cut serially from a single piece of rock. Thus, it is not unlikely that they constitute a single population of a single species of blue-green algae.

Modern algal mat communities usually contain from one or two to more than a dozen species of blue-green algae, of which only a few may be coccoid. Forty-five algal species have been described from Bitter Springs mats, including 18 spheroidal taxa. One can infer from Fig. 1 that the apparent discrepancy in diversity between the ancient and modern mats is due primarily to taxonomic treatment rather than to major differences in cyanophytic community structure between the Late Precambrian and the present. Precambrian fossils are similar to those of the Phanerozoic in that they must be treated as populations rather than as individuals. Similarly, they resemble younger fossils in that they are not preserved in perfect cytological detail. All Precambrian microfossils were probably subject to degradation prior to silicification, and it is essential to discuss them in this light.

Of greater significance to students of evolution is the bearing of this study on the origin of the nucleated cell. Because the rise of the eukaryotic cell from its prokaryotic ancestors was the single greatest quantum step in evolutionary history, it is important to attempt to ascertain when this transition occurred. Although the Bitter Springs fossils were unknown only a decade ago, it has become the conventional wisdom to assume that they include the earliest assured eukaryotic remains. This view has become so entrenched that it may seem heterodox to challenge it, but the fact is that there is simply no compelling evidence for the presence of nucleated cells in the Bitter Springs formation or in any other rocks which significantly antedate sequences containing Ediacaran assemblages. Pseudonuclei can be found in fossils from many Precambrian localities (*4*), some of which are nearly 2 billion years old (*8*) (see Fig. 1FF). The oldest such structures to be expressly considered as evidence of eukaryotic life are found in the 1300-million-year-old Beck Springs Dolomite of southern California (*9*); however, degradation studies of modern cyanophytes are applicable to these older microbiotas, as well as to the Bitter Springs flora. All are most reasonably interpreted as blebs of degraded protoplasm within undecomposed sheaths. The presence of pseudonuclei within filamentous cyanophytic sheaths of some Bitter Springs fossils (Fig. 1II) corroborates this view. If one infers that these blebs are in fact genuine nuclear remnants, the problem arises as to why the cytoplasm has disappeared completely, while molecularly similar nuclear material persists. Such a sequence of protoplasmic degradation is illogical. Figure 1EE shows a single field of *C. turgidus* cells which fortuitously presents all stages in classical cyanophytic cell division. This sequence is identical to that which has been hypothesized to be a mitotic sequence (*2*). Again, the assumption that the internal bodies present in the fossils chosen to illustrate petrified stages of mitosis are nuclear remnants leads to problems, for if these pseudonuclei are indeed organellar, the question arises why cytokinesis has preceded mitosis (*10*). If the pseudonuclei are interpreted as protoplasmic remnants, the fossil sequence can be considered to illustrate simple cyanophytic fission.

The tetrahedral structure shown in Fig. 1BB has been hypothesized to represent the product of meiosis, but in fact it is a member of a large cell population which conforms in every way to a population of *C. turgidus*. Although true cyanophytic tetrads have not been described in the literature, "pseudotetrads" caused by two successive cell divisions in different planes or cell slippage within a single sheath are common among blue-green algae (*11*). The "trilete mark" pictured in Fig. 1V is the product of a fortuitous relationship of folded protoplasm internal to the sheath. It does not occur on the outer cell surface where one would necessarily find a true trilete scar.

In short, there is no good evidence for the presence of eukaryotes in Bitter Springs cherts. Similarly, all reports of older eukaryotes do not withstand critical examination. It would be hazardous for us to state that eukaryotes did not exist 900 million years ago, but if they did, their remains have yet to be found. Alternatively, multicellularity as evidenced by the several known Ediacaran faunas may have evolved quite rapidly following the origin of the nucleated cell. That is, eukaryotic cells may not have existed until very near the end of the Precambrian. Only further paleontological investigation of Precambrian rocks can elucidate this problem.

ANDREW H. KNOLL
Department of Geological Sciences, Harvard University, Cambridge, Massachusetts 02138
ELSO S. BARGHOORN
Department of Biology and *Botanical Museum, Harvard University*

References and Notes

1. E. S. Barghoorn and J. W. Schopf, *Science* **150**, 337 (1965).
2. J. W. Schopf, *J. Paleontol.* **42**, 651 (1968).
3. _____ and J. N. Blacic, *J. Paleontol.* **45**, 925 (1971).
4. J. W. Schopf, *Annu. Rev. Earth Planet. Sci.* **3**, 213 (1975).
5. S. M. Awramik, S. Golubic, E. S. Barghoorn, *Abstr. Geol. Soc. Am.* **4** (7), 428 (1972). This study grew from observations made by S. Golubic on living algal communities.
6. A. H. Knoll, E. S. Barghoorn, S. Golubic, *Proc. Natl. Acad. Sci. U.S.A.* **72**, 2488 (1975); S. Golubic and H. Hofmann, *J. Paleontol.*, in press. These papers outline the sequence of degradation commonly observed in natural populations of blue-green algae and relate it to specific examples in the Precambrian fossil record.
7. H. C. Bold, *Ann. N.Y. Acad. Sci.* **175**, 601 (1970).
8. E. S. Barghoorn, *Sci. Am.* **140** (5), 30 (1971); H. J. Hoffman, *Nature (Lond.)* **249**, 87 (1974).
9. P. E. Cloud, Jr., G. R. Licari, L. A. Wright, B. W. Troxel, *Proc. Natl. Acad. Sci. U.S.A.* **62**, 623 (1969). This paper also compares certain microstructures with chrysophyte statospores; however, much evidence accumulated by Margulis (*11*) and Schopf (*4*) indicates that these structures are not fossil cysts and may not be biological in origin.
10. The second of four photographs which purport to demonstrate a mitotic sequence exhibited by Precambrian algae shows a cell in which cytoplasmic division is well advanced, yet the internal body exists as a single entity precisely in the plane of cell division.
11. L. Margulis, *Biosystems* **6**, 16 (1974). The possibility that the Bitter Springs tetrahedral structure is a meiotic tetrad is here considered to be "remote."
12. We thank S. Golubic and L. Margulis of Boston University and J. M. Kingsbury of Cornell University for helpful discussion. S. Golubic and D. E. Wodehouse (Cornell University) provided the cultures. Supported by NSF grant GA-13821 and NASA grant NGL-22-007-069 to E.S.B. and an NSF graduate fellowship to A.H.K.

21 May 1975

Freeze-Etching Nomenclature

Freeze etching (*1*) is now widely used as a preparatory technique for electron microscopy of biological materials. Observation of many freeze-etched prokaryotic and eukaryotic specimens have provided extensive views of their membranes. Because membranes are split during the fracture process used in freeze etching (*2*), two new fracture faces from the hydrophobic interior of the membrane are seen in addition to the true surfaces that can be exposed by etching alone. We wish to propose a simple, uniform nomenclature to describe and label these fracture faces and surfaces.

The recent publication of a symposium on freeze etching (*3*) shows the bewildering assortment of nomenclatures in current use:

1) The A, B, C, and D designations, which are hard to remember and are used in differing ways by different workers.

2) "Convex" and "concave" designations, which are useless for all but the simplest vesicle systems.

3) Descriptive designations (inner fracture face, outer fracture face, outer surface, inner surface), which are confusing, particularly when applied to complex tissues or membrane infoldings.

We propose that for any membrane that can be split, the half closest to the cytoplasm, nucleoplasm, chloroplast stroma, or mitochondrial matrix be designated the "protoplasmic" half, abbreviated P; the half closest to the extracellular space, exoplasmic space (*4*), or endoplasmic space be designated (as the case may be) the extracellular, exoplasmic, or endoplasmic half, abbreviated E. The concept of exoplasmic space, as proposed by de Duve (*4*), includes the interior of endocytic vacuoles, phagosomes, primary and secondary lysosomes, food vacuoles, ordinary plant vacuoles, and Golgi vesicles. The endoplasmic space would include the cisternae of the endoplasmic reticulum, the cisternae formed between inner and outer nuclear membranes, and the cisternae formed by the Golgi lamellae.

In any particular case a distinction between an extracellular half-membrane, exoplasmic half-membrane, or endoplasmic half-membrane can be avoided by referring simply to the E half. E is also used to designate the half-membranes closest to the space between the inner and outer membranes of mitochondria and chloroplasts as well as the intrathylakoid spaces. If mitochondria and chloroplasts are descendants of prokaryotic ancestors, these spaces could have been derived from extracellular or exoplasmic space. In any case, these spaces are external to the mitochondrial matrix or the chloroplast stroma and a commitment to a particular theory of evolution is avoided by using E in referring to the half-membranes closest to these spaces.

Once the half-membrane is labeled P or E, the particular aspect seen in the electron microscope can then be identified as a true surface of the membrane or as a fracture face. The surface, to be designated either PS or ES, is the hydrophilic portion of the membrane usually exposed by the etching process; the fracture face, to be designated PF or EF, is the hydrophobic portion of the membrane usually exposed by the fracture process. Thus, a single membrane will possess two true surfaces, PS and ES, and two fracture faces, PF and EF.

Figure 1 illustrates how this nomenclature would be used to label some typical freeze-etched membranes. Full labeling is shown to distinguish between the E or P faces exposed by fracture and the E or P surfaces which would be exposed by etching. However, in many studies, little or no etching is used, and only the P and E fracture faces are examined. In such cases, or in any case where a distinction between fracture faces and surfaces is unnecessary, illustrations could be labeled simply with P or E.

The nomenclature we propose generates

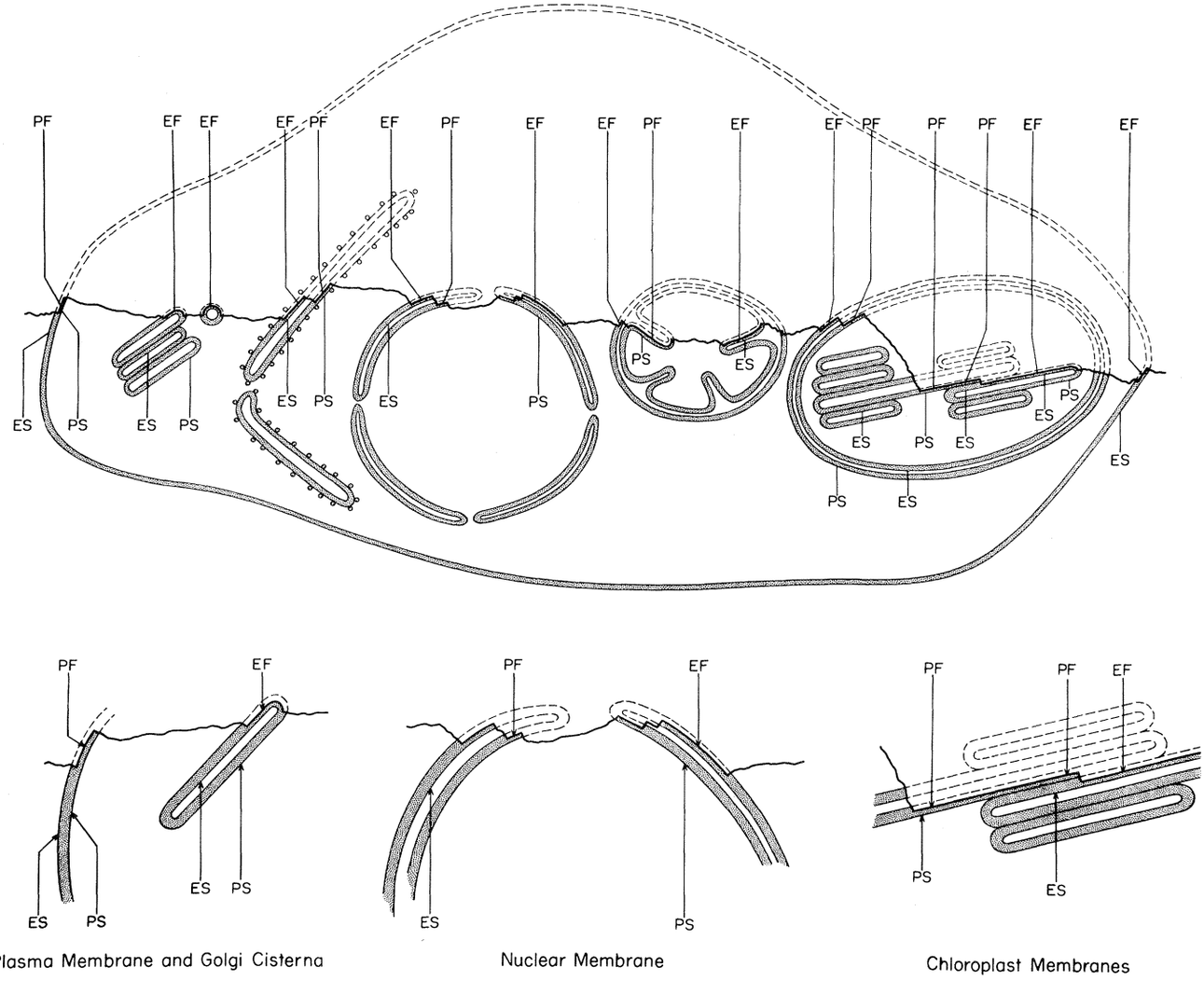

Fig. 1. Labels for some frequently studied membrane fracture faces and surfaces. The dark line through the cell and some of its organelles traces the course of a hypothetical fracture prior to etching. PF and EF labels are used here to distinguish the faces of the P and E halves of each membrane produced by fracture, while PS and ES indicate the true hydrophilic surfaces that could be exposed by etching. However, simple P and E labels would suffice when a distinction between fracture faces and surfaces is unnecessary.

a mnemonic labeling scheme that relates topologically equivalent portions of different membranes and that can be easily applied in a uniform manner to all freeze-etch images. We urge it be adopted by all who publish freeze-etch results.

DANIEL BRANTON
Biological Laboratories, Harvard University, Cambridge, Massachusetts 02138
STANLEY BULLIVANT
Department of Cell Biology, University of Auckland, Private Bag, Auckland, New Zealand
NORTON B. GILULA
Rockefeller University, New York 10021
MORRIS J. KARNOVSKY
Department of Pathology, Harvard Medical School, Boston, Massachusetts 02115
H. MOOR
K. MÜHLETHALER
Laboratory of Electron Microscopy, Swiss Federal Institute of Technology, 2 Universitatstrasse, Zurich, Switzerland
D. H. NORTHCOTE
Department of Biochemistry, University of Cambridge, Tennis Court Road, Cambridge, England
LESTER PACKER
Department of Physiology, University of California, Berkeley 94720
BIRGIT SATIR
PETER SATIR
Departments of Physiology and *Anatomy, University of California, Berkeley 94720*
VOLKER SPETH
Max Planck Institute for Immunobiology, Freiburg, Germany
L. A. STAEHLIN
Department of Molecular, Cellular and Developmental Biology, University of Colorado, Boulder 80302
RUSSELL L. STEERE
Plant Protection Institute, U.S. Department of Agriculture, Plant Industry Station, Beltsville, Maryland 20705
RONALD S. WEINSTEIN
Department of Pathology, Tufts University School of Medicine, Boston, Massachusetts 02111

References and Notes

1. R. L. Steere, *J. Biophys. Biochem. Cytol.* **3**, 45 (1957); H. Moor, K. Mühlethaler, H. Waldner, A. Frey-Wyssling, *ibid.* **10**, 1 (1961).
2. D. Branton, *Proc. Natl. Acad. Sci. U.S.A.* **55**, 1048 (1966).
3. L. Benedetti and P. Favard, Eds., *Freeze-Etching Techniques and Applications* (Societé Française de Microscopie Electronique, Paris, 1973).
4. C. de Duve, in *Lysosomes in Biology and Pathology*, J. T. Dingle and H. B. Fell, Eds. (North-Holland, Amsterdam, 1969), vol. 1, pp. 3–40.
5. We thank P. Garwood for help in preparing the figure.

29 April 1975

Enhancement of Mouse Cytomegalovirus Infection During Host-Versus-Graft Reaction

Abstract. *C_3H/He mice chronically infected with murine cytomegalovirus were given skin allografts from histoincompatible BALB/c donors. A significant increase in cytomegalovirus titers occurred within 3 days after placement of the graft in the spleens and kidneys of the allograft recipients as compared with control animals. No significant changes in virus titers were detected in the salivary gland, lung, liver, or blood of allograft recipients. These results indicate that the host-versus-graft reaction alone can enhance murine cytomegalovirus in a chronically infected host and may help explain the high incidence of cytomegalovirus infection seen after renal and other allograft transplantation in man.*

Since the description of cytomegalovirus (CMV) inclusions in the organs of renal transplant recipients at autopsy, numerous studies have documented the occurrence of CMV infections in these patients (*1–4*). Between 73 and 91 percent of renal allograft recipients develop CMV infection after transplantation, as judged by virologic, serologic, and histologic evidence. CMV infections are common in liver allograft recipients (*5*) and in patients given transplants of allogeneic bone marrow (*6*). CMV infection after blood transfusion has been reported frequently, both in patients undergoing extracorporeal circulation and in other transfusion recipients (*7*). While multiple putative risk factors for CMV infection may be operative in these various groups, the feature common to all, the interaction between a host and an allogeneic graft, has been postulated to be the inciting determinant (*8*).

Most CMV infections in transplant recipients appear to be of little consequence (*2, 3*). However, various clinical manifestations including fever, pneumonitis, leukopenia, mononucleosis, and hepatitis have been associated with CMV infection in renal transplant recipients (*3, 9*). Rifkind and his associates (*10*) first described the coincidence of allograft rejection with serologic evidence of CMV infection in two renal transplant patients. Other investigators noted that acute rejection episodes after renal transplantation appeared to follow infections caused by various viruses (*11*). In an extensive study of 61 renal transplant recipients, Lopez and his colleagues (*4*) found that the clinical triad of fever, leukopenia, and allograft rejection was associated with herpesvirus infections, particularly CMV. They concluded that either the virus infection triggered allograft rejection or that the rejection process activated a latent CMV infection. Since it is difficult to differentiate between these two possibilities by epidemiologic studies in man, the effect of a skin allograft on chronic CMV infection in the mouse was investigated.

To establish the course of murine cytomegalovirus (MCMV) infections, groups of mice were examined over a period of from 1 to 23 weeks after receiving virus. Five-week-old C_3H/He mice were inoculated intraperitoneally with 2×10^5 plaque-forming units (PFU) of Smith strain MCMV. At each sampling period, mice were killed by intracardiac exsanguination. The liver, spleen, kidneys, and salivary glands were dissected in toto, and 10 percent individual organ homogenates were made for virus titration. The titers of 10 percent whole, heparinized blood were also determined. Virus titration was performed by the plaquing method with tragacanth overlay (*12*). The virus titers were highest in the salivary gland, followed by that of the spleen and kidneys (Table 1). The salivary glands of all mice contained virus 1 week after infection. Virus titer peaked at 4 weeks and declined thereafter. All spleens were infected 1 week after infection; many were negative at 2 weeks, and no spleens containing virus were detected after 9 weeks. The proportion of positive kidneys was similar. MCMV was found in low titer (log PFU per 100 mg of tissue homogenate was 1.97 ± 0.40, S.E.) in the livers of about one-third of the mice from 1 to 6 weeks after infection. No virus was detectable in the liver from 9 weeks through 23 weeks after infection. Viremia was detected in low titer (< 10 PFU/0.1 ml) in two of six mice infected 1 week previously, but MCMV was not found in the blood thereafter.

The proportion of infected organs decreased at varying rates after the first weeks of infection (also see below). But titers of infected spleens and kidneys varied relatively little during the period from 2 to 6 weeks after infection. This characteristic permitted the use of these organ titers for detection of viral enhancement.

The effect of a skin allograft on chronic infection was first studied in C_3H/He mice inoculated 5 weeks previously with 2×10^5 PFU of MCMV. Normal 10-week-old BALB/c mice served as donors. Full-thickness donor skin grafts of approximately 15 mm in diameter were cut with a