

# DIATOMS ON EARTH, COMETS, EUROPA AND IN INTERSTELLAR SPACE\*

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**Abstract.** There exists a close correspondence between the measured infrared properties of diatoms and the infrared spectrum of interstellar dust as observed in the Trapezium nebula and toward the galactic center source GC-IRS 7. Diatoms and bacteria also exhibit an absorbance peak near 2200 Å, which is found to agree with the observed ultraviolet absorbance properties of interstellar grains. We review the observational data and consider the known properties of diatoms and bacteria. It is suggested that these characteristics are consistent with the concept of a cosmic microbiological system in which these or similar microorganisms might exist on comets, Europa and in interstellar space.

## 1. Introduction

An ever-increasing body of astronomical evidence suggests that life may be a cosmic (rather than a strictly terrestrial) phenomenon. Complex organic chemicals have been observed on comets and in vast regions of space, and the origin of these materials, as yet, remains unexplained. Carbonaceous chondrites have transported to the Earth tantalizing indications of not only a complex cosmic biochemistry but also an array of 'organized elements' which may represent an extraterrestrial microbiology. The chemical evolution models of the origin of life are encountering more difficult problems, as Pflug *et al.* (1979) discovered the microfossils *Ishuasphaera isua* in the 3.8 billion year old Isua quartzites of Greenland. Here, in the oldest sedimentary rocks known on Earth, is clear evidence of a complex microbiology constituted of photosynthetic microorganisms (Pflug, 1981). The time allowed for the origin of life on Earth by chemical evolution in a primordial soup has now been reduced to a small fraction of a billion years. The appearance of complex microorganisms roughly coincides with the formation of liquid oceans on Earth, which raises serious questions about the validity of a model requiring large time periods for chemical evolutionary processes to occur.

The interesting correspondence of the measured infrared spectral properties of diatoms to the observed infrared spectra of dust in the Trapezium nebula and

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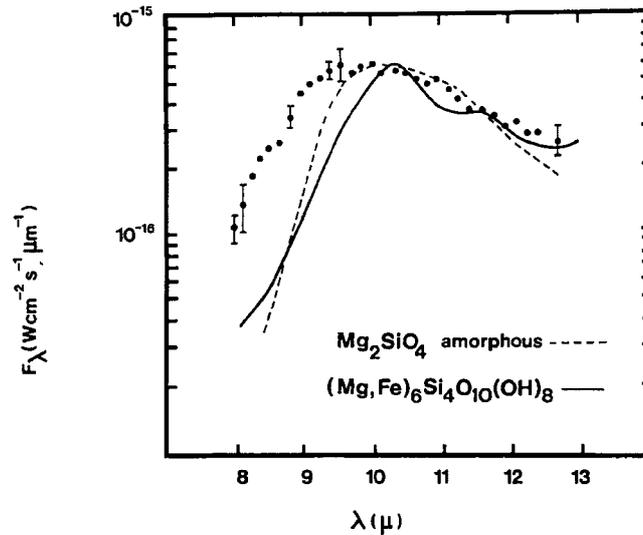


Figure 1. Observed flux from the Trapezium nebula (dots) compared with the theoretical flux calculated from models of silicate grains at a temperature of 175 K. Normalization is to  $F = 6 \times 10^{-16}$  Watt  $\text{cm}^{-2} \text{s}^{-1} \mu\text{m}^{-1}$  at  $\lambda = 9.5 \mu\text{m}$ .

toward the galactic center source GC-IRS 7 suggests that the ‘silicate’ component of interstellar dust is not like inorganic silicates but, instead, is like siliceous polymers produced by these types of microorganisms. Could diatoms or similar microorganisms form a component of the interstellar dust? What evidence is there for the existence of microbiology on a cosmic scale? These intriguing questions led us to re-examine the known properties and behaviour of terrestrial diatoms and bacteria and to consider their possible viability on comets and Europa as well as their potential for surviving long periods of exposure to the hard vacuum, thermal, and radiation environments of interstellar space.

## 2. Interstellar Grains and Diatoms

For over a decade, astronomers have attempted to match an absorption feature in the 8–12  $\mu\text{m}$  waveband produced by interstellar grains with various grain models. A mixture of naturally occurring silicates was first considered and the resulting correspondences with the data were far from satisfactory. Later attempts to improve this situation invoked properties of both amorphous as well as hydrated silicates, but even these refinements did not lead to a significant improvement. The results for the amorphous and hydrated silicates which best fit these data are shown in Figure 1.

At the present time, astronomers regard the 8–12  $\mu\text{m}$  absorption in grains as necessarily resulting from some form of silicate. Moreover, it has been inferred

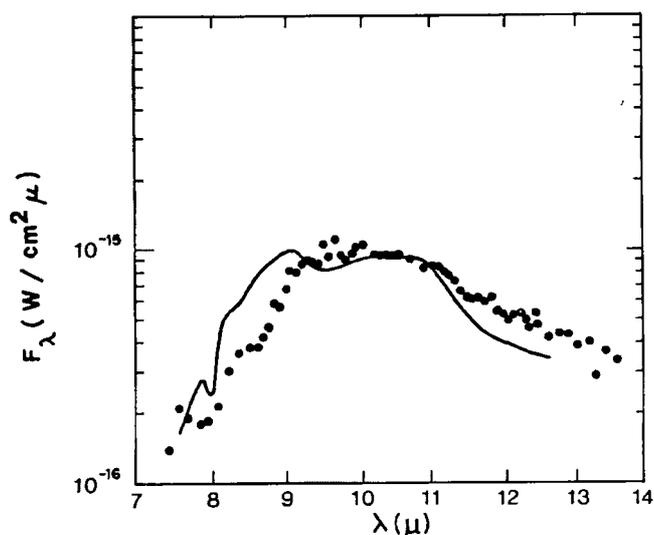


Figure 2. Observed flux from the Trapezium nebula (dots) compared with the theoretical flux from 445 K POM polymer grains.

that an opacity function  $\tau(\lambda)$  derived from the astronomical flux measurements from the dust surrounding the Trapezium nebula corresponds to the properties of some mythical 'silicate'. Thus when another astronomical source exhibits 8–12  $\mu\text{m}$  absorption or emission, comparison is not made with any known silicate, but rather with the properties of Trapezium nebula dust which has been designated 'silicate'.

Two of the present authors (F.H. and N.C.W.) have felt uncomfortable with this procedure and have looked for alternative explanations for some years. The 8–12  $\mu\text{m}$  absorptions, as shown in Figure 1, clearly do not lead unequivocally to any simple silicate model. Furthermore, absorptions near 10  $\mu\text{m}$  could arise from other functional groups besides  $\text{SiO}_2$ , notably C–O–C linkages in organic material. With the radioastronomical discoveries of numerous organic molecules in interstellar space, the possibility that interstellar dust was predominately organic needed to be investigated. Wickramasinghe (1974) and Hoyle and Wickramasinghe (1977) subsequently considered organic polymers as possible candidates for infrared absorption in grains, leading instantly to improved correspondence with the 8–12  $\mu\text{m}$  Trapezium data (Figure 2). While these agreements were significantly better than those seen in Figure 1, notable discrepancies remained.

Hoyle and Wickramasinghe (1979) began developing the theory that interstellar grains might include freeze-dried microorganisms and that such microorganisms included in comets may have led to the origin of life on our planet. Our first attempt to explain the 8–12  $\mu\text{m}$  feature in terms of intact biological material led to an astounding success. We obtained a mixed culture of diatoms from a local river, consisting of 60% diatoms and 40% purely carbonaceous microorganisms. A desiccated specimen of this culture exhibited infrared absorption characterist-

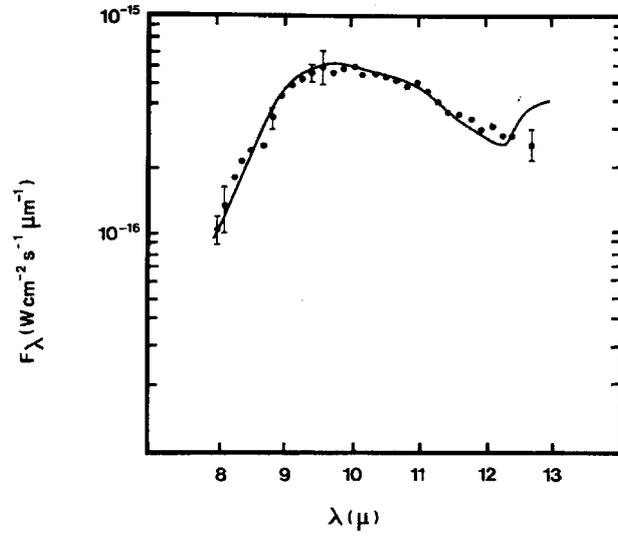


Figure 3. Observed 8–12  $\mu\text{m}$  flux from the Trapezium nebula compared with theoretical flux calculated from models of a mixture of diatoms (solid curve) at a temperature of 175 K. Normalization is the same as in Figure 1.

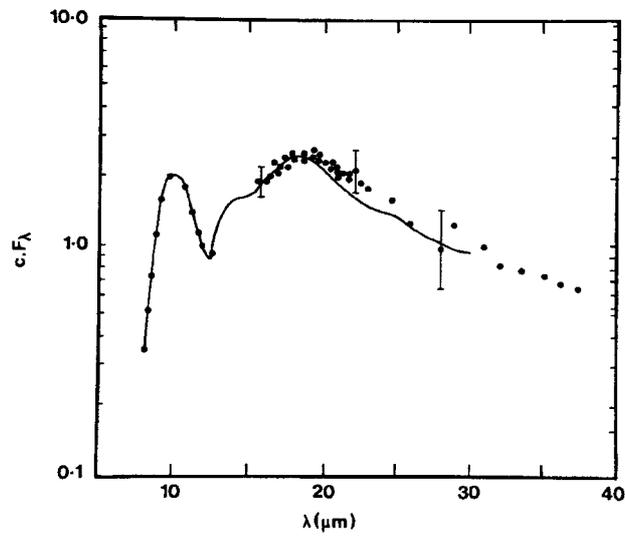


Figure 4. Infrared flux calculated for a model of a mixture of diatoms (solid curve) at 175  $\mu\text{m}$  waveband compared with infrared observations of the Trapezium nebula dust.

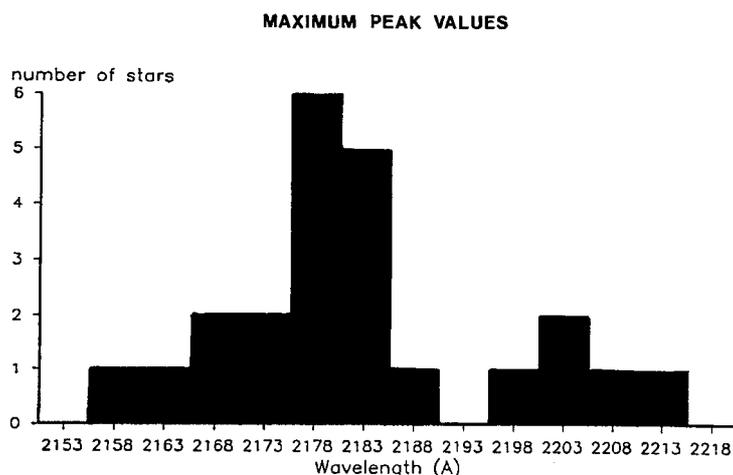


Figure 5. Histogram showing distribution of the peak ultraviolet absorption wavelengths of interstellar grains from observations of 25 reddened stars.

ics consistent with the observations of the 8–12  $\mu\text{m}$  and 8–30  $\mu\text{m}$  spectra of the Trapezium nebula; these comparisons are shown in Figures 3 and 4 (Hoyle *et al.*, 1982a,b).

Diatoms and bacteria possess absorptivities which match the observed properties of interstellar grains over a broad wavelength band of the infrared spectrum, and they also exhibit interesting properties in the ultraviolet spectrum.

### 3. The 2200 Å Interstellar Extinction Feature

The most striking property of the extinction curve of starlight is an absorption feature centered near 2200 Å with a half width of about 500 Å. Figure 5 shows the distribution of this absorption for 25 stars over widely distributed areas of the sky. The maximum occurs at a wavelength of 2178 Å, and as the number of stars in the sample is increased the peak sharpens further at this wavelength. Consequently, this central wavelength of the absorption feature is a highly reproducible property of interstellar grains. Until recently, Hoyle and Wickramasinghe (1963) have attempted to attribute the so-called 2200 Å absorption to small graphite spheres. However, this identification is dubious for the following reasons:

(1) Calculations show that the graphite grains must be spheres of radius  $0.02 \pm 0.01 \mu\text{m}$  in order to produce the required absorption profile centered on 2180 Å. Such a fine tuning of radius is difficult to explain as a natural occurrence.

(2) Departures from a spherical shape would be expected to lead to a substantial mismatch of the central wavelength from the astronomical peak, thus imposing an unrealistic constraint on particle shape for interstellar graphite grains.

(3) The presence of even a minute concentric core of refractive index different from graphite (or of a thin outer coating of non-graphitic material) would again destroy the wavelength correspondence.

However, we have found that these observational data are consistent with a model in which freeze-dried microorganisms are primary constituents of the interstellar grains. Our first experiments were carried out in Sri Lanka with bacterial suspensions in distilled water. The main absorption effect was found to be centered on  $\lambda = 2050 \text{ \AA}$ . However, water is a polar liquid which interacts optically with polar chromophores. To measure the wavelength of absorption associated with these organisms more precisely, subsequent experiments were performed with the bacteria suspended in a non-polar organic solvent. These measurements were carried out with a Beckman Model 25 Double Beam Spectrophotometer, balanced over the ultraviolet region with quartz cuvettes filled with 2-methylbutane (a non-polar liquid). The sample cuvette contained a suspension of the microorganisms also in 2-methylbutane, permitting the spectral characteristics of the diatoms and bacteria to be accurately measured (Hoyle *et al.*, 1984). These experiments revealed that the principal ultraviolet absorption peak for a variety of microorganisms occurs near  $2180 \text{ \AA}$ , with an average maximum absorption coefficient at this wavelength estimated at  $35\,000 \text{ cm}^2 \text{ g}^{-1}$ .

Pflug (1981) independently observed this absorption peak near  $2200 \text{ \AA}$  by in-situ vacuum spectroscopy of fossilized Precambrian microorganisms. Our own ultraviolet results for the bacteria *Escherichia coli* and diatoms are reproduced in Figure 6. The significance of these curves is that the most conspicuous spectral feature of interstellar dust is now seen to coincide with the vacuum absorption properties of bacteria and diatoms. This result was not entirely unexpected. Some years ago we had found that organic molecules occurring in biology have an average absorption profile that peaks at approximately this wavelength (Wickramasinghe *et al.*, 1977; Hoyle and Wickramasinghe, 1979). Hence, there is a striking degree of consistency relating the astronomical observations of interstellar grains in the ultraviolet and infrared portions of the spectrum with the observed properties of microorganisms. The  $2.9\text{--}3.9 \mu\text{m}$  features in the galactic center source GC-IRS 7 (Figure 7) may be due to CH linkages in the organic material of bacteria and diatoms, whereas the  $10 \mu\text{m}$  absorption shown in Trapezium dust (Figure 3) and GC-IRS 7 (Figure 8) may arise from the combined effect of  $(\text{HSiO})_n$  and  $(\text{H}_2\text{CO})_n$  type polymers.

#### 4. Diatoms on Earth

Diatoms are by far the most important siliceous microorganisms on Earth. They comprise a group of unicellular golden-brown algae which constitute the major component of the marine phytoplankton. These microscopic plants are responsible for approximately 25% of the total net primary production of organic material on

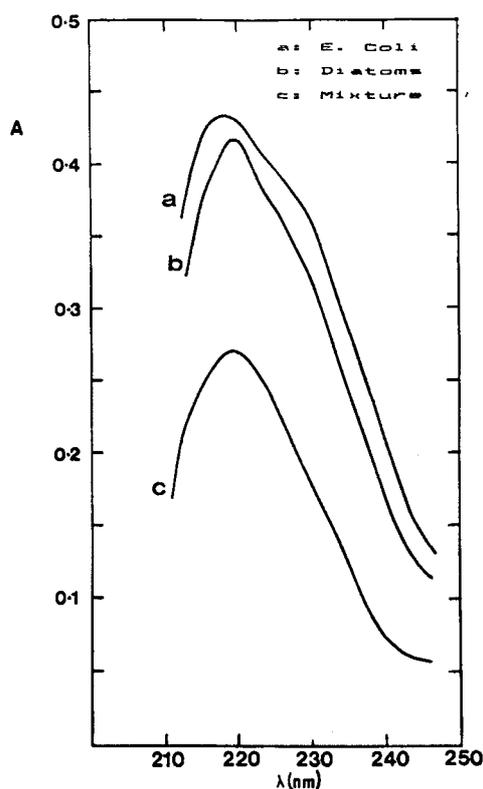


Figure 6. Ultraviolet absorbance spectra of dry microorganisms in 2-methylbutane: [A]. Curve for *E. coli*. [B]. Curve for a mixture of diatoms. [C]. Curve for a combination of diatoms and *E. coli* in a mass ratio of 2 to 1.

our planet, fixing some  $10^{13}$  kg of carbon in organic form each year (Werner, 1977). Siliceous cell wall structures are also encountered in other algal groups such as the Silicoflagellates, certain Chrysophytes and some Xanthophytes. Silica is also utilized by planktonic protozoans (e.g., Radiolaria) and it is found in plates, scales and spines secreted by several minor groups (e.g., Testacid Sarcodina, Heliozoans and loricate Choanoflagellates). Some foraminifera utilize an organic keratinoid cement to incorporate inorganic silicates such as quartz granules or flakes into their shells. This is entirely different from the silicification mechanisms utilized by diatoms (and radiolaria) for the formation of their cell walls, and the infrared properties of these siliceous foraminifera should resemble inorganic silicates.

Diatom silica is unusual because it is laid down molecule by molecule in a very complex manner. A membrane known as the silicalemma plays a crucial role in the construction of the diatom cell wall. Orthosilicic acid  $\text{Si}(\text{OH})_4$ , is apparently polymerized to silica gel due to either changes in pH, orthosilicic acid concentration or ionic interaction/hydrogen bonding which results in the binding of silica to specific

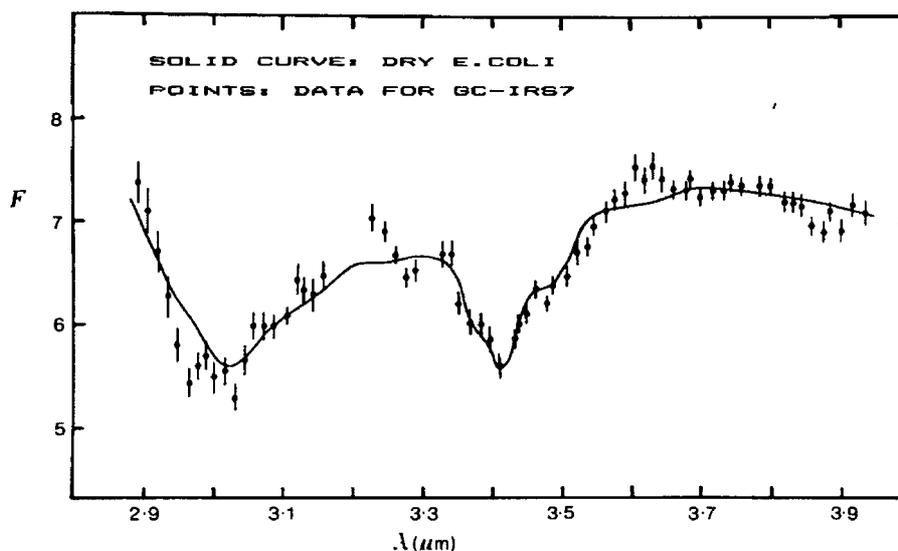


Figure 7. Normalized flux calculated for *E. coli* compared with the astronomical data for GC-IRS 7. Normalization procedure is described in Appendix A.

sites in the silicalemma (Volcani, 1981). Coombs and Volcani (1968) showed that there is a significant increase in protein synthesis during silica deposition. Hecky *et al.* (1973) indicate that the silicification process in diatoms is mediated by a mineralizing template protein (high in glycine and in the hydroxyl containing serine and threonine) which is on the proximal surface of the silicalemma. Their model of the layers in the diatom cell wall (Figure 9) involves a three part arrangement of the organic casing in which a polysaccharide layer (comprised of glucose, fructose, mannose and xylose) plays a fundamental role as the structural carbohydrates immediately above the protein template layer.

The intricate structural features of the diatom shell are repetitively reproduced with astonishing precision. Some species span approximately 100 million years (representing  $10^{10}$  to  $10^{11}$  generations) without detectable variations in morphological features. This clearly indicates the requirement for some form of highly stable protein template. Volcani (1981) asserts that in the biological mineralization (silicification) of diatoms, two closely interrelated biochemical systems are involved. One of these involves the translocation, polymerization and deposition of the siliceous portion of the shell, while the other governs the formation of the organic casing.

Recently, an entirely new amino acid (3,4-dihydroxyproline) was isolated from the proteinaceous material in the cell wall of the diatom *Navicula pelliculosa* Hilse. This amino acid has subsequently been found in the cell walls of diatoms such as the colorless heterotroph *Nitzschia alba* Lewin and Lewin, the thermophile *Nitzschia thermalis* Auerswald and several other species (Nakajima and Volcani, 1969).

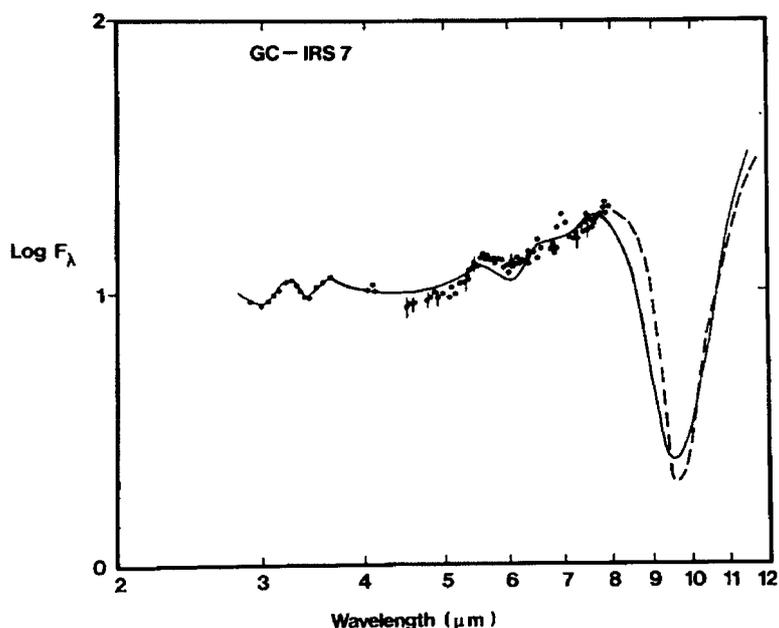


Figure 8. A composite of the observed infrared flux from the galactic center source GC-IRS 7 represented by the dots [data from Willner *et al.* (1979) and Allen and Wickramasinghe (1981)] and the dashed curve [(data from Woolf (1973)]. The solid curve is the predicted flux from the mixed diatom model.

It may be a determinant of the molecular structure of the organic matrix associated with silicification (as hydroxyprolines are in collagen).

The complex biochemistry of the organic siliceous polymers in the cell walls of diatoms provides a clear indication of the dramatic differences between diatom silica and inorganic silicates, which may account for the observed differences in the infrared absorption spectra. The sharp peaks at  $8.7 \mu\text{m}$  and  $12.7 \mu\text{m}$  encountered in inorganic silica (Figure 10) are not observed either in diatom silica or in interstellar dust.

Proteins and polysaccharides account for the majority of the organic matter in the diatom cell walls, with lipids and hexosamines representing minor components. Many diatoms produce extracellular polysaccharides in the form of gelatinous capsules, stalks, tubes or threads. The capsules encasing *Navicula pelliculosa* Hilse are composed of glucuronic acid residues (Lewin, 1955) whereas *Amphipleura rutilans* Cleve secretes mucilage tubes that consist of a polymer of xylose and mannose with traces of rhamnose and protein (Lewin, 1958).

*Thalassiosira fluviatus* Hustedt secretes long filaments that are a very pure crystalline form of chitin (Dweltz *et al.*, 1967). These organic materials affect the absorption characteristics of diatoms in portions of the infrared spectrum, particularly around  $3 \mu\text{m}$ . The organic casing provides protection to diatoms from

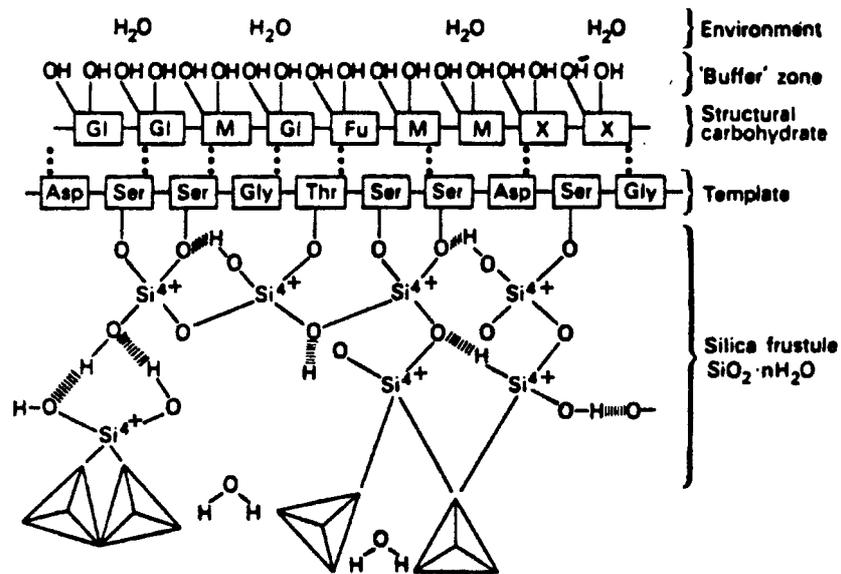


Figure 9. Model of the layers in the cell wall of a diatom. Complex siliceous biopolymer in the silica frustule of a diatom is overlain with a layer of protein template for polycondensation of  $\text{Si}(\text{OH})_4$ . The template contains serine (Ser), threonine (Thr), glycine (Gly) and aspartic acid (Asp). Upper layer is a polysaccharide consisting of fucose (Fu), glucose (G1), mannose (M), and xlyose (X), surrounded by a hydroxyl buffer zone in the aqueous environment. (Redrawn from Hecky *et al.*, 1973).

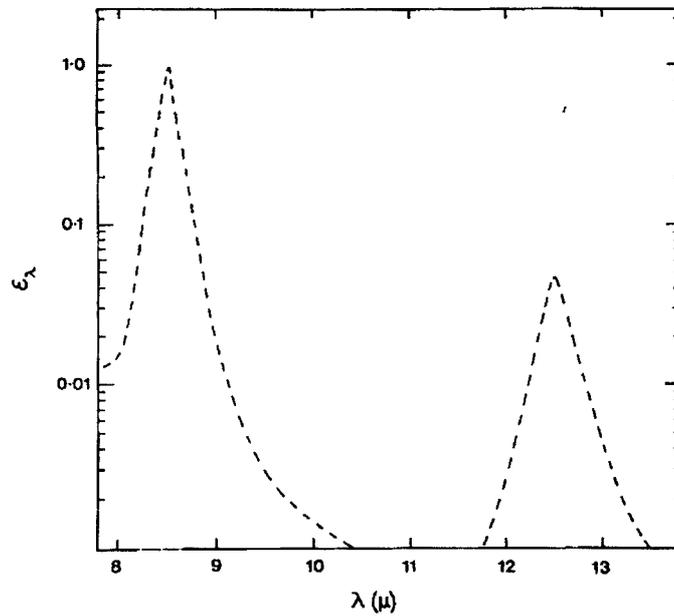


Figure 10. Infrared properties of inorganic silica showing sharp peaks at  $8.7 \mu\text{m}$  and  $12.7 \mu\text{m}$ .

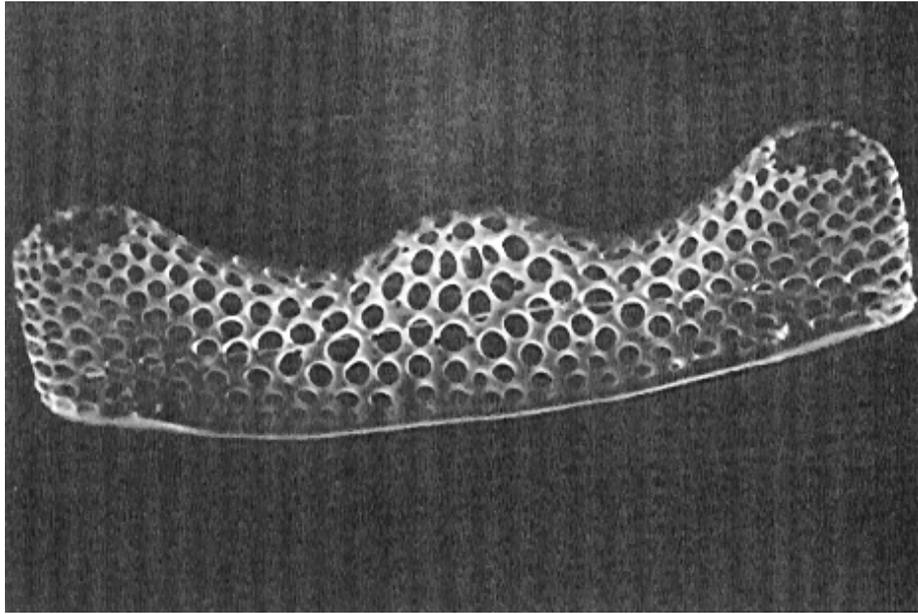


Figure 11. Resting spore of the Antarctic diatom cf. *Eucampia antarctica* Castracane from G Dallas Hanna. (SEM Photomicrograph by Richard B. Hoover and Daniel W. Gates).

death by desiccation, and could also provide protection from the effects of ultraviolet radiation in interstellar space. Even as little as a 1  $\mu\text{m}$  thick layer of graphite produced by carbonization of this external organic casing would provide shielding by attenuation of ultraviolet radiation in the 2200  $\text{\AA}$  region (Hoyle and Wickramasinghe, 1982). The mucilage surrounding many diatoms could produce graphite layers in excess of this requirement.

Although many diatoms are capable of forming spores and cysts (Figure 11) to remain viable when subjected to harsh environments, others are resistant to desiccation without structural alterations. Typically they are those which produce thick mucilaginous sheaths (Hoover, 1976) such as *Schizonema* (*Navicula*) and *Encyonema* (*Cymbella*). *Navicula contenta* Grunow, *N. krasskei* Hustedt and others are able to live on dry rocks, dry moss and in the soil (Patrick, 1977). Some diatoms prepare for long dry periods by storing large amounts of oil and building inner plates (e.g., *Melosira roseana* Rabenhorst).

Little is known about the ability of diatoms to survive very long periods of desiccation. One of us (R.B.H.) recently studied the type material at the Henri Van Heurck diatom collection of the Royal Zoological Society of Antwerp, Belgium and found diatoms to be alive after having been subjected to desiccation for almost a century and a half (Hoover, 1979). These were small naviculoid diatoms in gelatinous sheaths collected in France in 1834 by Lenormand, and mounted on an herbarium sheet as the type series for *Schizonema lenormandi* (Kützing,

1849). Since the herbarium was bound as a book kept in an opaque folder, the diatoms had remained dry and in the dark for this entire period. Distilled water was added to a small portion of this material on a clean microscope slide and many diatoms emerged from broken ends of the sheaths. They began exhibiting locomotion that is characteristic of living pennate diatoms. The revival was very rapid and a large percentage of the diatoms in the water was observed to be swimming of their own accord within minutes. These diatoms were preserved not as resting spores or cysts, but as intact dormant organisms which revived when reintroduced into water. Clearly, dessication over long periods is necessarily lethal to diatoms. Although this demonstrates diatom viability after dessication for 150 years, others have shown microorganisms to be capable of remaining viable over very long time periods.

For example, Dombrowski (1963) isolated living bacteria preserved in Middle Devonian, Silurian, and Precambrian salt deposits. These previously unknown microorganisms, which could be seen inside the ancient salt crystals, were released by introducing the salt crystals into sterile water. Many of the bacteria were found to be still viable and capable of being cultured after as much as 650 million years of dessication.

Many diatoms are the proper size required for safe entry into the Earth's atmosphere. Diatoms typically range in size from  $0.75\ \mu\text{m}$  for *Chaetoceras galvestonensis* Collier and Murphy to several hundred  $\mu\text{m}$ . (The giant *Ethmodiscus rex* Hendey sometimes exceeds  $2000\ \mu\text{m}$  in diameter.) Hoyle and Wickramasinghe (1982) have calculated the size range allowable for the safe entry of a microorganism into the Earth's atmosphere and found that it is approximately  $40\ \mu\text{m}$  diameter for rod shaped organisms, with no restriction on the length of the rod. Organisms with favorable aerodynamic design could safely enter singly with diameters up to  $100\ \mu\text{m}$ . The majority of the diatoms, particularly the ice diatoms, are found to lie within this size range. Of course, these size limits do not apply to microorganisms which might enter the atmosphere encased in cometary debris.

## 5. Comets, Chondrites, Europa and Ice Diatoms

Hoyle and Wickramasinghe (1982) have pointed out that the observed hydrogen, carbon, nitrogen and oxygen ratios on comets are quite different from the cosmic abundances of these elements and very similar to the ratios encountered in microorganisms. This suggests that microorganisms might be present in comets. Let us therefore consider what is known about comets and about diatoms to see if it is feasible that these microorganisms might find comets to be suitable habitats.

According to the 'icy-conglomerate' model of Whipple (1950) the nucleus of a comet consists largely of water ice with possibly non-volatile meteoritic matter. Ultraviolet, radio, optical and infrared observations have established the presence of a diverse array of chemical species on comets, including silicates, several polyatomic

TABLE I  
Amino acids in chondrites

Amino acid	Carbonaceous chondrite	
	Murray ( $\mu\text{g g}^{-1}$ )	Murchison ( $\mu\text{g g}^{-1}$ )
Glycine	6.1	3.0
Glutamic acid	3.1	1.6
alanine	3.5	1.3
Aspartic acid	1.7	1.6
Valine/isovaline	1.6	0.9
Proline	1.3	0.4
$\alpha$ -Aminoisobutyric acid*	2.5	11.4
$\alpha$ -Amino-N-butyric acid*	1.1	0.5
$\beta$ -Alanine*	0.4	1.2
$\beta$ -Aminoisobutyric acid*	0.7	0.3

\* Amino acid analogues are not found in proteins. Data from Cronin and Moore (1975).

organic molecules and some amino acids. Dobrovolsky and Kajmakov (1977) have tabulated numerous amino acids, carbonic acids, nitrils and amides that may be present in cometary nuclei and A'Hearn (1984) has recently provided a review of the chemistry of comets.

Additional information may be inferred from the chemical composition of the carbonaceous chondrites, which are believed to be meteoritic material from the non-volatile fractions of comets. The Type 1 chondrites are heavily laced with complex organics, including amino acids, fatty acids, polysaccharides, alcohols, purines and pyrimidines. Lawless *et al.* (1972) concluded that the Orgueil meteorite, a Type 1 chondrite which fell in France in 1864, contained several amino acids of extraterrestrial origin. Using gas chromatography to separate the D, L enantiomers, they isolated the D-isomers of alanine, proline and aspartic acid. (It is well known that virtually all amino acids produced by terrestrial life forms are the L-isomer.) They also found isomers of several unusual amino acid analogues;  $\alpha$ -aminoisobutyric acid,  $\beta$ -aminoisobutyric acid, N-methylglycine, N-methylalanine, and others, which are not found in proteins and are seldom associated with living plant and animal tissues.

Table I shows the concentrations of selected amino acids found in the Murchison and Murray chondrites by Cronin and Moore (1975). The heavy concentration of the unusual  $\alpha$ -aminoisobutyric acid is most interesting; in the Murray it is almost four times as abundant as glycine. Kotra and Ponnampereuma (1980) found this substance to also be present in the Alien Hills and Yamato chondrites discovered

in Antarctica and they found it to be more abundant than glycine in the interior of the Murchison meteorite.

Hodgson and Baker (1964) found porphyrins in Orgueil, but virtually no chlorins. The porphyrins are complex organic molecules which are the foundation of chlorophyll and the oxygen carrying pigments in animal blood. The absence of the closely related chlorins indicates that porphyrins are indigenous, since if the organics were terrestrial contaminants the chlorins should be far more abundant than the porphyrins.

Carbonaceous chondrites also contain organized elements, which many now consider to be indigenous fossilized microorganisms. (See *Ann. New York Academy of Sciences* **108**, 339–616, 1963, for an early review of the subject.) Many of the organized elements resemble Chrysophytes (related to diatoms) and Hystrichospheres (fossilized dinoflagellates, cysts and spore cysts of dinoflagellates). Some workers have also found diatoms (*Nitzschia acicularis*) and diatom-type structures in chondrites (Claus *et al.*, 1963).

If diatoms are to find comets habitable they must certainly be able to live on ice and in the absence of light. The diatom assemblage most relevant to this question is the sea-ice diatom community of the polar regions. Ice diatom studies by Bunt (1963), Bunt and Wood (1963), and Meguro *et al.* (1967) have shown that the great majority of the primary productivity of the Arctic and Antarctic seas is the result of diatoms frozen in the ice. Diatoms frequently account for 90 to 99.5% of the phytoplankton in the polar regions. The concentrations of diatoms in the polar ice can be a full two orders of magnitude greater than that in the surrounding waters. These diatoms actively carry out photosynthesis at temperatures below  $-1.86^{\circ}\text{C}$  with light levels reduced to less than 1% that at the surface. Sullivan and Palmisano (1981) examined the chlorophyll- $\alpha$  content of several ice cores from McMurdo Sound, Antarctica and measured a mean value of  $1.14\text{ mg m}^{-2}$ . This result is astonishing when one considers that the maximum quantity of chlorophylls in the euphotic zone of a freshwater lake is only 2 or 3 times this value.

There are distinctly different communities of ice diatoms in these ice ecosystems. One lives in the ice crystals and the interstitial water of the ice matrix at the bottom surface of ice layers that may be many meters in thickness. The other inhabits the layer between the new fallen snow and the old pack ice. There are approximately 60 species known in the Arctic and Antarctic ice diatom floras. The primary components are the small rod shaped, pennate diatoms such as *Nitzschia seriata* Cleve, and *Amphiprora kjellmani* Cleve. These are minute (average size ranging from 2 to 20  $\mu\text{m}$  length) benthic and epiphytic forms. However, some of the larger centrics such as *Coscinodiscus subtilis* Ehrenberg and *Biddulphia weisflogii* Janisch were also found growing in and on the ice.

Diatoms are by far the dominant form of plant life in the ice ecosystems. The ice diatoms are present in such great numbers that they give a brown color to bands in vast areas of polar sea ice, particularly near the water/ice interface associated with cracks in the ice. The brown coloration which diatoms produce on the Antarctic sea

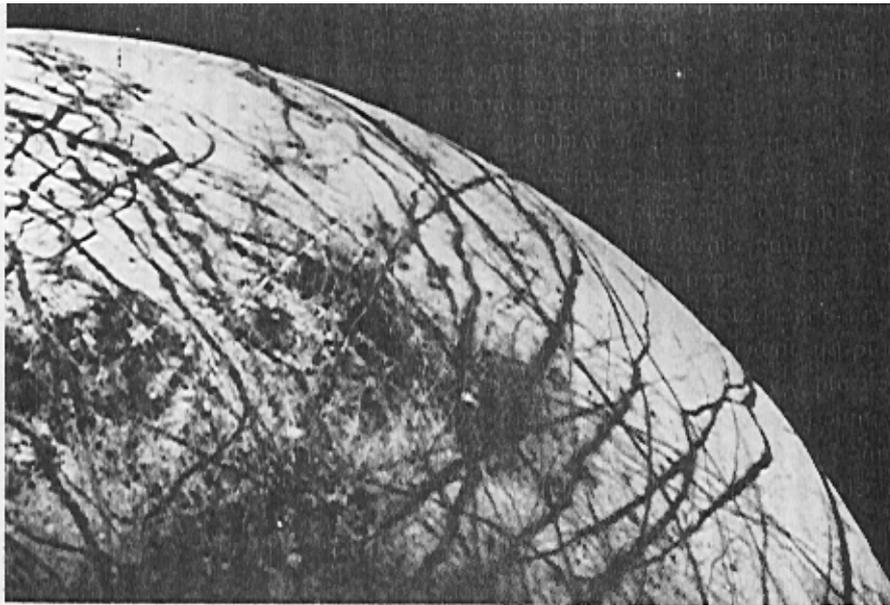


Figure 12. Voyager 2 image of the Jovian satellite Europa. The dark lines are brownish in color and transect the white, icy crust.

ice is intriguingly similar to features recently observed by the Voyager spacecraft as it flew near Europa, one of the four Galilean satellites of Jupiter. Reynolds *et al.* (1983) considered the sea-ice diatom communities as a possible analog for the observed European features. In the high resolution pictures from Voyager, Europa is seen to have a very bright surface *transected by a network of long linear features of lower albedo and of Brownish color* (Figure 12).

Europa's density is  $3.03 \text{ g cm}^{-2}$  indicating that it is roughly 6% water by mass. Squyres *et al.* (1983) show that the observed features of Europa are consistent with a liquid water ocean overlaid by a thin ice crust. Using the characteristics of the Antarctic sea-ice diatoms, Reynolds *et al.* have estimated the maximum biomass that could be supported by oxygenic photosynthesis in an ocean on Europa. Assuming an efficiency for conversion of absorbed energy to organic carbon of 0.5% and the energy required to produce carbohydrate photosynthetically as  $112 \text{ kcal mol}^{-1}$ , they compute a maximum carbon production of  $2.6 \times 10^8 \text{ g y}^{-1}$ . This is based on the available energy inflow of photosynthetically active radiation at the surface of Europa. Since the mean carbon content of diatoms is 53%, a maximum biomass production rate of  $4.8 \times 10^8 \text{ g y}^{-1}$  is implied.

Burkholder and Mandelli (1965) have calculated that the diatoms trapped in the 2.6 million square kilometers of brown ice that surrounds Antarctica in the summer should produce about one half million tons of carbon fixed per day. This is the equivalent of  $1.64 \times 10^{14} \text{ g y}^{-1}$ . Of course the light levels on Europa are much

lower than at the surface of the Earth. In these considerations of the habitability of Europa, diatoms were considered solely as photosynthetic organisms. However, terrestrial diatoms exhibit some interesting properties, which would clearly be of great value to life on comets or Europa.

For example, the ice diatoms *Fragilaria sublinearis* Van Heurck and *Chaetoceras fragilis* Meunier can grow after several months of exposure to total darkness in the austral winter. These cryophilic species carry out respiration at extremely low rates when exposed to darkness at low temperatures. They 'hibernate' as intact organisms rather than entering some type of resting spore state. They photosynthesize rapidly when light and nutrients are available and then survive without added organic nutrients when light is absent.

Although diatoms are normally photosynthetic microorganisms, certain species can live entirely without light. During a study of the aphotic zone of the Indian Ocean, Nel (1968) regularly found living oceanic diatoms at depths ranging from 500 to 3000 m. Since these species were unlike those in the overlying waters, it was possible to verify that they were not merely sinking. Malone *et al.* (1973) recovered living diatoms from the red clay sediments taken from the bottom of the North Atlantic at a depth of 6150 m. These diatoms live heterotrophically on the rich organic sediments in total darkness.

Some diatoms are capable of carrying out photosynthesis when light is available and then growing heterotrophically when necessary. *Cyclotella cryptica* Reimann, Lewin and Guillard has almost no ability to transport glucose in high light levels, but rapidly acquires this ability when grown in the dark (White, 1974). When these diatoms are transferred from a high light level into the dark, their growth terminates abruptly and resumes 1 to 2 days later. This is thought to be the time required for the development of the glucose uptake transport mechanism. *Nitzschia laevis* Hustedt can switch from an autotrophic to a heterotrophic mode rapidly, indicating that it maintains the transport mechanism continually. It can also live on several amino acids, including glutamate, alanine and arginine.

The colorless diatom *Nitzschia alba* Lewin and Lewin is totally incapable of photosynthesis, but consumes a wide variety of foods. This diatom can even consume cellulose. Linkins (1973) has shown that when given microcrystalline cellulose, the diatom produces an extracellular acid sulfate containing a polysaccharide to surround the crystals. It then secretes  $\beta$ -1,4-glucanases to hydrolyse the macromolecules. This diatom is also capable of growing heterotrophically on alginate, agar, glucose, fructose, gluconate, acetate, lactate, succinate, chitin, rhamnose and mannose. Many of these amino acids, sugars and polysaccharides have been found in carbonaceous chondrites and may exist on comets.

The widely distributed benthic marine diatom *Melosira nummuloides* Agardh, utilizes a number of amino acids (including valine) extensively as nitrogen sources for growth in the absence of inorganic nitrogen (Hellburst and Guillard, 1967). It also actively consumes the amino acid analogue  $\alpha$ -aminoisobutyric acid, which is the dominant amino acid in many chondrites (Hellburst and Lewin, 1977).

Consequently, based upon our present knowledge of diatoms and the nature of comets as inferred from chondrites it is not inconceivable that ice diatoms and other microorganisms could indeed find these regions suitable for habitation. Many are sufficiently small that they could enter the Earth's atmosphere safely after being boiled off the comet with other volatiles as it nears the Sun. Many diatoms and bacteria also form resting spores and cysts that could be far more resistant to long periods of dessication and the radiation levels encountered in the space environment.

It is interesting that *Thalassiosira antarctica* Comber and *Porosira glacialis* Grunow have been found at both poles, although neither diatom has as yet been recorded from latitudes lower than 58 degrees in either hemisphere. Several other species exhibit a similar bipolar distribution, which is very disconcerting to diatom ecologists. However, this bipolar distribution is consistent with an injection model in which these microorganisms arrive from space, either individually or associated with cometary debris.

## 6. Interstellar Space, Radiation and Microorganisms

The ultraviolet and soft X-ray components of the radiation environment of interstellar space are undoubtedly most hazardous to microorganisms over very long exposures. Of course, it is known that certain microorganisms are extremely resistant to high doses of ionizing radiation. In 1960, Fowler *et al.* reported a species of *Pseudomonas* living in a research nuclear reactor where the average dose was estimated to be more than a million rads. Another bacterium, *Micrococcus radiodurans*, can survive exposures of several megarads. Nassim and James (1978) experimented with exposures estimated to cause of the order of 10 000 breaks in the DNA of these bacteria, yet the bacteria repaired this immense damage by an intricate process of snipping and inverse base-copying.

It has been established that many diatom species are capable of living in environments containing extremely high concentrations of usually lethal radioisotopes such as plutonium, americium, strontium, etc. Diatoms thrive in highly radioactive waste ponds, including U-pond and the Z-19 trench (containing over 8 kg of various radioisotopes of plutonium according to official records) at the Hanford facility which has been processing plutonium since 1944. Not only do diatoms live in this environment, but they seem to have a remarkable affinity for plutonium. Emery *et al.* (1974) report that the algae of these ponds (of which diatoms are by far the dominant form) concentrate americium<sup>241</sup> three million fold and certain isotopes of plutonium are accumulated to 400 million times the concentration in the surrounding water. The plant life in these radioactive ponds contain more than 95% of the total plutonium burden. Diatoms and *Potamogeton* alone contain more than 99% of the plutonium in plants (Emery *et al.*, 1980). In this environment,

diatoms grow in great abundance while continuously subjected to high levels of X-rays, gamma rays, alpha and beta particles.

Therefore, it is feasible that individual intact diatoms may be capable of surviving exposure to the deep space radiation environment for long periods of time. Furthermore, virtually nothing is known of the radiation resistance of diatom cysts and spores. Also, since many diatoms can live or remain viable when encased in ice, comets might well provide a suitable habitat with adequate protection from the ultraviolet and soft X-ray radiation environment.

Hagen *et al.* (1971) have investigated the effect of temperature on the survival of microorganisms in a deep space environment. In the NASA Jet Propulsion Laboratory Space Molecular Sink Research Facility (MOLINSK) bacteria and bacterial spores were exposed to a simulated deep space environment. These organisms included *Bacillus subtilis* var. *niger*, *Staphylococcus epidermidis* and a species of *Micrococcus* isolated from Apollo 11 before launch. The specimens were subjected to hard vacuum ( $10^{-10}$  torr) and temperatures ranging from  $-124$  °C to  $+59$  °C. Hagen *et al.* found that the '*bacterial survival was better in the test environment at all temperatures than cells held at ambient room conditions (760 torr at 25 °C.)*' In calculating the survival fraction, they were forced to compare the number of organisms surviving to those originally placed on the platform rather than to the control group of microorganisms surviving ambient room conditions for the same time period. This was necessary because after a 14 day exposure the control group showed only 2.9% of the original *Micrococcus* sp. survived and no Staphylococci remained viable. After 14 days of exposure to the deep space environment, over 90% of the *Bacillus subtilis* var. *niger* remained viable independent of temperature over the range from  $-124$  °C to  $+34$  °C, while even as much as 30% of the  $59$  °C sample remained viable. The *Micrococcus* sp. tested at  $-105$  °C diminished in number to about 20% of the original during the first week but then levelled out as no more organisms lost viability with time. These results clearly show that the effects of hard vacuum and low temperature such as are encountered in deep space are not lethal to these microorganisms. On the contrary, it seems to greatly improve their survivability as contrasted to the conditions encountered at the surface of the Earth!

The question of the survivability of terrestrial type microorganisms in alien environments is not new. Seckbach and Libby (1970) investigated the survivability of algae on Venus. They performed experiments with algae in pure CO<sub>2</sub>, at elevated temperatures ( $50$  °C) and pressures ( $50$  atm) in acid. The green alga *Scenedesmus* sp. produced larger cells and showed higher activity in the simulated environment than in the laboratory control. The *Cyanidium caldarum*, a thermophilic/acidophilic alga collected from the acid sulphate springs of Yellowstone National Park literally thrived in the simulated Cytherean atmosphere. It also produced larger cells than the control.

Ponnamperuma and Molton (1973) studied the survivability of terrestrial microorganisms in a simulated Jovian atmosphere. They found that *Escherichia coli*,

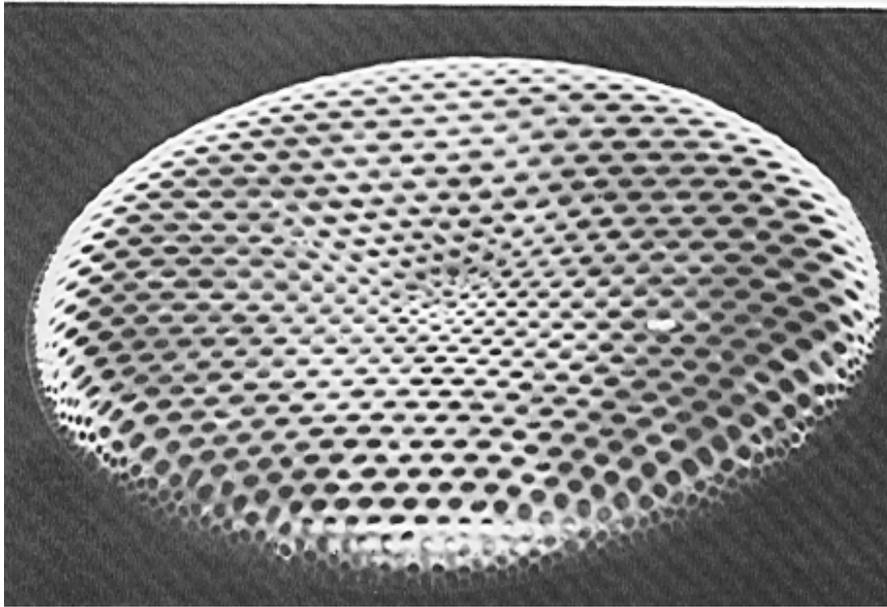


Figure 13. SEM photomicrograph of a recent *Coscinodiscus lineatus* Ehrenberg from McMurdo Sound, Antarctica. The structural features of *C. lineatus* have undergone virtually no changes from the Cretaceous to the present. (G. Dallas Hanna specimen. Photomicrograph by Richard B. Hoover and Daniel W. Gates).

*Serratia marcescens*, *Aerobacter aerogenes*, and *Bacillus subtilis* remained viable after 24 hour exposures to the 102 atmosphere pressure, 20 °C environment and synthesized atmosphere that might be encountered on Jupiter.

Indeed, the terrestrial microorganisms that have been considered in these studies have been found to do phenomenally well in simulated 'alien' environments and deep space conditions. These results are also difficult to understand from strictly terrestrial evolutionary considerations. Conversely, this unexpected viability in these 'alien' environments leads credence to the concept that terrestrial microorganisms may indeed have had an extraterrestrial origin.

## 7. The Origin of Diatoms

The origin of terrestrial diatoms is very puzzling. Diatoms appear abruptly in the fossil record in the Cretaceous as a very highly developed and diversified group of organisms. The oldest known diatoms are from the Lower Cretaceous Albian phosphorites found near Hannover, Germany. Forti and Schulz (1932) described 10 species of these 112 million year old diatoms, which were reported to be well preserved. Many upper cretaceous deposits have been found in several regions of the world; some 32 genera and 135 species and varieties have

been found in Campanian deposits of western Siberia. A considerably different suite of 45 genera and 130 species are encountered in the Maastrichtian deposits described by Hanna (1927) from material discovered by Anderson and Pack in the Moreno Shale of Panoche Hills, California. These superbly preserved diatoms exhibit extremely intricate morphology. Many of the more unusual forms, such as *Glorioptychus callidus* Hanna, *Sphinctoletus monstrosus* Hanna and *Benetorus fantasmus* Hanna are known only from this deposit, while other species are found in more recent deposits. Many of these fossils are strongly silicified, some of which are diatom cysts or resting spores (Figure 11). There are approximately 70 genera and 300 species of Cretaceous diatoms. These diatoms are intricately organized and the structure of the shells of some species does not differ from that of presently living forms. Indeed, several of the Cretaceous species found in the USSR deposits (i.e., *Stephanopyxis turris* Talfs, *Coscinodiscus lineatus* Ehrenberg, *Melosira sulcata* Kutzing, etc.) can be found living today (Figure 13) and are morphologically identical to their ancestors, which preceded them by some  $10^{10}$  or more generations.

The great difficulty exists in explaining this intricate diversity of highly evolved structural features of the known Cretaceous diatoms without a long line of evolutionary predecessors. Strelnikova (1974) suggests that: 'The great systematic variety of Cretaceous diatoms and their complicated morphological structures indicate a long path of preceding evolutionary development. The predecessors of diatoms should be looked for in pre-Jurassic deposits.' Strelnikova and many other diatomists have been troubled by the total absence of pre-Cretaceous diatoms in the fossil record and the highly complex morphology observed in the earliest Cretaceous forms. As Burkle (1978) has emphasized: 'Cretaceous forms show as much variation in shell structure as do the tertiary forms. Because of this it is difficult to use stage of evolutionary development as a gross stratigraphic tool.' Several workers have argued that diatoms must be much more ancient than the Cretaceous, and that their absence from the fossil record must be because their shells dissolved. However, this theory fails to explain why the siliceous shells of radiolarians (which are very similar in nature to those of diatoms) did not also dissolve.

Indeed, there are very few fossil groups in geological history with as complete a record as the radiolarians (Kling, 1978). These organisms have been reported from rocks as old as the Precambrian. Excellent, well preserved radiolarian assemblages are known from the Ordovician to the present. From the Cretaceous on these microfossils are very commonly found in diatomites. Hence, it seems unlikely that the absence of pre-Cretaceous diatoms was in reality caused by their shells dissolving, otherwise the much more ancient radiolarian assemblages should likewise be absent.

Round and Crawford (1981) have advanced the theory that diatoms evolved from a 'pre-diatom' stage in which a naked photosynthetic cell acquired a coating of siliceous scales. This was presumably followed by a stage when the siliceous scales differentiated to form valves and girdle bands to give a recognizable but

extremely simple diatom. They also emphasize that the diatoms appear suddenly in the fossil record as quite elaborate organisms and that 'many of the earliest fossil examples appear as, or more complex than the modern species, and little subsequent evolution is evident'. Hence, the evolutionary history must go very far back, perhaps even to the Precambrian. These observations are very curious, as the hypothetical pre-diatoms have never been found. It seems unlikely that diatoms, which were naked protoplasm prior to the Cretaceous, would immediately adopt such complicated and intricate structures that we find when they first began to construct shells.

Rather, the fossil record is far more suggestive of an external injection of a highly evolved assemblage of diatoms rather than a long period of evolution on Earth. This is also emphasized by the fact that several forms that can be found living in the polar waters today, are essentially identical to their Cretaceous ancestors, with little or no evolutionary changes observable.

## 8. Summary

The infrared and ultraviolet absorbance properties of diatoms and bacteria are consistent to a high degree of accuracy with the observed spectral characteristics of interstellar dust grains. Carbonaceous chondrites, which are thought to be the non-volatile fractions of comets, have transported to Earth evidence of a complex cosmic biochemistry. Indeed, the 'organized elements' found in the chondrites may well represent our first real contact with extraterrestrial life. The abundance of these microfossils within the chondrites provides strong indications that comets may serve as suitable habitats for microbiological communities. Diatoms, which constitute the most abundant form of plant life on Earth, seem to be well suited for survival and reproduction on comets, which are largely composed of ice. In the Arctic and the Antarctic ice, diatoms constitute by far the dominant life form. These microorganisms thrive on and in the ice and they exhibit interesting capabilities for coping with long periods of total darkness. This is accomplished by switching from food production by photosynthesis (autotrophic nutrition) to consumption of organic materials directly (heterotrophic nutrition). It is fascinating that many of the organics that these diatoms can directly consume are either found on comets or have been discovered in carbonaceous chondrites. These include unusual amino acid analogues, such as  $\alpha$ -aminoisobutyric acid. Some diatoms, such as those found in the deep North Atlantic sediments live and reproduce entirely in total darkness.

The Jovian satellite Europa is thought to possess a water ice crust. Brownish discolorations of cracks in the ice, which were observed during the Voyager flyby mission, are strongly reminiscent of those observed in Antarctica. In the terrestrial case, it is known that these discolorations are due to heavy coatings of the golden brown diatoms of the sea-ice community.

Some bacteria have exhibited a capability of living in the highly radioactive environment associated with the cores of nuclear reactors. Diatoms have been found to thrive in highly radioactive wastes found in the ponds where diverse radionuclides have been deposited. Some species actively concentrate radionuclides. The organic casings found in many diatoms could provide protection from exposure to ultraviolet and soft X-rays, as well as protection from dessication. Indeed, some microorganisms have been found to be capable of remaining viable after long periods of dessication. Bacteria have been found to be viable after preservation by dessication in salt deposits for two thirds of a billion years.

The abrupt appearance of diatoms as a highly developed, morphologically diverse group in the Cretaceous is believed to be consistent with the external injection model. The attempts to explain this phenomenon in terms of these organisms suddenly learning to build silicate shells is seen as artificial. The concept that all pre-Cretaceous diatom shells have dissolved seems erroneous in view of the vastly longer history of well preserved and similar siliceous shells of the radiolarians.

It is felt that the answer to many of these intriguing questions may be available in the near future as astronomical observations become more sophisticated. It is hoped that this year's return of Halley's comet, which will be studied extensively by ESA's space mission GIOTTO (Fechtig and Rahe, 1984) and by the Soviet VEGA spacecraft (Blamont and Sagdeev, 1984), will provide interesting new scientific data of potential relevance to these hypotheses. The Space Telescope should provide a wealth of new information regarding the far ultraviolet absorptivity of interstellar grains with high spectral resolution to permit even more extensive evaluations of the microbiological model. Extremely exciting plans are currently underway for a visit to a comet by the mid-1990's with the goal of extracting a sample of cometary ice for return to Earth. This mission could provide definitive information regarding the concept that life may be a cosmic (rather than a strictly terrestrial) phenomenon.

### **Appendix A. The 3.4 $\mu\text{m}$ Band and Biology**

The astronomical data for the galactic centre source GC-IRS 7 reached three of us in June 1981, with the points laid on a graticule which permitted a better reading of the numerical values than the diagram (without graticule) subsequently published (Allen and Wickramasinghe, 1981). Whenever a diagram without graticule is enlarged to read-off numerical values with respect to fiducial marks, a risk is taken that small drafting inaccuracies are amplified. We avoided this possibility by enlarging the original plot provided by the observers and then placing black letraset points directly over the original data points. Drawing the bacterial curve then presented a manipulative problem, as the scale of our enlargement did not have units that were round numbers of centimeters or inches. Inaccuracies resulting from this execution were generally of the order of the width of ink lines in our diagrams.

We were informed that the minimum near  $3.4 \mu\text{m}$  was at  $3.408 \mu\text{m}$  and that the wavelength spacing between data points was a constant value of  $0.014 \mu\text{m}$ . Hence, the wavelengths of the points on the observer's graph did not require reading, but were considered known. It was necessary to read the flux values, which were given to us on a relative scale,  $cF_\lambda$  with  $c$  a constant such that  $cF_\lambda = 5.65$  at  $\lambda = 3.408 \mu\text{m}$  (the unit of  $cF_\lambda$  was  $10^{-10} \text{ erg s}^{-1} \text{ cm}^{-2} \mu\text{m}^{-1}$ ). During the ensuing months the value of  $c$  was determined by the observers, so that  $F_\lambda$  was given in the published graph rather than  $cF_\lambda$ . This is simply a matter of normalization with no relevance of the fit of the observational points to the bacterial curve.

We were also subsequently supplied with several further points in the difficult wavelength region around  $2.9 \mu\text{m}$ . Except for a withdrawal of six of these points (four near  $3.2 \mu\text{m}$  and two near  $3.3 \mu\text{m}$ ), no substantive changes to what we had received were subsequently made. Table II shows wavelengths  $\lambda(\mu\text{m})$ , observed relative fluxes  $cF_\lambda$  transmittances  $e^{-\tau}$  read from our laboratory measured penchart spectrum of *E. coli* at the wavelengths required, and our calculated values obtained in the following manner.

Imagine a collimated beam of radiation from GC-IRS 7 with intensity distribution  $I(\lambda)d\lambda$  directed toward the Earth. Due to the scattering and absorption which occurs *en route* to the Earth, a terrestrial observer determines the spectrum

$$\exp[-\tau_{\text{sca}}(\lambda)] \exp[-\tau_{\text{abs}}(\lambda)] I(\lambda) d\lambda,$$

$\tau_{\text{sca}}(\lambda)$  and  $\tau_{\text{abs}}(\lambda)$  being the wavelength dependent scattering and absorption optical depths integrated along the line of sight. The source of  $I(\lambda) d\lambda$  is inferred from studies of CO absorption at  $\lambda \cong 2.4 \mu\text{m}$  and from near-infrared filter photometry to be an M supergiant with an effective temperature near 3200 K, so that  $I(\lambda) d\lambda$  is much like the Planck distribution for this temperature. Multiplication by  $\exp[-\tau_{\text{sca}}(\lambda)]$  has the effect, over a limited wavelength range of yielding an intensity distribution  $I(\lambda) \exp[-\tau_{\text{sca}}(\lambda)] = B(\lambda, T_c)$ , where  $B(\lambda, T_c)$  is the Planck distribution for this temperature. Multiplication by  $\exp[-\tau_{\text{abs}}(\lambda)]$  has the effect, over a limited wavelength range, of yielding an intensity distribution  $I(\lambda) \exp[-\tau_{\text{sca}}(\lambda)] \exp[-\tau_{\text{abs}}(\lambda)] = B(\lambda, T_c) \exp[-\tau_{\text{abs}}(\lambda)]$ , where  $B(\lambda, T_c)$  is the Planck function for a suitably chosen color temperature  $T_c$ . From the known scattering properties of interstellar grains  $T_c$  can be shown to be likely to lie in the range from 1000 K to 1500 K. In our former work we took  $T_c = 1100 \text{ K}$ , which was consistent with the envelope of the observation of Allen and Wickramasinghe. Hence, Equation (1) can be written as

$$AB(\lambda, 1100) \exp[-\tau_{\text{abs}}(\lambda)] d\lambda \quad (2)$$

where  $A$  is a constant depending on the intrinsic emission and distance of the source. If  $\tau_{\text{abs}}(\lambda)$  arises from the absorption values appropriate for our laboratory sample of *E. coli*, then Equation (2) takes the form

$$AB(\lambda, 1100) \exp[-\alpha\tau(\lambda)] \quad (3)$$

TABLE II

$\lambda(\mu\text{m})$	$cF_\lambda$	Observed	$AB(\lambda, 1100) \exp -\alpha\tau$
		$e^{-\tau(\lambda)}$	$\alpha = 1.300$ Calculated
2.890	7.4	0.620	7.090
2.904	7.1	0.605	6.853
2.918	6.7	0.596	6.706
2.932	6.3	0.580	6.458
2.946	5.8	0.570	6.299
2.960	5.45	0.558	6.112
2.974	5.60	0.549	5.969
2.988	5.75	0.541	5.841
3.002	5.5	0.532	5.700
3.016	5.56	0.531	5.670
3.030	5.3	0.530	5.640
3.044	5.66	0.536	5.707
3.058	6.0	0.543	5.786
3.972	6.0	0.553	5.907
3.086	6.0	0.566	6.069
3.100	6.1	0.570	6.105
3.114	6.45	0.574	6.141
3.128	6.35	0.579	6.190
3.142	6.3	0.583	6.224
3.156	6.5	0.594	6.355
3.170	Withdrawn	0.605	6.486
3.184	Withdrawn	0.610	6.532
3.198	Withdrawn	0.615	6.579
3.212	Withdrawn	0.620	6.623
3.226	7.05	0.621	6.612
3.240	6.95	0.622	6.600
3.254	6.7	0.624	6.602
3.268	6.47	0.628	6.631
3.282	6.5	0.631	6.646
3.296	Withdrawn		
3.310	Withdrawn	0.638	6.687
3.324	6.7	0.638	6.660
3.338	6.75	0.637	6.619
3.352	6.2	0.624	6.416
3.366	6.0	0.598	6.045
3.380	6.0	0.590	5.915
3.394	5.85	0.583	5.798
3.408	5.65	0.569	5.593
$\sim 3.429^*$	5.9	0.590	5.824
3.436	6.05	0.605	6.004

TABLE II  
(continued)

$\lambda(\mu\text{m})$	$cF_\lambda$	Observed	$AB(\lambda, 1100) \exp -\alpha\tau$
		$e^{-\tau(\lambda)}$	$\alpha = 1.300$ Calculated
3.450	6.15	0.623	6.209
3.464	6.35	0.638	6.374
3.478	6.2	0.640	6.371
3.492	6.35	0.641	6.354
3.506	6.45	0.661	6.582
3.520	6.7	0.688	6.901
3.534	6.75	0.698	6.999
3.548	6.95	0.708	7.095
3.562	7.1	0.711	7.100
3.576	7.25	0.716	7.130
3.590	7.3	0.719	7.134
3.604	7.55	0.727	7.202
3.618	7.45	0.730	7.205
3.632	7.55		
3.646	7.45		
3.660	7.3	0.745	7.287
3.674	7.3		
3.688	7.45		
3.702	7.25	0.760	7.365
3.716	7.3		
3.739	7.3		
3.744	7.4		
3.758	7.35	0.770	7.337
3.772	7.3		
3.786	7.35		
3.800	7.35		
3.814	7.2		
3.828	7.2		
3.842	7.15		
3.856	6.95		
3.870	6.9		
3.884	7.15		
3.898	6.9		
3.912	7.2	0.790	7.150
3.926	7.1		

\* Although the prescription given to us was that  $\lambda$  changed by  $0.014 \mu\text{m}$  from one data point to the next, a careful examination of the diagram of Allen and Wickramasinghe (*loc. cit.*) shows an exceptionally large wavelength step at this particular point.

with  $\exp[-\tau(\lambda)]$  as given in Table II and  $\alpha$  the factor by which the quantity of absorbing material along the astronomical line of sight exceeds the amount used in the laboratory sample.

It is worth noting that  $B(\lambda, 100)$  is nearly flat over the wavelength range from 2.8  $\mu\text{m}$  to 3.6  $\mu\text{m}$ , varying by only about 10%, so that the situation is nearly the same as if the interstellar grains were in the laboratory with a flat source function used to obtain their spectrum. This is a favorable situation for using the astronomical observations to infer  $\tau(\lambda)$  (i.e., for making the present comparison).

$A$  and  $\lambda$  must be specified before explicit numbers can be calculated from Equation (2). The constant  $A$  disappears when Equation (2) is normalized with respect to the scale used for  $cF_\lambda$ , with  $\alpha$  remaining as a disposable constant. The calculations were made by requiring Equation (1) to be equal to  $cF_\lambda$  at two values of  $\lambda$ , one the minimum at  $\lambda = 3.408 \mu\text{m}$ . The other point was chosen at 3.324  $\mu\text{m}$ , due to the fact that in June, 1981 there was much puzzlement concerning two seemingly narrow absorptions: one near 3.2  $\mu\text{m}$  and the other near 3.3  $\mu\text{m}$ . It then seemed reasonable to choose  $\alpha$  so that Equation (2) agreed with an observational point near 3.3  $\mu\text{m}$ . With this reason now gone (following the removal of the apparent absorptions) we have followed a simpler procedure. Instead of the former  $\alpha = 1.3588$  we have founded the disposable parameter to  $\alpha = 1.3000$ . All that remains is to decide on the scale factor  $A$ , which can obviously be chosen to agree with the observed flux at any one wavelength, *but only at one wavelength*. The sensitive region for comparing the calculation of Equation (2) with the observed fluxes is the range 3.3–3.5  $\mu\text{m}$ , and the comparison will be all the stronger if we avoid choosing  $A$  so as to normalize to one of the data points in the critical range. Explicitly, we have chosen to normalize with respect to the data point at  $\lambda = 3.562 \mu\text{m}$ . The resulting flux curve together with the observational points is shown in Figure 4.

The error bars given by the observers differ at different wavelengths; typical error bars for the data points in various wavelength ranges are shown in Figure 2. It seems significant that where the deviations of points from the curve are largest the error bars are also the largest. A similar remarkable agreement to that shown in Figure 4 has been found for other microorganisms studied under similar conditions, including diatoms and mixtures of diatoms with *E. coli*.

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