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*ELECTRON MICROSCOPY OF THE GUNFLINT MICROFLORA: PRELIMINARY RESULTS**

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The discovery of the approximately 2-milliard-year-old Gunflint microbiota, announced in 1954 by Tyler and Barghoorn,[1] at first did not attract much attention. Interest increased, however, with the establishment of its great age by Hurley *et al.*[2] and with growing realization of the probable relations between biologic and atmospheric evolution.[3, 4] Recently Barghoorn and Tyler[5] and Cloud[6] have stressed the morphologic diversity of the Gunflint assemblage and its possible bearing on the history of the atmosphere. And now, as the oldest structurally preserved microflora yet known, having a morphology and associated biochemical characteristics resembling that of simple living autotrophs, it has come to constitute a critical datum in considerations of atmospheric and biotic evolution.[7]

Studies to date of the Gunflint organisms have involved their morphology as revealed at high magnifications under the optical microscope, as well as aspects of the geochemistry of the enclosing rock. Of much interest is the announcement by Oró *et al.*[8] of the presence in this rock of the isoprenoid alkanes, pristane and phytane, interpreted by these authors as "chlorophyll degradation products." Pristane and phytane, however, are found not only as derivatives of green-plant chlorophylls but in bacteria and rather widely among animals as well,[9] so the possibility is not excluded that the Gunflint microflora may be dominantly bacterial.

Study under the electron microscope, therefore, has been undertaken in the hope of obtaining additional evidence as to the structure and affinities of these organisms. Not only the Gunflint fossils, but a number of other Precambrian microfossils deserve similar study. Paleontology at a molecular level will be approached when we have embedded these remarkable fossils, made microtome sections, and studied their internal structure. The results here reported are strictly preliminary.

Preparational Methods.—A variety of techniques was tried in preparing for electron microscopy samples of chert from the Gunflint Iron Formation at the previously described Schreiber Beach locality,[5, 6] 6.5 km west-southwest of Schreiber, Ontario.

First we tried putting a high polish on the chert and stripping a nitrocellulose peel from which palladium-carbon replicas were prepared. Where the surface was first lightly etched with hydrofluoric acid, crystal structure obscured other features, if any, and we got no useful results. The unetched polished surfaces, however, yielded a few problematical filamentous structures which stripped free with the peels (Fig. 2).

All other preparations involved some variation of standard palynologial maceration procedures.[10] Blocks of chert were sawed into small pieces and fresh material was steeped in Schulze's solution (15 gm $KClO_3$ + 150 ml H_2O + 300 ml HNO_3) to remove any adherent organic matter.

This was then oven-dried and pulverized with a sterilized tool-steel mortar and pestle. The powdered material was immersed in concentrated hydrofluoric acid until digested. The residue was repeatedly washed and centrifuged in distilled water until neutral. Concentrates were then suspended in glycerine jelly, ethanol, or methanol, or dried. Others were again briefly treated with Schulze's solution, then washed until neutral. Fossils were obtained from the latter (e.g., Figs. 3 and 4), but Schulze's solution breaks up many specimens even with brief immersion.

The richest preparations were those taken directly from the washed HF residues in ethanol to the mounting grids. Many specimens of both filamentous and globular bodies were obtained in this manner (Figs. 8–24).

Microscopical Methods.—Preparations as described above were scanned under the Philips North American model 100B electron microscope, operated at 80 kv, and selected objects were photographed.

Several different techniques were tried in preparing samples for ultramicroscopic observation. At first a suspension of HF residues in ethanol was vaporized ultrasonically; the vapor was caught on a grid with a carbon-coated formvar film and the sample scanned under the electron microscope. The fossils, however, were disintegrated by the ultrasonic treatment. A second technique involved the use of oven-dried samples. The dried residues were spread in ethylene glycol between glass slides and the formvar-coated grid slipped into this suspension. This also gave unsatisfactory results because the residues clumped in the ethylene glycol.

Best results were obtained when a drop from a suspension of HF residues in alcohol was allowed to dry on the formvar-coated grid. The residues concentrated toward the edge of the grid, but gave a relatively open distribution toward the center.

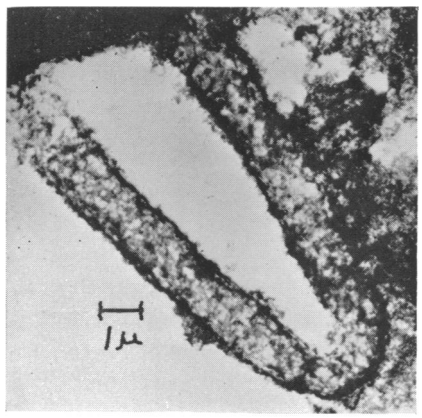
Recognition of Contaminants.—Many pitfalls attend an investigation of this sort. The microbiota observed in a rock may contain foreign elements derived from reworking of older material, downward movement from overlying rocks, or infiltration from the present surface. Structures of nonvital origin may mimic the morphology of organisms. Optical "artifacts" can be photographed to resemble structures of biologic origin. And, even with extreme care, contamination can enter at various stages in preparation outside a sterile chamber. In thin sections the mounting medium can be a source of foreign bodies. While washing HF macerations, microorganisms can be introduced from the atmosphere or even from distilled water.

Prepreparational contamination of the fresh rock is eliminated by the observed distribution of the microfossils in thin section. They occur only in dark, laminated segments of chalcedonic chert that are separated from each other by unfossiliferous detrital rock.[5, 6] Within these laminated, biologically organized parts of the chert they occur in greatest abundance along particular upwardly convex laminae which represent growth stages in the development of the laminated structure. Finally, individual fossils can be traced across contacts between adjacent enclosing mineral grains, showing that they were there before the minerals crystallized. They show no preference for postdepositional fractures in the rock, and the possibility of such introduction is eliminated not only by their localization in particular types of matrix, but also by their abundance in areas without fractures.

Real contamination during preparation, however, was detected and is illustrated in Figures 5–7. To make sure that we deal here with fossils surely endemic to the Gunflint chert, *preparations known or suspected to include contaminants are eliminated from the discussion of Results.* The endemism of the remaining organisms is further assured by their similarity to forms observed in thin section.

To detect contamination in HF macerations, several devices were employed. Blank preparations were run on large quartz crystals from a granite. Blank formvar-coated mounting grids were exposed to the atmosphere and then observed for airborne particles. And preparations containing objects that looked too well preserved or too unlike forms recognizable in thin section were eliminated. Figure 5 illustrates a known contaminant, the stalked bacterium *Blastocaulis* sp.,[11] found abundantly in various growth stages attached to minute pyrite crystals in one preparation. Evidently it entered the preparation during washing and utilized the residues as nutrients.

The objects illustrated in Figure 6 settled from the atmosphere onto a bare mounting grid. They resemble Figure 7, a "too-well-preserved" *Gallionella*-like structure that was common in one of our early preparations. Perhaps Figure 7 and like objects are not contaminants, but the safest thing is to exclude them. Figure 2 is also excluded as not surely endemic.



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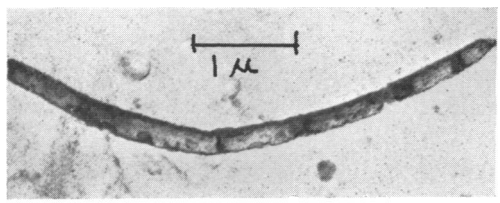

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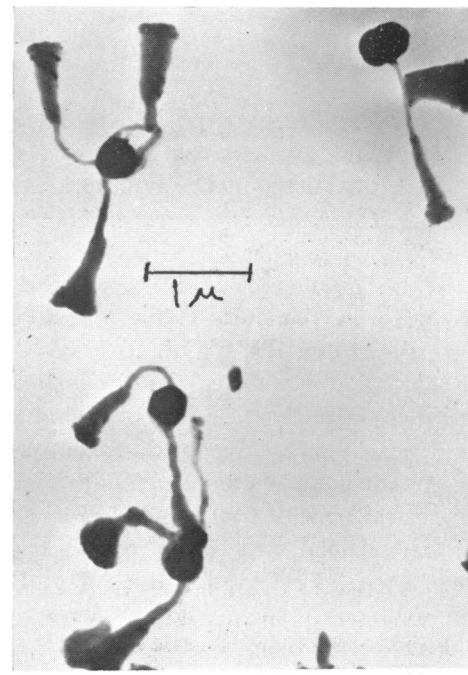

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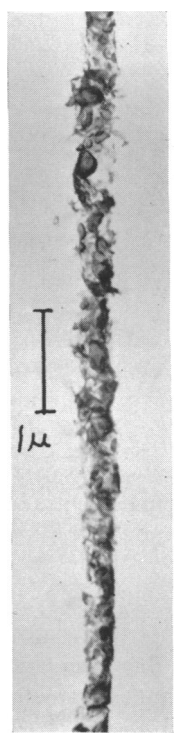

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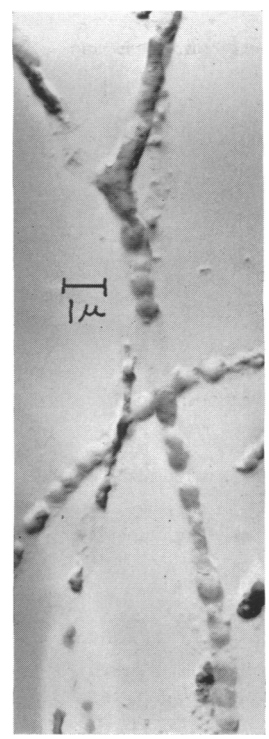

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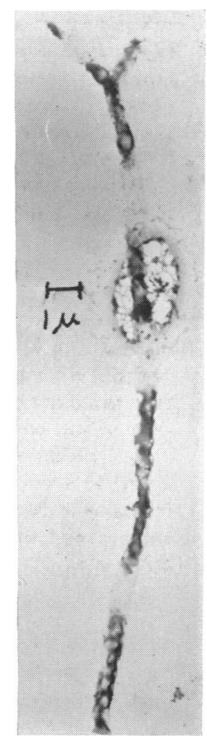

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FIGS. 1, 3, and 4.—Electron micrographs of filaments endemic to Gunflint chert. Bar scales for 1 μ.

FIGS. 2, 5–7.—Types of contaminants and possible contaminants encountered in some preparations. Bar scales all 1 μ. *Fig. 2:* Thread from polished surface of chert. *Fig. 5: Blastocaulis* sp. A living stalked bacterium attached to minute pyrite grains in HF residues. *Figs. 6–7:* Known and suspected contaminants. Fig. 6 is the known contaminant, from dust settled onto a bare formvar-coated grid. Compare this with Fig. 7, a probable contaminant found in early preparations.

Results.—All microorganisms recognized under the electron microscope as endemic to the Gunflint chert can be classed as (*a*) filaments with a central opening, (*b*) fine-textured ovoid bodies, and (*c*) reticulate, globular to ellipsoidal bodies.

(a) *Filaments with a central opening* (Figs. 1, 3, 4, and 8–14): This category includes threads of two different size groups. The largest and most abundant type, assignable to the taxon *Gunflintia minuta* Barghoorn, includes discrete threads 0.7–1.8 μ in diameter, having a septate or multicellular structure (Figs. 8–11 and probably 1 and 12). With advanced degradation some such threads begin to suggest a spiral structure. In one instance (Fig. 13) threads of this size meet in a manner suggestive of microcolonial growth. Whether this was a real microcolony of five individual threads or a fortuitous crossing of two threads with a third touching at the point of crossing remains a moot point. Figure 14, presumably representing a fragment of *Gunflintia minuta*, shows a vesicular surface that is reminiscent of the vacuolar structure of certain bacteria and blue-green algae. It may represent a partially degraded alveolar wall or an artifact produced by minute crystals impinging on the degrading tissue.

Smaller threads, measuring only 0.15–0.37 μ in diameter (e.g., Figs. 3 and 4), may or may not have a septate structure. Figure 2, of an object stripped from a polished surface of the chert, may belong to the same category, but it could be a contaminant.

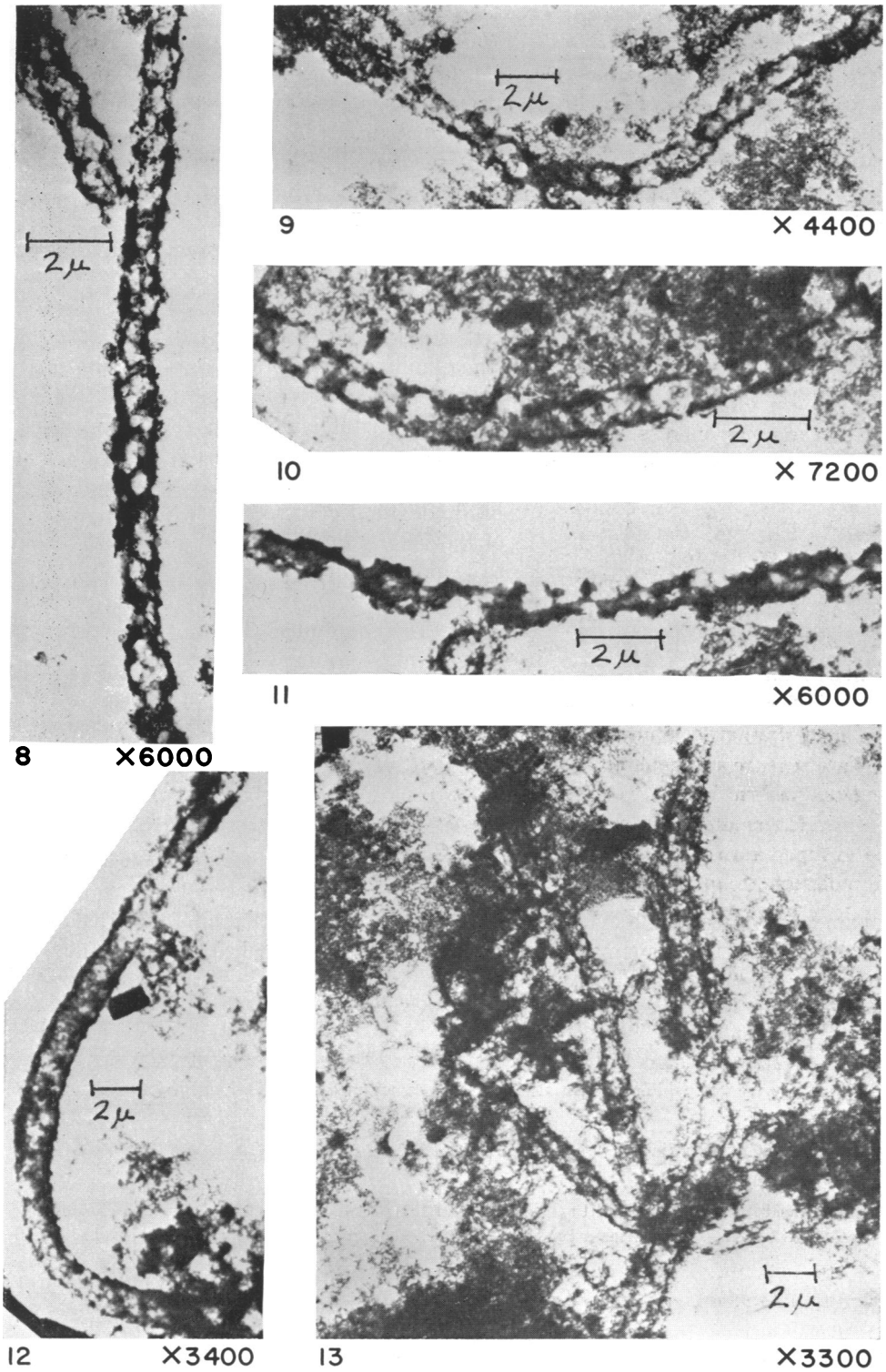
(b) *Fine-textured ovoid bodies* (Fig. 15): Here is included an object 2.6 by 5.7 μ in diameter somewhat like Barghoorn's *Huroniospora psilata*. It has the surface texture and general morphology of certain bacteria, for instance *Gallionella*[12] or *Desulfovibrio*.[13] Only one specimen was found, but we believe it to be endemic.

(c) *Reticulate, globular to ellipsoidal bodies* (Figs. 14–24): These structures can be grouped into two or three size classes, in which the dimensions of the surface reticulations tend to vary inversely with the size of the body. The larger bodies, 10–12 μ in diameter, have the smallest-scale reticulation (Figs. 23–24). They are assignable to the taxon *Huroniospora microreticulata* Barghoorn. The smaller bodies, 1–5.6 μ in diameter, have a large-scale reticulation (Figs. 16–22). Spheres of 1–2.6 μ diameter (Figs. 16, 19), however, have larger reticulations than an intermediate group 3.5–5.6 μ in diameter (Figs. 17, 18, and 20–22). Both these groups (Figs. 16–22) are assignable to the taxon *Huroniospora macroreticulata* Barghoorn.

Of interest is the indication of encapsulation suggested by Figures 16 and 19. This recalls the spores of bacteria whose reticulate coats are wrapped in an outer covering with mirroring reticulations, for instance *Bacillus meganterium* in Figure 14 of Robinow (1960).[14]

Interpretations.—Some interpretations are now in order.

We have one group of threads about 0.7–1.8 μ in diameter (Figs. 8–14) that show a structure reminiscent both of the thread bacteria and the blue-green algae. Cloud[7] has considered the difference unimportant and treated the association simply as one of early prokaryotic thallophytes. If a distinction were to be made on the basis of the present inconclusive evidence, however, we would favor assignment of these minute filaments to the thread bacteria on the basis of size alone. This is fortified by the presence of even smaller threads, only 0.15–0.36 μ in diameter (Figs. 3 and 4). The macroreticulate sporelike bodies in the range of 1–2.6 μ in diameter



FIGS. 8–13.—Electron micrographs of filaments endemic to the Gunflint chert. Bar scales all 2 μ. *Figs. 8–12:* Various stages of degradation of 0.7–1.8-μ-diameter septate threads assignable to the taxon *Gunflintia minuta* Barghoorn. The extensively degraded thread of Fig. 11 hints at a spiral structure, perhaps as an artifact of degradation. *Fig. 13:* Conjunct or overlapping filaments suggesting a microcolony of the above. The individual threads resemble Barghoorn's *Gunflintia minuta* while the complex as a whole resembles his *Eoastrion simplex*. As no other assemblages such as this were observed, it could be a fortuitous juxtaposition of filaments.

could be spores of the 0.7–1.8-μ filaments. The complex of threads illustrated in Figure 13 may or may not represent a colonial association.

Larger filaments, 2.5–6 μ in diameter, not found under the electron microscope, but described from thin section by Barghoorn[5] as *Gunflintia grandis* and by Cloud[6] as "*Oscillatoria*-like," may well be cyanophycean algae. Possibly they relate to some of the larger sporelike bodies, or those ellipsoidal structures may be independent organisms.

The form illustrated in Figure 15 suggests a eubacterium, although its endemism is of a lower order of certainty than categories (*a*) and (*c*).

It appears, then, that we have in the Gunflint microflora an association of early and simple prokaryotes. Both bacterial and cyanophycean stages of development may be present. Reproduction was probably asexual, with radiation-induced diversity contravening the selective advantages which sex was later to offer, as suggested by Sagan.[15]

Present findings, therefore, support and amplify previously expressed and independently arrived at conclusions of Barghoorn and Tyler[5] and of Cloud.[6] They also suggest some interesting uses of the electron microscope.

The findings here reported also do not indicate any major changes in previous interpretations of the possible relevance of the Gunflint microflora to the evolution of the atmosphere.[6] However, it seems germane to mention some work by others which bears on this theme, and which was unknown to Cloud at the time of the report in reference 6. In particular, he would like to acknowledge that part of what he proposed in reference 6 had been anticipated in an earlier paper by Nursall.[3] In the same vein, Carl Sagan, in an unusually thoughtful discussion of life origins,[15] has added strong support to the concept of ferrous iron as an oxygen sink by emphasizing (along with Calvin) the probable prevalence under primitive conditions of ferrous iron and iron-chelated tetrapyrroles in prokaryotic metabolism, as well as the potential importance of the catalysis of peroxide reduction by aqueous ferric oxide in advance of the evolution of catalase as a peroxide inhibitor. Even now, catalase is absent in many anaerobes, and when it is supplied to them, they can tolerate oxygen.

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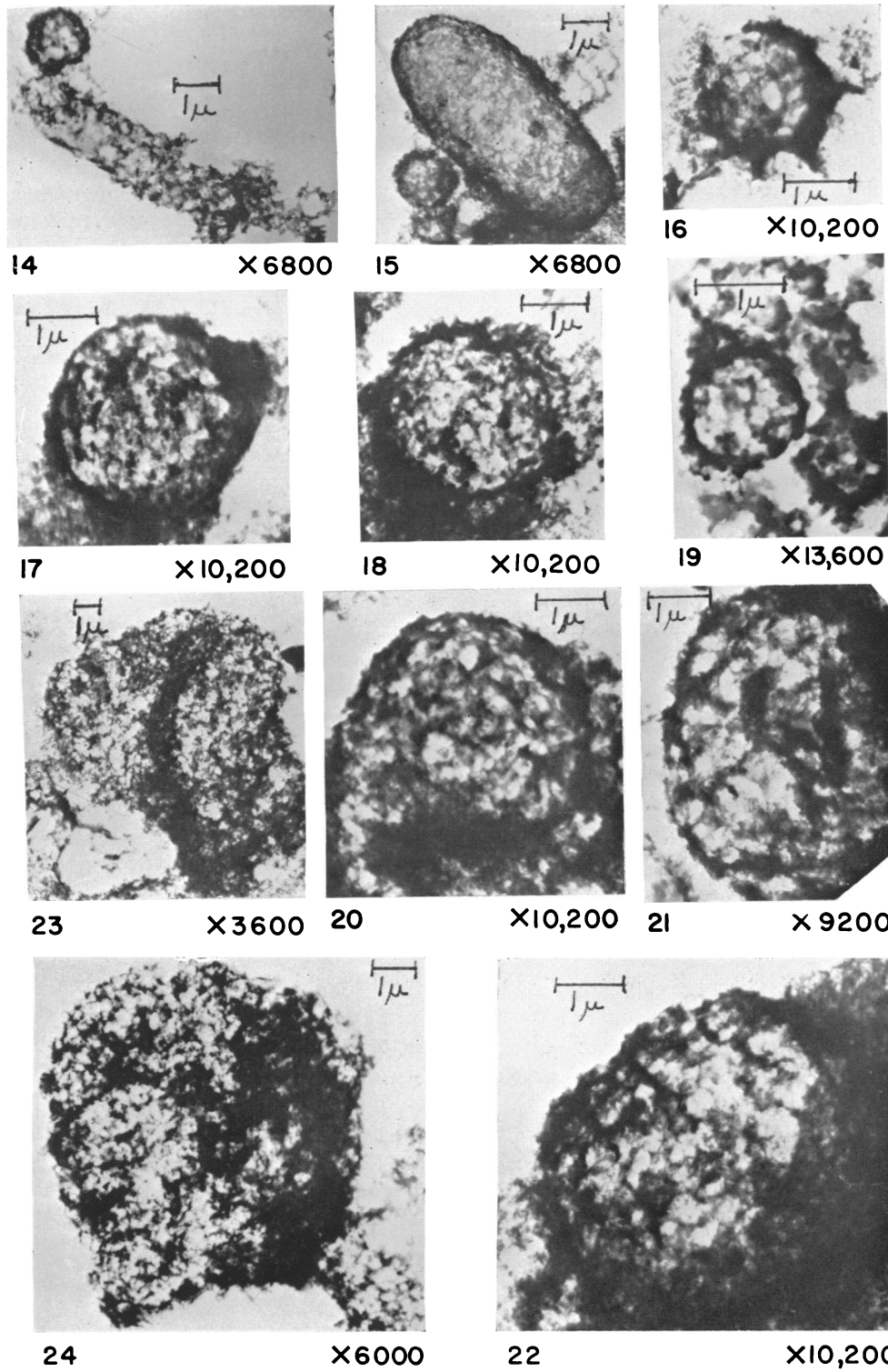

FIGS. 14–24.—Electron micrographs of a fragment of a filament, an ovoid body, and a number of globular to ellipsoidal objects endemic to the Gunflint chert. Bar scales all 1 μ. *Fig. 14:* Fragment of a 1.3-μ-diameter thread having the dimensions of *Gunflintia minuta* Barghoorn, but with a conspicuously reticulate wall structure. At upper left is a reticulate spheroid of same diameter. *Fig. 15:* Ovoid body 5.7 μ by 2.6 μ (also a reticulate spheroid like that of Fig. 14). This object has roughly the shape, size, and surface texture of certain Eubacteria. *Figs. 16–22:* Reticulate-surfaced globular to ellipsoidal bodies, 1.3–5.6 μ in diameter, assignable to the taxon *Huroniospora macroreticulata* Barghoorn. Figs. 16 and 19 suggest a reticulate sphere encapsulated within a ridged outer covering of some sort. *Figs. 23 and 24:* Microreticulate subellipsoidal bodies 10.3–11.7 μ in diameter, assignable to the taxon *Huroniospora microreticulata* Barghoorn.

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RECONNAISSANCE OF THE SOMALI CURRENT DURING THE SOUTHWEST MONSOON

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Previous observations off the coast of East Africa are inadequate in density or extent to identify the Somali Current as a true western boundary current, or to establish its width, velocity, dynamic topography, etc. In August and September 1964, the research vessel *Argo* was engaged with *Discovery* on a joint Anglo-United States expedition to the Somali Current; our purpose is to present here a brief notice of some of the *Argo* observations. Detailed discussions and complete results, including presentation of the oceanographic station data and the results of direct measurements of currents by various meters will be reported elsewhere.

Our work in the Somali Current was of an exploratory nature. We were uncertain as to whether we would be able to trace the axis of the current at all by the topography of the temperature field: indeed, similar work in the Guiana Current had proved disappointing in the past. Some results of *Atlantis II* in 1963 suggested that the Somali Current might be broad and diffuse and difficult to pinpoint. We found, on the contrary, that it is a clearly marked, definite, intense, narrow stream, easily measurable and identifiable.

The Somali Current as we found it appears to be of theoretical interest because, although like the Gulf Stream and Kuroshio it flows toward the north as an intense boundary current along the western coast of a great ocean basin, it differs from them in fundamental ways: (1) it is present during only part of the year, since the driving wind stresses reverse with the monsoons (a period rather short theoretically for baroclinic equilibrium), (2) it flows across the equator (where geostrophy breaks