants of the frustule wall or may be artefacts; note the more electron-dense aspect of the silica; × 69000.



WESTALL *et al.*, Experiment 3

Probably, during the gradual crystallization of the silica, the non-mineralized organic matter is expelled and migrates through the still porous and hydrated precipitate and concentrates at the edges of the silica deposit. In fact, SEM studies invariably show the botryoidal siliceous masses coated with a biofilm of stringy, denatured EPS (Pl. 10, figs 1, 4).

The gradual change in crystallinity of the silica with time, although not crystallographically measurable, was evident in two ways. In the first place the precipitated silica had a less porous and more electron-dense texture when observed with the TEM and, secondly, its morphology changed. Although spheres of relatively electron-dense silica are present in all samples after one week of fossilization (even at 1 atmosphere), most of the early silica precipitates occurred as porous disseminated or loosely aggregated spherules, or as a porous crust around the microorganisms. After a few weeks the disseminated or loosely aggregated spherules in the groundmass consolidated to form botryoidal-surfaced masses and larger spheres, and the uniform porous crusts around the microorganisms became crusts of fused spherules to which other spherules became attached. The size of these spherules gradually increased, resulting in a rough mammillated crust, similar to the bacteria crusts observed in the Oligocene to middle Miocene sediments from the south-eastern Atlantic (Pl. 10, fig. 2). Other studies of silicified microorganisms also noted the mammilated morphology of the mineralized organisms (Oehler 1976) and the mineralizing silica (Knoll 1985).

Silicification artefacts

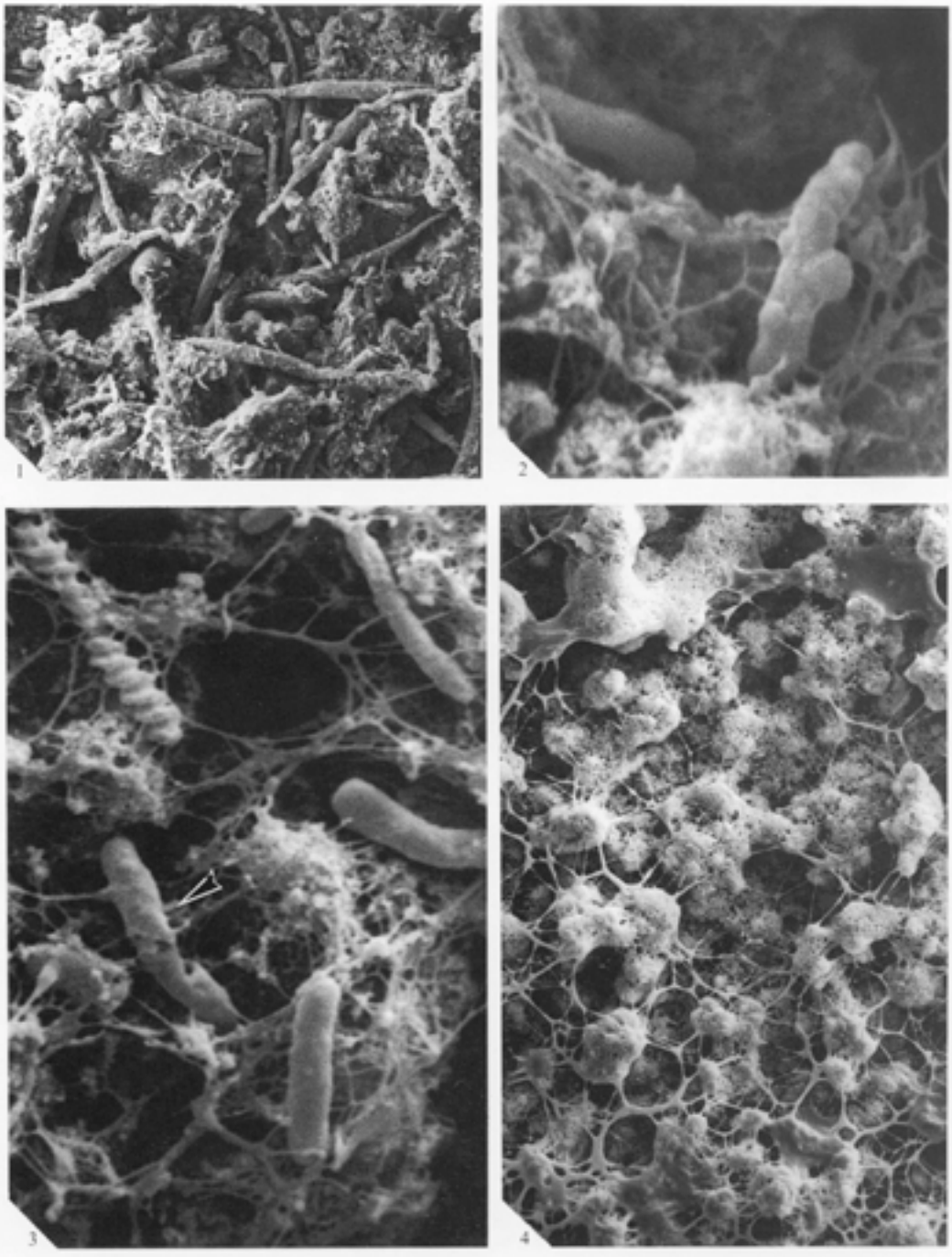
One of the most interesting observations from this experiment relates to the effects of continued fossilization with unlimited supplies of dissolved silica. Both the SEM and TEM studies demonstrate that, with time, and especially under pressure, the precipitated silica aggregates engulfed mineralized and unmineralized microorganisms. These aggregates were characterized by the botryoidal surfaces typical of opaline silica. The engulfed organisms were not distinguishable within the silica aggregates with the SEM. Moreover, the TEM sections showed a gradual merging of the silicified microorganisms with the engulfing silica such that, in Experiment 3 (500 atmospheres) after four weeks, bacteria could not be distinguished even in TEM section. In the long-term pressure Experiment 5 (one to three months at 500 atmospheres), even the robust diatom frustules lost their identity and only the clay moulds which used to coat bacteria remained to document the former presence of the relatively abundant microbial community in this deep-sea sediment sample.

Another silicification artefact was the formation of a silica core in the mineralized bacteria, which looked like an artificial nucleus, resulting from the mineralization of the shrunken, degraded cytoplasm. The occasional presence of an organic membrane around the false 'nucleus' could be due to the adsorption of the excluded organic matter from the silicified cytoplasm onto the siliceous deposit. If such a phenomenon were preserved in the natural fossil record, it could lead to misinterpretation and confusion with eukaroytes. In fact, the formation of artificial 'nuclei' was also noted by Francis *et al.* (1978*a*), who discussed the implications of such artefacts in the determination of the timing of the appearance of the eukaryotes during the Proterozoic.

EXPLANATION OF PLATE 10

Figs 1–3. Experiment 3, Sample 2 (500 atmospheres, 3 weeks). 1, all the organic structures appear to be encrusted and the EPS no longer has a loose fibrous aspect; × 1000. 2, even the bacteria begin to look encrusted and mineralized; this individual displays a remarkable resemblance to the encrusted bacteria in the upper Oligocene–middle Miocene samples from the SE Atlantic (Pl. 1, fig. 3); × 19000. 3, the bacteria here have smoother surfaces but the bacterium centre left is broken showing the hollow interior (arrow); note the granular silica deposits still associated with the EPS in the groundmass; × 14000.

Fig. 4. Experiment 3, Sample 2 (500 atmospheres, 4 weeks); individual organic structures are no longer recognizable in the mammillated silica deposit; the EPS has taken on a smoother, stringy appearance; × 19000.



WESTALL *et al.*, Experiment 3

Other non-microbial structures which formed during the course of our experiments included (1) individual silica spheres (they have previously been misinterpreted as epiphytic bacteria attached to other silicified bacteria, e.g. Monty *et al.* 1991, pl. 4, fig. 6) and strings of spheres attached to the surfaces of the silicified microorganisms, (2) regularly and irregularly shaped objects occurring in diatoms and in the groundmass of Experiment 3, resulting from the mineralization of the degradation products of the microorganisms, as well as (3) the hollow or full spheres in Experiment 5. The last may represent the silicified blobs of the degradation products of the microbial cells, or even the interface along which dissolved organic matter accumulated between a water droplet and TEOS. The mineralization of liquid or gas droplets in nature is not an unusual phenomenon (Gerdes *et al.* 1994).

Comparison of the experimentally produced microfossils with the silicified bacteria in upper Oligocene to middle Miocene sediments from the south-eastern Atlantic

There are a number of similarities and differences between the silicified structures obtained from these experiments and those observed in the upper Oligocene to middle Miocene sediments of the south-eastern Atlantic. In the first place, the botryoidal morphology of the surface of the silicified bacteria is similar. Although most of the Oligocene–Miocene bacteria displayed a rough mammillated crust-like surface, other individuals appeared to be smooth and looked 'unfossilized' and 'fresh' (Monty *et al.* 1991). Our experiments show that, in the latter case, the fossilization process was probably not very advanced and that the bacteria were probably impregnated by hydrated porous silica, similar to that in the early weeks of the fossilization experiment at atmospheric pressures, or at one week at pressures of 500 atmospheres. The SEM-visible rugged crusts coated with spherical/hemispherical structures, the size of those in the ancient sediments, only formed after a few months of fossilization at atmospheric pressures and after a few weeks at deep-sea pressures.

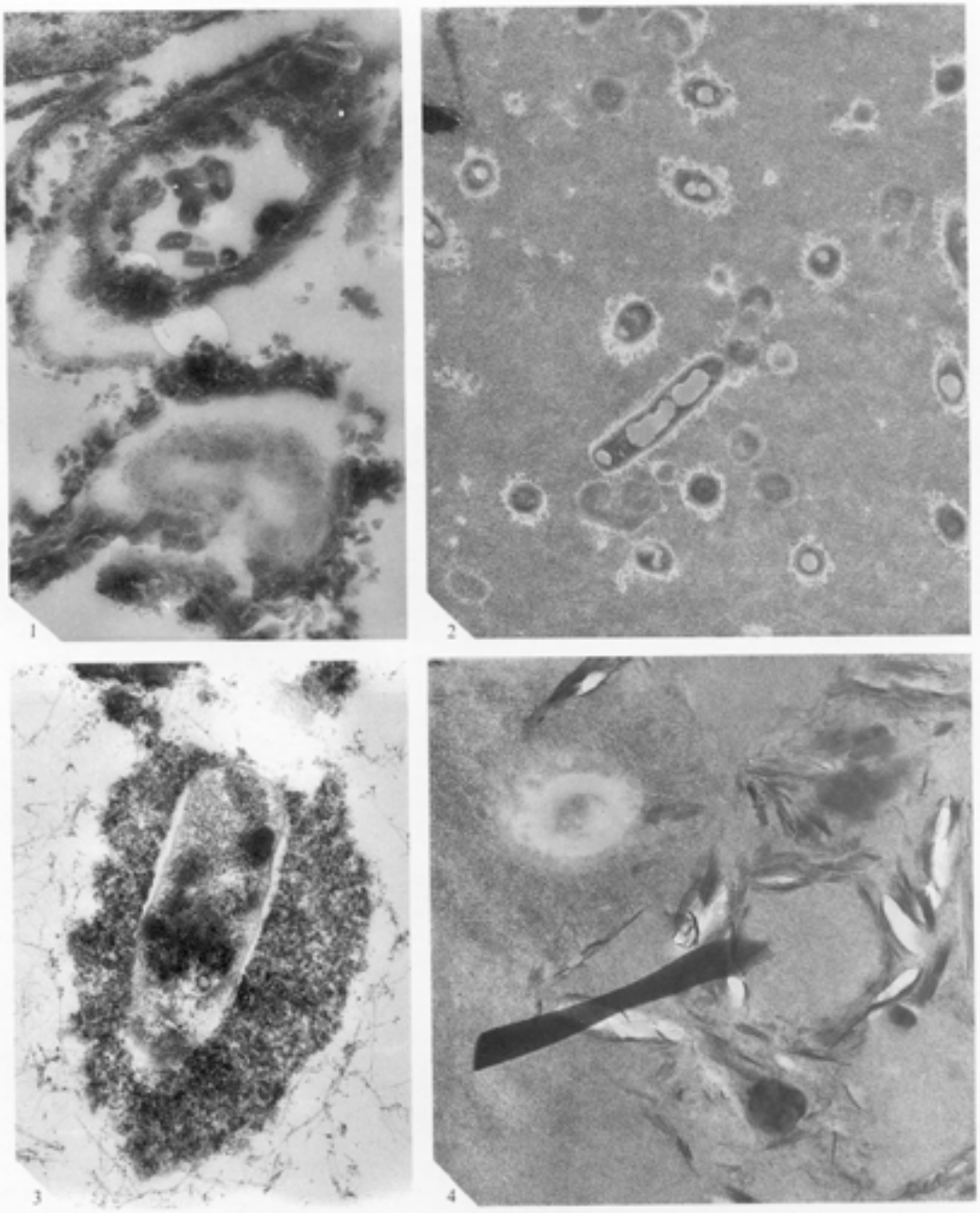
From this observation it can be concluded that, within a given fossil bacteria-bearing horizon in the upper Oligocene to middle Miocene sediments from the south-eastern Atlantic, bacteria exhibiting a rugged crust were subjected to a longer period of fossilization than those having a smooth surface. Our experiments showed that, for those bacteria recalcitrant to degradation and last fossilized (apparently Gram negative forms), the whole wall was not permineralized by very fine silica. Instead, silica spheres grew at specific locations on their surfaces. This observation may indicate that the smooth-surfaced fossil bacteria of the deep-sea sediments represent permineralized younger bacteria.

As the sediments in which the fossil bacteria were found contained a small amount of other minerals (< 10 per cent.), including clay minerals, and since the pore waters would be carrying many dissolved ions, there is a strong possibility that certain types of bacteria in the microbial mat might have immobilized Fe ions on their surface, which would have retarded their degradation and increased their chances of fossilization (cf. Ferris *et al.* 1988).

EXPLANATION OF PLATE 11

Fig. 1. Experiment 3, Sample 2 (500 atmospheres, 4 weeks); uncontrasted TEM section showing rod-shaped silica crusts in a diatom which could represent specific organelles or artefacts; ×41000.

Figs 2–4. Experiment 4, Sample 3 (500 atmospheres, 1 week), contrasted TEM sections. 2, showing many bacteria and other unidentified organic structures embedded in a finely granular silica deposit; the bacteria seem to be in the form of casts outlined by an organic membrane; Gram negative bacteria are outlined by an empty halo; ×16500. 3, a Gram negative bacterium with a thick glyocalyx; both glycocalyx and bacterium have been silicified; faint remnants of the layered cell wall may be seen; ×41000. 4, the silicified glycocalyx around a bacterium represented by an empty space containing an artificial 'nucleus' (upper left); at the centre right a circle of clay flakes forms a mould which marks the position of a completely degraded bacterium (compare with Pl. 12, fig. 3); ×28000.



WESTALL *et al.*, Experiments 3 and 4

Whereas in the experiments the fossilization process continued until most of the fossilized microorganisms were engulfed in botryoidal silica masses and the bacteria lost their identity, silicification in the ancient deep-sea sediments stopped after the mineralization of the bacteria and the complete coating of all mineral particles in the horizon. Small filamentous structures (part of the original reticular structure of the biofilm?) were engulfed in the botryoidal-surfaced silica coating and bacteria bases were embedded in it, but silica precipitation ceased before the bacteria were engulfed. The cessation of silica precipitation would have occurred when the amount of dissolved silica in the pore waters dropped below a critical concentration.

The formation of artificial nuclei in the experimentally silicified bacteria was noted. Similar structures in clay moulds were observed in the ancient deep-sea sediments from the south-eastern Atlantic (Pl. 2, fig. 4). However, despite innumerable hours of searching, very few of the fossilized bacteria could be identified with certainty in these samples using the TEM. Those that were identified had a completely permeated aspect and were not hollow (Pl. 2, fig. 2).

CONCLUSIONS

The experiments demonstrated the following.

(1) Not all microorganisms silicify with the same facility; the Gram negative bacteria (especially spirochetes) in Experiments 1 and 2 seemed to be recalcitrant to fossilization, whereas the Gram positive bacteria and eukaryotes (diatoms and fungi) silicified very readily.

(2) The EPS of the groundmass, including organelles such as the chloroplasts of diatoms, was particularly susceptible to silicification.

(3) The resulting morphology of the fossilized microorganisms depended upon the fossilization time and, consequently, the amount of silica available. In the samples observed after only a few weeks of fossilization (those at atmospheric pressures and after one to two weeks at 500 atmospheres), most of the bacteria appeared 'fresh' and 'unmineralized' when observed with the SEM, whereas the TEM studies showed that they were, in fact, impregnated by very finely textured, hydrated silica. Continued fossilization (two to four months at atmospheric pressures and three weeks at 500 atmospheres) gave the microorganisms an encrusted, mammillated, surface morphology, whereas further fossilization resulted in the complete embedding of the microorganisms in a siliceous precipitate and the eventual loss of individual cellular identity.

(4) Artefacts such as silica cores looking like artificial nuclei in the bacteria were common. Other artefacts formed after longer periods of silicification at deep-sea pressures.

(5) Silicification occurred more rapidly under deep-sea pressures than at atmospheric pressures.

Interpretation of the process of fossilization of the upper Oligocene to middle Miocene silicified microbial mats in the south-eastern Atlantic sediments was aided by the observations obtained from the experiments: comparison with the results of the experiment indicated that the microbial

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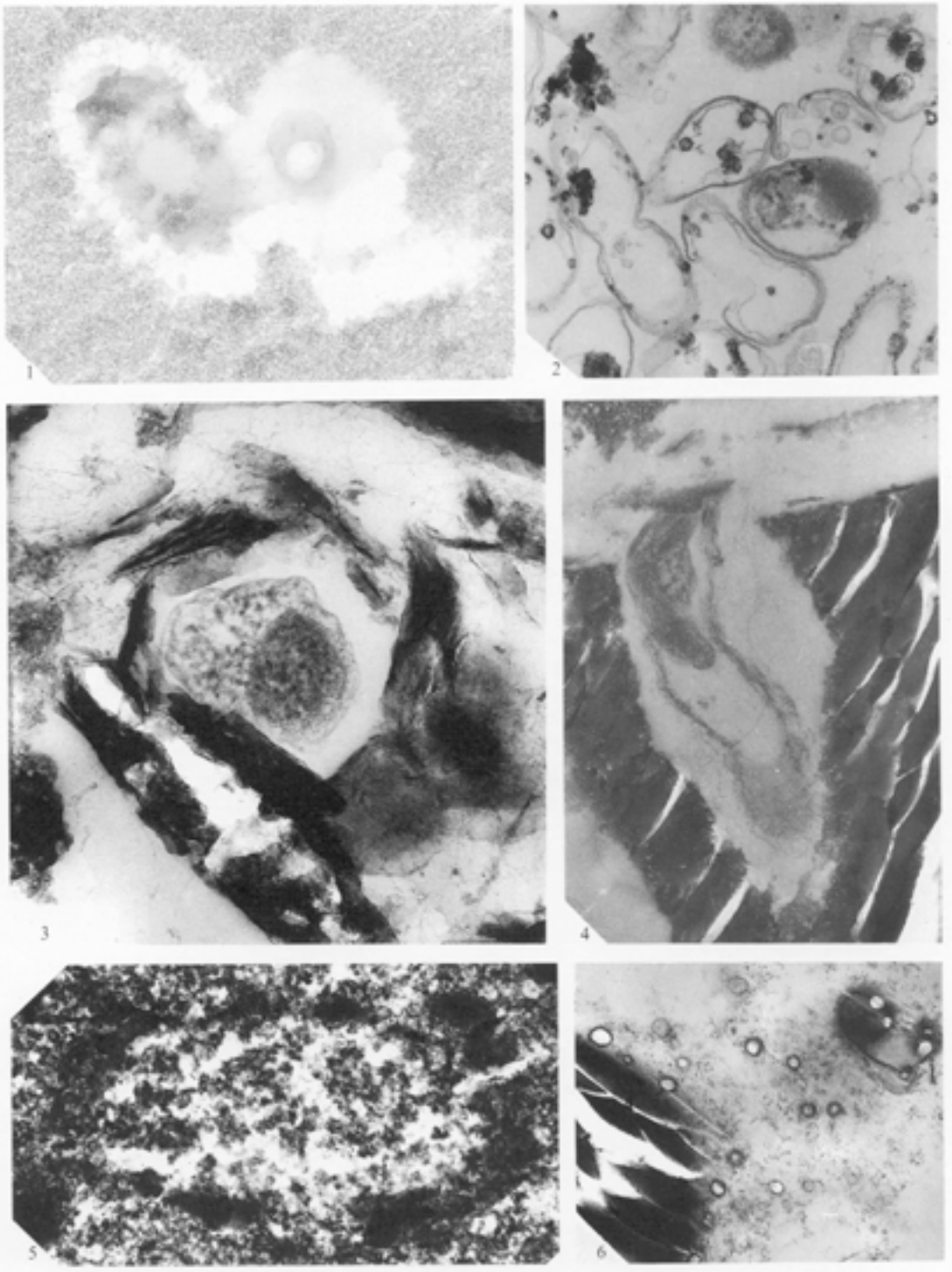
EXPLANATION OF PLATE 12

Fig. 1. Experiment 4, Sample 3 (500 atmospheres, 3 weeks); uncontrasted TEM section of the moulds of two bacteria containing artificial 'nuclei'; ×41000.

Fig. 2. Experiment 4, Sample 3 (500 atmospheres, 4 weeks); contrasted TEM section of very degraded Gram negative bacteria and other unidentified organic structures with silica spheres growing within the structures and on the walls; ×41000.

Figs 3–4. Experiment 5, Sample 4 (untreated); contrasted TEM sections. 3, partly distorted bacterium within a clay particle mould; distortion of the bacterium could be a result of the change in pressure between the deep-sea and the laboratory; ×69000. 4, bacterium within the aureola of a diatom frustule; ×41000.

Figs 5–6. Experiment 5, Sample 4 (500 atmospheres, 1 month); uncontrasted TEM sections. 5, faint outline of a diatom frustule in the silica deposit; ×28000. 6, artificial silica spheres, possibly formed around liquid droplets; ×41000.



WESTALL *et al.*, Experiments 4 and 5

community within individual fossiliferous microbial horizons seems to have been silicified continuously during the life of each individual community; older, thickly encrusted bacteria coexisted with younger, smooth individuals. However, the supply of silica was not unlimited because the organic structures were impregnated and encrusted but not completely engulfed by the siliceous deposits.

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APPENDIX

All samples are stored at the laboratory of the Sezione di Chimica e Tecnologia degli Alimenti of the Dipartimento di Protezione e Valorizzazione Agroalimentare at the University of Bologna. The SEM sample stubs are kept in a dessicator and are listed below.

Sample	Silicification time	Pressure atm	Stub no.
2	1 wk	1	208
	2 wk		215
	3 wk		209
	4 wk		216
	1 wk	500	211
	2 wk		219
	3 wk		220
	4 wk		221
3	1 wk	500	212+222
	3 wk	500	213
4	1 mth	500	214

TEM sample blocks and sections in numbered boxes are as follows.

Sample	Silicification time	Pressure atm	Resin block	Ultrathin section (* contrasted)
1	0	1	Box 3: 1C	Box 3: 2E, 3A*
	2 mth		Box 3: 1A	Box 3: 6C*, 6D, 6E, 7A
	4 mth		Box 3: 1B	Box 3: 7B*, 7C, 7D
2	1 wk	1	Box 4: 1J, 1K	Box 3: 19E*, 20A, 20B, 20C
	2 wk		Box 4: 1L	Box 4: A1*, A2, A3, A4
	3 wk		Box 4: 1M, 1N	Box 4: A5*, B1, B2, B3
	4 wk		Box 5: 1A, 1B, 1C	Box 3: 14A*, 14B
	1 wk	500	Box 4: 2A, 2B	Box 3: 12B*, 12C, 12D
	2 wk		Box 4: 2C, 2D	Box 3: 12E*, 13A, 13B
	3 wk		Box 4: 2E, 2F	Box 3: 13C*, 13D, 13E
	4 wk		Box 5: 1D, 1E, 1F, 1G	Box 3: 14C*, 14D, 14E, 15A
3	1 wk	500	Box 4: 2G	Box 3: 15B*, 15C, 15D
	2 wk		Box 4: 2H	Box 3: 15E*, 16A, 16B, 16C
	3 wk		Box 4: 2J	Box 3: 16D*, 16E. 17A, 17B
	4 wk		Box 4: 3L, 3M, 3N	Box 3: 17C*, 17D, 17E, 18A
4	0	500	Box 4: 2K	Box 3: 18B*, 18C, 18D, 18E
	1 mth		Box 5: 1H, 1J	Box 3: 19A*, 19B, 19C, 19D
	3 mth		Box 3: 2A, 2C, 2D	Box 4: C2*, C3