Reports

Electron Microscopy of Fossil Bacteria Two Billion Years Old

Abstract. *Occurrence of well-preserved rod-shaped and coccoid bacteria in the Precambrian Gunflint chert (1.9 × 10^9 years old) has been demonstrated by electron microscopy. This appears to be the oldest definite occurrence of bacteria in the fossil record. The organisms are morphologically comparable with certain modern iron bacteria.*

The Gunflint chert, a sediment in southern Ontario aged about 2 × 10^9 years, contains the oldest known structurally preserved evidence of life (*1*). The diversity and complexity of this Precambrian microfossil assemblage and the occurrence of possible biogenic remnants in older sediments (*2*) leave little doubt that biological systems must have originated long before Gunflint time. It might be hypothesized, on the basis of speculation concerning the nature of the primitive environment, that anaerobic heterotrophic bacteria were among the earliest forms of living organisms. This hypothesis would be consistent with the morphology and metabolism exhibited by living bacteria. Moreover, it has been suggested that chlorophyll *a*, the dominant photosynthetic pigment of algae and higher plants, may be the evolutionary derivative of bacteriochlorophyll, a photosynthetic pigment present in purple and brown bacteria (Thiorhodaceae, Athiorhodaceae) (*3*). Thus the beginnings of life, the origin of the photosynthetic mechanism, and the effects of these events on the history of the earth may be intimately related to the origin and evolution of the bacteria. We now report the occurrence of ancient fossil bacteria in the Gunflint chert.

Fossil bacteria have been reported in fecal pellets, coprolites, petrified bones, silicified and calcified plant tissue, salt deposits, oil shales, coal, limestones, cherts, and iron ores (*4*). The rod-shaped and coccoid bacteria present in the Gunflint chert lie stratigraphically below, and are hence older than, the oldest previously reported generally accepted fossil bacteria, filamentous forms from the Precambrian of northern Minnesota (*5*).

Mineralogists and metallurgists have used electron microscopy to great advantage in studying alloys and crystallization phenomena, but very few fossils and fossiliferous rocks have been investigated (*6*). Recently, remarkably well-preserved imprints of iron bacteria in pyrite 300 million years old were reported by Schopf, Ehlers, and others (*7*); our application of electron microscopy to the study of Precambrian life was stimulated by their demonstration of its important paleontological potentials (*8*).

Three techniques were used in studying the Gunflint chert. Surface replicas were prepared in the following manner. A hand-sized specimen of Gunflint chert from the Schreiber locality (*9*), known by study of thin sections to contain a variety of microfossils, was cut to obtain seven 1-cm cubes of dense black chert. The cubes were mounted in Bakelite, ground with Carborundum grit and finally polished with 0.05-μ γ-alumina on a nylon lap. Six of the polished samples were either etched by immersion, polished-face up, in a 4.91-percent solution of hydrofluoric acid (reagent grade) in distilled water, or fume etched by suspension, polished-face down, over the same warmed solution.

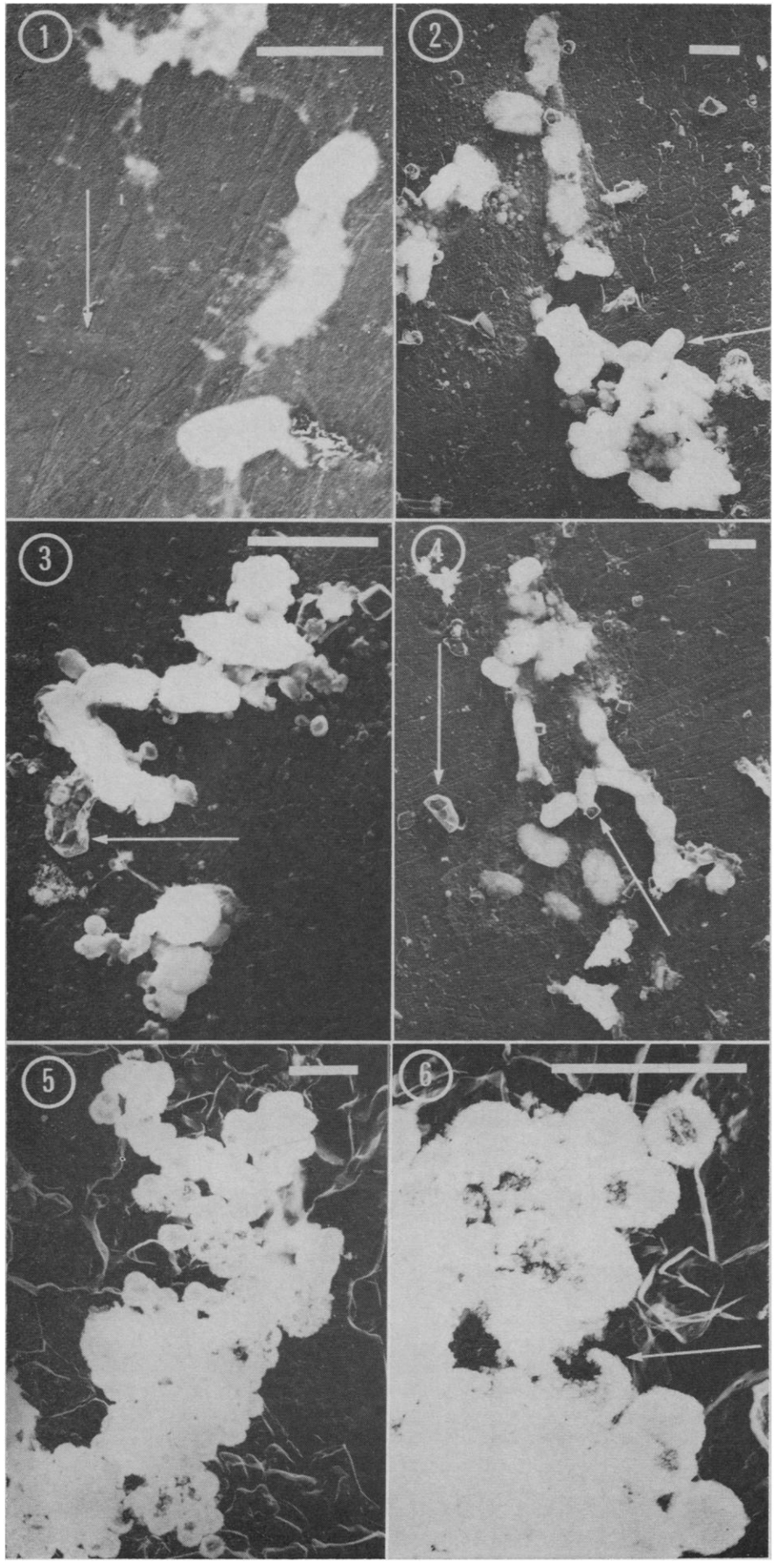
Five of the seven samples contained bacteria; the surfaces of these five samples were prepared as follows: sample 1, not etched; samples 2, 5, and 6, etched by immersion for 60, 90, and 180 seconds, respectively; sample 7, fume etched for 30 seconds over the same solution held at 60°C.

In a vacuum evaporator, the surfaces of the samples were then shadowed with platinum at about a 2 : 1 angle and replicated with a film of evaporated carbon. The surfaces were then scored to 0.5-cm squares; the platinum-carbon replicas were floated off in a dilute hydrofluoric acid solution and picked up on microscope grids. Micrographs were taken with an RCA-EMU-3F electron microscope.

In addition to surface replicas, hydrofluoric acid macerations and ultrathin sections of the Gunflint specimen were studied by electron microscopy. The acid-resistant residue from hydrofluoric acid maceration was prepared for study by placing drops of the macerated suspension on coated grids, blotting off the excess liquid, and shadowing the grids with platinum. Numerous septate filaments (*Gunflintia* spp.) and reticulate spheroidal bodies (*Huroniospora* spp.) were observed in these preparations (*10*). This method of preparation has the advantage of concentrating the organic material for rapid examination. This advantage is more than offset, however, by the destruction of microstructure resulting from the dissociation of the organic residues from the mineral matrix, which destruction accompanied even very slow maceration in dilute acid.

Ultrathin sections (0.05μ to 0.15μ thick) of the specimen were cut with a diamond knife on a Sorvall MT-2 ultramicrotome, for which purpose a thin slice of the specimen was embedded in Epon 812. Although it is possible to cut sections in this manner, microconchoidal fracturing of the chalcedonic matrix and the low concentration of organic material in a section of such thinness make examination difficult and relatively unrewarding.

A variety of microfossils has been observed in the Gunflint chert in each of the three types of preparation described. Of particular interest are rod-shaped (Figs. 1–4) and coccoid (Figs. 5 and 6) bacterial remains which are best studied by the replica method. The photographs presented are negative prints of micrographs of surface replicas. The electron density of both bacterial forms shown indicates that the organic particles have been pulled from the inorganic matrix during replication, and that we are observing in most cases the organic material itself and not a replica of it. In some instances (Fig. 2) the bacterial cells have separated slightly from the replica, producing electron-transparent edges; these electron-transparent areas should not be confused with the more-electron-dense shadowed structures.



Rod-shaped bacterial cells occur in the five samples listed. Measurements of 20 of these elongate bacteria, oriented parallel to the plane of the replica, show that they are approximately 1.1 μ long and 0.55 μ wide. Orientations of the many other rod-shaped cells present, passing through the plane of the replica, make meaningful measurement difficult (see Figs. 2–4). In size and shape the cells closely resemble certain modern rod-shaped bacteria. The fossil microorganisms sometimes occur as apparently isolated cells (Fig. 1), but more frequently are clumped in groups of six or eight (Fig. 2) or are in chains up to seven cells long (Figs. 3 and 4). Amorphous organic material, which may be the remnant of an encompassing (possibly) mucilaginous sheath, often surrounds and connects the cells (Figs. 3 and 4).

False branching appears to be present in at least one chain (Fig. 4). Rod-

Figs. 1–6 (left). Bacteria in Gunflint chert; line in each figure represents 1 μ. Fig. 1. Well-preserved, rod-shaped, apparently isolated bacteria in surface replica of sample 1. Arrow points to bacillary imprint in rock surface. Subparallel lineations oriented approximately vertically are polishing scratches (about $\times$ 19,500). Fig. 2. Clumped and isolated rod-shaped bacteria in surface replica of sample 2. Arrow points to electron-transparent edges (dark in micrograph) where organic material (light in micrograph) has separated from carbon-platinum replica (about $\times$ 7800). Fig. 3. Sample 2: Surface replica showing poorly preserved rod-shaped bacteria arranged in a filament. Note organic continuity between cells and the encompassing sheath-like residue; arrow points to cellular remnant in which only this surrounding amorphous material is present (about $\times$ 19,700). Fig. 4. Sample 2: Rod-shaped bacteria, both isolated and apparently arranged in a branched (false branching) filament. Arrows point to organic material interpreted as bacteria seen in transverse section; several similar rod-shaped cells, apparently passing through the plane of the replica, appear in Fig. 2 (about $\times$ 7100). Fig. 5. Sample 6: Large group of coccoid bacteria composed of more than 100 individual cells. The cells, approximately 0.35 μ in diameter, appear to be randomly distributed with respect to one another. Chalcedony grain boundaries (irregular white lines) are clearly visible in this replica of a deeply etched surface (about $\times$ 10,600). Fig. 6. Sample 6: Surface replica showing chalcedony grain boundaries and numerous coccoid bacteria. Note the pronounced thickness and irregular surface of the cell walls; arrow points to a ruptured bacterial cell (about $\times$ 30,100).

shaped bacterial cells enclosed in a sheath and showing occasional false branching are the typical growth habit of the modern iron bacterium *Sphaerotilus natans* (*11*). A possible relation between the fossil bacteria and this extant form is questionable, however, because of the considerably smaller size of the Gunflint organism.

Coccoid bacteria-like objects occur in samples 2 and 6. These nearly spherical, rough-surfaced bodies are approximately 0.35 μ in diameter (Figs. 5 and 6). The cell walls are sometimes broken and may show surficial folding (Fig. 6). The thickness of the cell wall, up to 0.15 μ in some instances, may result from concentration of various minerals, such as iron compounds, either by metabolic activity or by diagenetic alteration. These fossils resemble in size and morphology certain iron bacteria of the genera *Siderocapsa* and *Siderococcus* (*12*), members of which metabolically concentrate iron compounds; this similarity suggests that the pronounced thickness of the cell walls is metabolic rather than diagenetic. Thimann (*13*) has brought to our attention the additional similarity in size, morphology, and general organization of these fossil coccoids to Winogradsky's "microcolonies in soil" (*14*).

That these minute fossils are both organically preserved and relatively undistorted is not surprising when one considers that many other members of the Gunflint assemblage are preserved in this manner and that complex organic molecules are present within the rock (*1, 15*). That these bacteria are indigenous to the rock rather than being laboratory contaminants is supported by the following three considerations: (i) the forms occur in the replicas both as organically preserved structures, and as imprints in the rock surface (Fig. 1); (ii) they are oriented in various positions not only parallel with but passing into the prepared rock surface; and (iii) the forms are consistently present in several samples prepared by different methods.

There seems little doubt that the forms we describe and portray are bacteria; their morphology, size, complexity of structure, and association with more-complex organisms are consistent with this interpretation. Rod-shaped and coccoid forms are widely distributed throughout the bacteria and occur in anaerobic and aerobic and heterotrophic and autotrophic types. Morphology offers, therefore, limited aid in the assignment of taxonomic position to fossil bacteria. A more satisfactory assignment should be based upon physiological processes in addition to morphology. In the absence of sufficient biochemical information on these bacteria, the conferring of taxonomic status by giving them generic and specific names seems unwarranted.

The electron microscope in conjunction with optical microscopy and other traditional methods of micropaleontology provides a powerful tool for morphological investigation of ancient life. Organic and inorganic geochemistry offer means for the investigation of ancient physiological processes. The occurrence of porphyrin derivatives of chlorophyll in Precambrian sediments, as demonstrated by organic geochemical analysis (*16*), may permit determination of the time of origin of the photosynthetic mechanism. Metabolic concentration of sulfur, iron, and other elements in bacterial and other fossils may be detectable by electron probe–x-ray microanalysis. Coordinated application of these and other techniques to the investigation of ancient sediments promises elucidation of diverse aspects of the morphology and physiology of Precambrian life.

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17. We thank Roger Branson, Harvard University, for assistance. Work supported by NSF grants GP-2794 and G-19727 and by PHS grants CA-06018 and GM-06637; one of us (J.W.S.) is a NSF graduate fellow.

14 July 1965

Sands of the Mid-Atlantic Ridge

Abstract. *Sands collected at 24 locations along the crest of the Mid-Atlantic Ridge between 57°S and 38°N consist predominantly of olivine, diopsidic augite, hypersthene, enstatite, amphibole, quartz, plagioclase, and volcanic glass, suggesting an olivine tholeiitic source. Eight cores contain relatively pure mineral sands; three of these cores reflect local volcanic activity. In 16 cores the manganese-coated mineral grains are mixed in a current-winnowed foraminiferal sand or ooze.*

The petrology of the Mid-Atlantic Ridge has been inferred from the petrology of the nine groups of islands and from about 20 dredge hauls (*1–5*). The rocks of the islands, predominantly composed of alkali basalt or its derivatives, are generally olivine-bearing but sometimes olivine-free (*6*). St. Paul's Rocks, the only exception, are composed of serpentinized dunite (*7*). Shand (*1*) and Quon and Ehlers (*2*) described the petrography of ten dredge hauls from the Mid-Atlantic Ridge near 30°N. The main rock types were serpentine, gabbro, and fine-grained basalt with, and occasionally without,