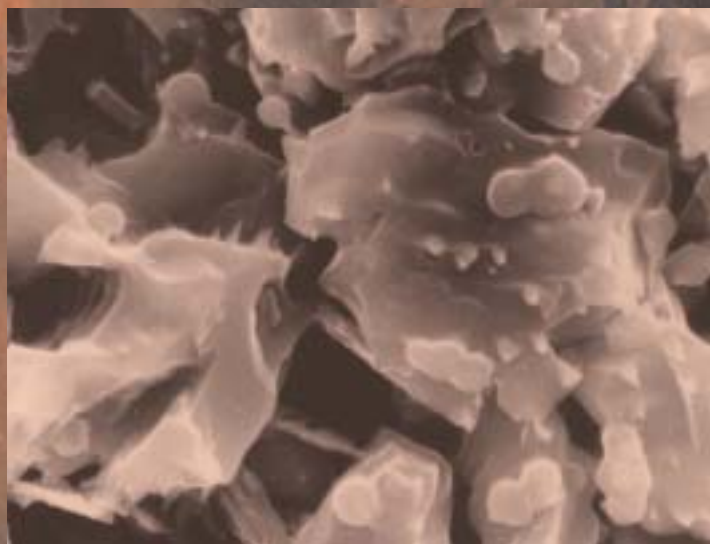


# Exobiology in the Solar System & The Search for Life on Mars



Report from the  
ESA Exobiology Team Study  
1997-1998

# **EXO BIOLOGY IN THE SOLAR SYSTEM AND THE SEARCH FOR LIFE ON MARS**

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*Cover*

Fossil coccoid bacteria, 1  $\mu\text{m}$  in diameter, found in sediment 3.3-3.5 Gyr old from the Early Archean of South Africa. See pages 160-161.

*Background:* a portion of the meandering canyons of the Nanedi Valles system viewed by Mars Global Surveyor. The valley is about 2.5 km wide; the scene covers 9.8 km by 27.9 km centred on 5.1°N/48.26°W. The valley floor at top right exhibits a 200 m-wide channel covered by dunes and debris. This channel suggests that the valley might have been carved by water flowing through the system over a long period, in a manner similar to rivers on Earth. (*Malin Space Science Systems/NASA*)

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# FOREWORD



The Exobiology Science Team was established in September 1996 by Dr. P. Clancy of ESA's Directorate of Manned Spaceflight and Microgravity. The task of the Team was to survey current research in exobiology and related fields and then to make recommendations to ESA on the nature of a future search for life elsewhere in the Solar System. In carrying out that task, the Team has benefited considerably from the contributions provided by many other experts, as listed below.

From a scientific point of view, it is probably the first time in recent years that the various relevant aspects involved in a potential search for life elsewhere have been brought together and examined in such detail in Europe. Likewise, this is the first attempt to outline what exobiology space experiments might reasonably be planned, given the limited resources currently available in Europe for new ventures, however exciting they may be.

The results of this first study are presented in Part I. For this part, the Team comprised:

*Chairman:* André Brack, Centre de Biophysique Moléculaire, CNRS, Orléans, France.

Patrick Forterre, Institut de Génétique et Microbiologie, Université de Paris Sud, Orsay, France.

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A major outcome was the recommendation to seek evidence of extinct life below the surface of Mars, using a group of six instruments. All of these are adaptations of instruments that are either under development or have already flown on other space missions. Together, they form an integrated package of analysis equipment that

should be able to provide a convincing answer to the question, 'Is there evidence here, at this particular landing site, of previous or existing life?' That instrumentation system is provisionally named 'Pasteur', to commemorate a great scientist who proved that life existed where others saw only chemistry. If the Pasteur package can prove the same on Mars, then the effort and cost to Europe will have been well rewarded. Should the result be conclusively negative, then that too is of value. But more than that, the enormous amount of information on the subsurface geochemistry and mineralogy, which will be acquired as part of the search for extinct life, will in itself be a major scientific achievement.

To accommodate this instrument package, a Mars Lander capable of a soft touch-down is required. The possibility of adding it to ESA's Mars Express mission, scheduled for launch in June 2003, is being investigated.

In addition to the proposed Mars experiments, the Team also recommended continuing support for programmes that are concerned with understanding the basic mechanisms leading to life, the survivability and effects of the space environment on microorganisms, the adaptation of life to extreme environments, and the search for meteorite samples of Mars sedimentary rocks.

Hence there is a strong interest in ESA's Rosetta mission to Comet Wirtanen, which offers the prospect of determining the range of organics in bodies that may have contributed the precursors of life on Earth. The Cassini/Huygens mission to sample the atmosphere and surface components of Titan is also of interest to exobiology, for its insight into a world rich in hydrocarbons. Likewise, the current experiments on Mir and future International Space Station experiments concerned with the response of microorganisms to space conditions, together with the photochemistry of organics under space irradiation, are of continuing fundamental importance to exobiology.

### **Life and its Requirements**

Any discussion on how to search for life must start from a definition of what actually constitutes life. Probably the simplest statement is that life, to a scientist, is basically a chemical system that is able to transfer its molecular information via self-replication and to evolve via mutations.

For life as we know it to emerge, there must be liquid water. Not pure water, for along with the necessary reduced organic molecules there will need to be an association with C, N, H, O, S and P atoms. In any case, life has continued to develop very well in water that is very acidic, alkaline or is a strong brine solution. It has also survived and flourished in water at temperatures above 100°C.

The fact that evidence of complex microbial life is to be found on Earth stretching back some 3800 million years – to within about 700 million years of its formation – shows that it emerged very early and that once established it proved to be adept at evolving and adapting to a wide range of changing physical and chemical conditions. The discovery of an active biosphere several hundred metres below the Earth's surface is witness to this incredible diversity and adaptability of life.

Other biomarkers – signatures of life – common to all life as we know it at present include the preferential isotopic fractionation of carbon. The  $^{13}\text{C}/^{12}\text{C}$  ratio in sedimentary organic carbon, as compared to the same isotopic ratio for coexisting carbonate carbon, provides a remarkably consistent signal of biological carbon fixation which has been shown to stretch back over some 3500 million years of the fossil record of microbial life on Earth. The origin of this fractionation in favour of  $^{12}\text{C}$  in the biological pathway of carbon fixation derives for the most part from a kinetic isotope effect imposed on the first  $\text{CO}_2$  – fixing enzymatic carboxylation reaction of the photosynthetic pathway. The importance of this biomarker, and of other potential isotopic biomarkers (H, N, O and S), is one of the reasons for the inclusion of a gas chromatograph/mass spectrometer in the suite of instruments recommended for any exobiology package.

Another reason for its inclusion is to identify the organic compounds released by

pyrolysis, or similar techniques, from any fossil residue of biological matter that might be found during a search. On Earth, the bulk of such 'kerogen' material is made up of highly polymerised aliphatic and aromatic hydrocarbons, representing the end product of diagenetic alteration of the primary biogenic substances in the sediments.

Among that residue of ancient life can be found trace amounts of quasi-pristine organic molecules (mostly pigments or discrete hydrocarbon chains) that have preserved their identity throughout the process of decay and evolution of the kerogen. The record of such biomarker molecules goes back about 1000 million years on Earth. Apart from their possible detection by evolved gas analysis (GCMS), which can also determine the molecular distribution patterns in a homologous series of remnant compounds, such as lipids, as a characteristic indication of biological action, further information may also be obtained by IR and Laser Raman spectroscopy, which yields details of characteristic bonding and structure.

An analysis of the elemental composition of these residues has also provided an insight into the origin and habitat of the original source organisms. The GCMS instrumentation, together with the proposed Alpha-Proton-X-ray (APX) elemental analysis instrument, is intended to supply that information. The APX instrument will also be used for elemental analysis of the mineralogical component of samples.

The chemical products of metabolism are a key indicator of extant life, and formed the basis for the exobiology experiments on the Viking lander on Mars. Culturing and subsequent biochemical analysis, e.g. by nucleic acid sequencing, and protein, lipid and sugar content determination are classic routes to identification of living organisms. However, their use under space conditions would be extremely challenging.

Microscopic examination forms the other principal means of detecting both extant and extinct life and equipment for that purpose is proposed in the instrument package. Two levels of observation are foreseen. The first is an optical microscope which would be used to search for groups of microfossil structures. It could also be used for petrological studies and to indicate possible residual carbonaceous material. If extant microbial life was present it would also be observable.

In order to observe the detailed structural components of any microfossil structures, or extant equivalents, it will be necessary to achieve a resolution in the nanometre range. This can be done using an Atomic Force Microscope. In this, a probe scans the surface field structure of the sample, yielding a 3D image with a resolution of a few nanometres.

### **Life: Where Else Might it Exist?**

From the evidence that life arose very early in the young Earth, we might reasonably expect to find evidence of life wherever water has existed continuously over a period comparable with the 700 million years that, at the very maximum, it took for life on Earth to develop.

We have clear evidence that water existed in substantial amounts on the surface of Mars at some earlier epoch. How long it was present at the surface is unknown. Nor do we know if water exists still in subsurface aquifers, although there are clear indications of large permafrost regions and of old water flows out of associated areas. It remains a matter of urgency to carry out a radar survey from orbit to determine the extent of any geothermally heated subsurface water table, lying below the cryosphere. Similarly, high-resolution orbital surveys to isolate regions of hydrothermal activity and of surface water vapour release are essential for any future search for extant life on Mars.

Given the discovery of a flourishing biosphere a kilometre below the Earth's surface, it is possible that a similar vast microbial community is still present below the surface of Mars, having long ago retreated into that ecological niche following the disappearance of a surface water environment.

The possibility that life may have evolved on Mars during an early period when water existed on the surface and that life may still exist deep below the surface, marks the planet as a prime candidate in a search for life beyond the Earth. It was for that

reason – and the prospect of Mars missions in the foreseeable future – that it was recommended a search for life be focused on the planet.

In stating that life may have begun on the surface of Mars, we reveal a natural inclination to consider life as a planet's surface phenomenon. The assumption may yet be proved wrong, with life perhaps able to develop comfortably within a warm wet subterranean world.

In that sense, therefore, life may have actually evolved in the subsurface world of Mars and of other planets and satellites. For example, Europa, a satellite of Jupiter heated by gravitational tidal forces, has an icy carapace enveloping the surface and possibly many kilometres thick. Indications of cryovolcanic flows at the surface point to a possible water or brine subsurface region which might harbour a basic lifeform. NASA may mount a mission to Europa to investigate that possibility.

### **The Search for Life on Mars**

As an extension to the initial general study, the ESA Exobiology Science Team was then asked by ESA to consider more specifically the case of Mars. This is presented below, in Part II. The constitution of the Science Team was slightly modified and expanded from the team that undertook the original broader study on 'The Search For Life In The Solar System'. Focusing on Mars, together with the need to concentrate on the practical and technical aspects, inevitably called for some changes in the Team's overall constitution. The ESA Exobiology Study Team of 1998 comprised:

*Chairman:* André Brack, Centre de Biophysique Moléculaire, CNRS, Orléans, France.

Beda Hofmann, Naturhistorisches Museum, Bern, Switzerland.

Gerda Horneck, Institute of Aerospace Medicine, DLR, Porz-Wahn, Germany.

Gero Kurat, Naturhistorisches Museum, Vienna, Austria.

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*Secretary:* Brian Fitton, European Science Consultants, Noordwijk, Netherlands.

This second study was carried out primarily through the efforts of three subgroups of the Science Team, aided by other specialist advisors. It also benefited from presentations and discussions during an extended meeting of the Team, which was attended by a wider group of advisors and specialists, together with ESA scientific and technical personnel.

The principal tasks of the subgroups – Teams I, II and III – are outlined below, together with the list of contributors to Part II of this report:

#### **TEAM I**

*To define those surface and near-surface environments of Mars where evidence of past life might best be sought and to establish a shortlist of preferred landing sites for a future exobiology mission.*

*Leader:* Gian G. Ori, Dipartimento di Scienze, Università d'Annunzio, Pescara, Italy.

Ignasi Casanova, Institute for Space Studies, Univ. Politecnica Catalunya,  
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Germany.

Gero Kurat, Naturhistorisches Museum, Vienna, Austria.

Nicolas Thomas, MPI für Aeronomie, Lindau, Germany.

## TEAM II

*To study specifically the chemical analysis aspects of an exobiology multi-user package. This includes the chemical analysis of surface and subsurface minerals and organics, to search for microbial life, past or present, in the Martian soil.*

*Leader:* François Raulin, LISA, Université de Paris, Créteil, France.

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Harald Strauss, Institut für Geologie, Ruhr University, Bochum, Germany.

Nicolas Thomas, MPI für Aeronomie, Lindau, Germany.

## TEAM III

*To define a set of imaging and spectroscopic systems which will allow a search for evidence of extinct microbial life at all scales down to 0.01  $\mu\text{m}$ , whilst also providing for the study of the mineralogy and petrography, as a function of depth, in the near-subsurface region of Mars and in surface rocks.*

*Leader:* Nicolas Thomas, MPI für Aeronomie, Lindau, Germany.

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Wojtek Markiewicz, MPI für Aeronomie, Lindau, Germany.

Harald Strauss, Ruhr Universität, Bochum, Germany.

Frances Westall, Università di Bologna, Italy.

In this volume, the three separate contributions from these Teams are introduced sequentially. From them, the Exobiology Science Team drew up conclusions on the desired strategy for searches and the set of sample acquisition, observation and analysis instrumentation to fulfil the scientific requirements. These conclusions were then adapted so that the resulting exobiology package and the operational strategies are reasonably consistent with the major constraints of potential future Mars lander missions. That has been achieved without a major loss of scientific potential.

Later, another subgroup (Team IV) embarked upon an analysis, from an exobiology viewpoint, of the potential value of human exploration of Mars. Its conclusions and recommendations on this critical subject are presented in Annex 1.

## TEAM IV

*To analyse the proposed ideas for a manned mission to Mars from an exobiology viewpoint in order to advise on the advantages offered and the precautions that need to be taken.*

*Leader:* André Brack, Centre de Biophysique Moléculaire, CNRS, Orléans, France.

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Gerda Horneck, Institute for Aerospace Medicine, DLR, Porz-Wahn, Germany.

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Wolfram Zillig, MPI für Biochemie, Martinsried, Germany.

Part II develops from Part I and provides the reasoning that leads to the definition of the specified multi-user instrument package. The technical design of that package is now being undertaken by ESA.

In developing these ideas, the ESA Exobiology Team has been greatly assisted by the following Advisors, whose contributions have also eased the task of preparing this Report:

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Michael Fernandes, Max Planck Inst. für Aeronomie, Lindau, Germany.

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Alexandra MacDermott, Chemistry Department, Cambridge University, Cambridge, UK.

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Harald Strauss, Institut für Geologie, Ruhr Universität, Bochum, Germany.

The Team would also like to thank the following ESA personnel for their contributions and assistance: Augustin Chicarro, Pierre Coste, Elena Grifoni, Gerhard Kminek, Franco Ongaro, Peter Schiller and Günther Seibert.

This study of the search for life on Mars, together with the earlier study on the possibility of life elsewhere in the Solar System, was undertaken on the initiative of ESA's Directorate of Manned Spaceflight and Microgravity. The Team is grateful for the Directorate's support and, in particular, for the work of Paul Clancy in developing that initiative, establishing the Team and guiding their activities.

# **I**

## **AN EXOBIOLOGICAL VIEW OF THE SOLAR SYSTEM**



# I.1 Introduction

Exobiology, in its broad definition, includes the study of the origin, evolution and distribution of life in the Universe. This volume's study, which was organised by P. Clancy and supported by the ESA Directorate of Manned Spaceflight and Microgravity, is restricted to the search for life in the Solar System, considered as an open system with pre-existing material. Although it is difficult to define succinctly exactly what is meant by 'life', for the purpose of this study we consider as living any chemical system able to transfer its molecular information via self-replication and to evolve. The concept of evolution implies that the system normally transfers its information fairly faithfully but makes a few random errors, leading potentially to a higher complexity and possibly to a better adaptation to the environmental constraints.

Contemporary living systems use organic molecules made of carbon, hydrogen, oxygen, nitrogen, sulphur and phosphorus atoms to store and to transfer their chemical information. The two functions (information storage and information transfer) are carried out by two sets of distinct organic polymeric molecules: nucleic acids and enzymes, respectively. Their basic constituents, nucleotides and amino acids, have at least one carbon atom that is asymmetric and therefore exists in two mirror-image forms. The proteins are based exclusively on left-handed (L) amino acids, whereas DNA and RNA are based exclusively on right-handed (D) sugars. Nucleic acids and enzymes themselves form asymmetric helical structures and superstructures. On the other hand, any chemical reaction producing asymmetric molecules in statistically large numbers that is run in a symmetrical environment yields equal quantities of right- and left-handed molecules. In view of the importance of the one-handedness in present-day life, it is difficult to imagine a primitive life form using simultaneously biomonomers of both handedness in the same protocell.

By analogy with contemporary life, it is generally believed that primitive life originated from the processing of reduced organic molecules by liquid water. Life may also have started with mineral crystals but this interesting hypothesis still needs to be supported by experimental results. Detectable living chemical systems must probably develop within a liquid in order to allow the ready diffusion of the constituents. Terrestrial life uses liquid water. Water molecules are widespread in the Universe as grains of solid ice or as very dilute water vapour. Normally, liquid water can persist only above 0°C but this temperature can be lowered when salts or organic compounds are dissolved. In addition, the atmospheric pressure must be higher than 6 mbar. Therefore, the size of a planet and its distance from its parent star are two basic characteristics that will determine the presence of liquid water. If a planet is too small, like Mercury or the Moon, it will not be able to retain any atmosphere and, therefore, liquid water. If the planet is too close to the star, the mean temperature rises. Any sea water present would evaporate, delivering large amounts of water vapour to the atmosphere and thus contributing to the greenhouse effect, which causes a further temperature rise. Such a positive feedback loop could lead to a runaway greenhouse. All of the surface water would be transferred to the upper atmosphere, where photodissociation by UV light would break the molecules into hydrogen and oxygen. Loss of the atmosphere would result from the escape of hydrogen to space and the combination of oxygen with the crust. If a planet is far from its star, it may permit the existence of liquid water, provided that it can maintain a constant greenhouse atmosphere. However, water could provoke its own disappearance. The atmospheric greenhouse gas CO<sub>2</sub>, for instance, could be dissolved in the oceans and finally trapped as insoluble carbonates. (by rock-weathering). This negative feedback could lower the

surface pressure of CO<sub>2</sub> and consequently the temperature to such an extent that water would be largely frozen. The size of the Earth and its distance from the Sun are such that the planet never experienced either a runaway greenhouse heating or a divergent glaciation.

According to its molecular weight, water should be a gas under standard terrestrial conditions, as are CO<sub>2</sub>, SO<sub>2</sub>, H<sub>2</sub>S, for example. Its liquid state derives from its ability to form hydrogen bonds. The hydrogen bonds form a very tight polymeric network of water molecules and raise the boiling temperature to the relatively high value of 100°C. Hydrogen bonds are also formed between water molecules and the biomolecules and between the biomolecules themselves. For instance, base-pairing via hydrogen bonds is a key feature of the biological information transfer. Hydrogen bonds are not restricted to water molecules – alcohols exhibit a similar behaviour. Hydrogen-rich liquids such as alcohols, liquid hydrogen sulphide and liquid ammonia therefore merit attention as possible media for non-terrestrial living systems.

Unrestricted diffusion in a liquid would probably reduce the efficiency of the information-transfer processes. Terrestrial life restricts the extent of the diffusion process by using membranes. Any other way of limiting the dispersion of the organic molecules, such as mineral surface metabolism, must also be considered.

Several sources have been examined for the prebiotic organic building blocks: terrestrial primitive atmosphere (methane or carbon dioxide), deep-sea hydrothermal systems and cometary and meteoritic grains. The import of extraterrestrial organic molecules gains increasing interest.

By analogy with contemporary living cells, it was generally believed that primitive life required at least boundary molecules, catalytic molecules and informative molecules. Until now, it has been impossible to produce significant amounts of informative RNA molecules under prebiotic conditions. Some scientists are now tempted to consider that primitive replicating systems must have used simpler autocatalytic informational molecules. However, the clues that may help to understand these very early forms of life on Earth about 4000 million years ago have been erased.

Evidently, the basic conditions that generated terrestrial life appear not to be restricted to the Earth. This report examines possible sites for life on other bodies in the Solar System, as well as the possible transfer of life from one body to another.

## I.2 Chemical Evolution in the Solar System

Primitive terrestrial life probably emerged in water, with the first chemical systems able to transfer their molecular information and to evolve. Unfortunately, the direct clues that may help chemists to identify the molecules that participated in the emergence of life on Earth about 4000 million years ago have been erased by the effects of plate tectonics, the permanent presence of running water, the unshielded UV radiation from the Sun and the oxygen produced by life itself. By analogy with contemporary life, it is generally believed that primitive life originated from the processing of reduced organic molecules by liquid water. Primitive life may also have started based on mineral crystals. This idea has been developed by Cairns-Smith (1982) but still needs to be supported by experiments.

### I.2.1 Terrestrial Prebiotic Chemistry

#### I.2.1.1 Terrestrial Production of Reduced Organic Molecules

Oparin (1924) suggested that the small reduced organic molecules needed for primitive life were formed in an early atmosphere dominated by methane. The idea was tested in the laboratory by Miller (1953) when he exposed a mixture of methane, ammonia, hydrogen and water to electrical discharges. In his initial experiment, he obtained four of the 20 naturally occurring amino acids, via the intermediary formation of hydrogen cyanide and formaldehyde. Miller's laboratory synthesis of amino acids occurs efficiently when a reducing gas mixture containing significant amounts of hydrogen is used. However, the true composition of the primitive Earth's atmosphere remains unknown.

The source of the primitive terrestrial atmosphere can be found in a combination of the volatiles  $\text{CO}_2$ ,  $\text{N}_2$  and  $\text{H}_2\text{O}$  trapped in the rocks that comprised the bulk of the planet's mass (internal reservoir) and a late-accreting veneer of extraterrestrial material (external reservoir). The formation of the internal reservoir is a natural consequence of the planet's accretion. Wetherill (1990) suggested that collisions with planetary embryos in eccentric orbits should have thoroughly homogenised the rocky composition of Venus, Earth and Mars. The late-accreting veneer contributed by some combination of volatile-rich meteorites and comets should also have been uniform from planet to planet. A volatile-rich veneer may have replaced the atmosphere produced by the planet's early accretion, which was subsequently blown off by the giant impact between the Earth and a Mars-sized planetary embryo that generated the Moon and caused the early atmosphere massive hydrodynamic loss. The abundance and isotopic ratios of noble gases neon, argon, krypton and xenon suggest that meteorites alone or in combination with planetary rocks could not have produced the Earth's entire volatile inventory and that a significant contribution from icy planetesimals was required (Owen & Bar-Nun, 1995).

The surface temperature of the early Earth was very much dependent upon the partial pressure of  $\text{CO}_2$  in the atmosphere. The dominant view in recent years has been that the early atmosphere was a weakly reducing mixture of  $\text{CO}_2$ ,  $\text{N}_2$  and  $\text{H}_2\text{O}$ , combined with lesser amounts of CO and  $\text{H}_2$  (Kasting, 1993). An efficient greenhouse effect induced by a high  $\text{CO}_2$  partial pressure was the most likely mechanism capable of compensating for the faint young Sun, the luminosity of which was about 30% lower than today. The dominant sink for atmospheric  $\text{CO}_2$  is silicate weathering on land and subsequent formation and deposition of carbonate sediments in the ocean. However, a negative feedback system probably maintained the Earth's surface temperature above freezing: if the temperature dropped below freezing, silicate weathering would slow down and volcanic  $\text{CO}_2$  would accumulate in the atmosphere. Walker (1985) suggested that the  $\text{CO}_2$  partial pressure could have been as high as

10 bar before the emergence of the continents because of limited silicate weathering and continental carbonate rocks storage. Under these conditions, the early Earth could have been as hot as 85°C. Recent analysis of samarium/neodymium ratios in the ancient zircons indicate that the continent grew rapidly, so a dense CO<sub>2</sub> atmosphere may have been restricted to the first few hundreds million of years of the Earth's history.

Recently, Wächtershäuser (1994) has suggested that the carbon source for life was carbon dioxide. The energy source required to reduce the carbon dioxide is proposed to be provided by the oxidative formation of pyrite (FeS<sub>2</sub>) from iron sulphide (FeS) and hydrogen sulphide. Pyrite has positive surface charges and bonds the products of carbon dioxide reduction, giving rise to a 2D reaction system, a surface metabolism. Experiments are being performed in order to test this new hypothesis.

Deep-sea hydrothermal systems may also represent likely environments for the synthesis of prebiotic organic molecules. Experiments have been carried out in order to test whether amino acids can be formed under conditions simulating the hydrothermal alteration of oceanic crust (Wetherhill, 1990).

#### **1.2.1.2 Extraterrestrial Delivery of Organic Molecules to the Earth**

The import of extraterrestrial organic molecules is a subject of increasing interest. The study of meteorites, particularly the carbonaceous chondrites, containing up to 5% by weight of organic matter, has allowed close examination of extraterrestrial organic material. Eight proteinaceous amino-acids have been identified in the Murchison meteorite amongst more than 70 amino acids (Cronin & Pizzarello, 1983). These amino acids are asymmetric and the two handedness forms L and D, are generally found in equal proportions. However, Engel (1990) reported that L-alanine was 18% more abundant than D-alanine in one sample of the Murchison meteorite. This rather surprising result has been recently confirmed by Cronin (1996). The latter found that one-handedness was in excess of about 10% for isovaline, α-methyl norvaline and α-methyl isoleucine. These excesses found in the Murchison meteorite may help us to understand the eventual emergence of a one-handed (homochiral) primitive life. Indeed, homochirality is now believed to be not just a consequence of life, but also a prerequisite for life, because stereoregular polymers such as α-sheet polypeptides do not form with mixtures of amino acids of both handedness. The excess of one-handed amino acids found in the Murchison meteorite may, however, result from the processing of the organic mantles of interstellar grains by circularly polarised synchrotron radiation from a neutron star remnant of a supernova (Bonner & Rubenstein, 1987).

A large collection of micrometeorites has been recently extracted from Antarctic old blue ice and analysed by Maurette (1995). A high percentage of unmelted chondritic micrometeorites of 50-100 μm size has been observed, indicating that a large fraction crossed the terrestrial atmosphere without suffering drastic thermal treatment. In this size range, the carbonaceous micrometeorites represent 80% of the samples and contain 7% of carbon. They may have brought about 10<sup>20</sup>g of carbon to the Earth over a period of 300 million years, corresponding to the late terrestrial bombardment phase. This delivery represents more carbon than that engaged in the surficial biomass, i.e. about 10<sup>18</sup>g. Amino acids such as α-amino isobutyric acid have been recently identified in these Antarctic micrometeorites, which may have functioned as tiny chondritic chemical reactors when reaching oceanic water.

For many decades, it was believed that primitive life emerged as a cell, thus requiring boundary molecules such as phospholipids, catalytic molecules such as protein enzymes and informative molecules such as nucleic acids. Vesicle-forming fatty acids have been identified in the Murchison meteorite (Deamer, 1985). Primitive membranes could also have initially been formed by simple isoprene derivatives (Ourisson & Nakatani, 1994). Selective condensation of amino acids in the presence of liquid water has been experimentally documented. When hydrophobic and hydrophilic amino acids coexist within the same polypeptide chain, the duality generates interesting topologies such as stereoselective and thermostable α-sheet structures. Short peptides have also been shown to exhibit catalytic properties (Brack, 1993).

A weakness in the theory of the cellular origin of life appeared when nucleotide chemists failed to demonstrate that accumulation of significant quantities of natural nucleotides, the building blocks of RNA, was a plausible chemical event on the primitive Earth. Intense experimental work is presently occurring in the field of RNA analogues, of which Eschenmoser's pyranosyl-RNA is a prime example (Eschenmoser, 1994). Since RNAs have been shown to be able to act simultaneously as informative and catalytic molecules, it is tempting to consider RNAs as the first living systems on the primitive Earth (RNA world). One should, however, remember that their synthesis under prebiotic conditions remains an unsolved challenge. Many chemists now consider that primitive life was supported by simpler informative molecules and large efforts are now devoted to autocatalytic systems, including simple organic molecules and micelles.

As discussed in detail in Chapter I.7, the geological record also provides important information. The isotopic signatures of the organic carbon of the Greenland metasediments bring indirect evidence that life may be 3850 million years old (Mojzsis et al., 1996). This conclusion is fully consistent with the remarkable diversity of the 3465 million year old fossilised microflora reported by Schopf (1993).

### I.2.2.1 The Icy Bodies

#### *Comets*

Comets show a substantial content of organic material. According to Delsemme's analysis (1991), Comet Halley dust particles ejected from the nucleus contain 14% organic carbon by mass. About 30% of cometary grains are dominated by light elements C, H, O, N and 35% are close in composition to carbonaceous chondrites (Kissel & Krueger, 1987; Langevin et al., 1987). Among the molecules identified in comets are hydrogen cyanide and formaldehyde. The presence of purines, pyrimidines and formaldehyde polymers has also been inferred from the fragments analysed by the Giotto PIA and the Vega PUMA mass spectrometers. However, there is no direct identification of the complex organic molecules likely to be present in the dust grains and in the cometary nucleus. As already mentioned, cometary grains may have been an important source of organic molecules delivered to the primitive Earth (Oro, 1961; Chyba et al., 1990; Delsemme, 1992; Greenberg, 1993).

Many chemical species of interest for exobiology were detected in Comet Hyakutake in 1996, including ammonia, methane, acetylene ( $C_2H_2$ ), acetonitrile ( $CH_3CN$ ) and hydrogen isocyanide ( $HN=C$ ) (Irvine et al., 1996; Bockelée-Morvan, 1997). In addition to hydrogen cyanide and formaldehyde ( $H_2CO$ ), seen in several earlier comets, Comet Hale-Bopp was also shown to contain methane, acetylene, formic acid ( $HCOOH$ ), acetonitrile, hydrogen isocyanide, isocyanic acid ( $HNCO$ ), cyanoacetylene ( $HN-C\equiv C-CN$ ) and thioformaldehyde ( $H_2C=S$ ) (Crovisier, 1998).

#### *Europa*

Europa is one of the most enigmatic of the Galilean satellites. With a mean density of about  $3.0\text{ g cm}^{-3}$ , the jovian satellite should be dominated by rocks. Surprisingly, it exhibits an icy surface. Among the three models proposed for Europa's interior (Oro et al., 1992), the two most likely are: (i) dehydrated silicates covered with a 100 km-thick layer of water ice, and (ii) dehydrated silicates covered with a 100 km-thick liquid water layer, itself covered with a water ice layer 10 km thick. The heat source that may maintain liquid water within Europa is the dissipation of tidal energy, which results from the variation of the gravitational field across the moon's body. Heat transfer from the core to the bottom of the ocean, similar to thermal vents in terrestrial oceans, is another possible source of thermal energy. Although the existence of such an ocean is still uncertain, several clues, including the most recent data from Galileo, support the presence of a water ocean below the icy surface. Moreover, the surface of Europa indicates that it has undergone substantial resurfacing and tectonic activity.

If liquid water is present within Europa, it is quite possible that it includes organic matter derived from chondritic material. The impact of large meteorites, several

## I.2.2 Chemical Evolution on Other Bodies of the Solar System



kilometres in diameter, would have periodically partially melted the icy cover down to the ocean, thus seeding the water with exogenous organic material. Terrestrial-like prebiotic organic chemistry and primitive life may therefore have developed in Europa's ocean (Reynolds et al., 1983). If Europa has maintained tidal and/or hydrothermal energy in its subsurface so far, it is possible that microbial activity is still present. Thus, the possibility of extraterrestrial life present in Europa's hypothetical ocean must be seriously considered (Carr et al., 1998).

#### *Ganymede*

An internal structure similar to that of Europa has been proposed for Ganymede, the largest satellite of Jupiter. However, the model is less documented and appears as less likely (see I.3.2.2).

### **I.2.2.2 The Non-Icy Bodies**

#### *Titan*

Titan, the largest satellite of Saturn, has a dense  $\text{N}_2\text{-CH}_4$  atmosphere rich in organics in both gas and aerosol phases and perhaps hydrocarbon oceans on its surface (Owen et al., 1992; Coll et al., 1996; Gautier & Raulin, 1997). It represents, therefore, a natural laboratory for studying the formation of complex organic molecules on a planetary-scale and over geological times. Despite the fact that Titan's surface temperatures are much lower than those found at the Earth's surface and that liquid water is totally absent, the satellite provides a unique milieu to study, *in situ*, the products of the fundamental physical and chemical interactions driving a planetary organic chemistry. Titan also serves as a reference laboratory to study, by default, the role of liquid water in exobiology.

The NASA/ESA Cassini/Huygens mission will deliver an orbiter to Saturn and Titan and a probe into Titan's atmosphere. The mission, launched in 1997, will arrive in 2004. It will systematically study the organic chemistry in Titan's 'geofluid'. *In situ* measurements will provide detailed analysis of the organics present in the air, in the aerosols and at the surface (Gautier & Raulin, 1997).

#### *The Giant Planets*

The outer-planet atmospheres, from Jupiter to Pluto, contain methane as the main carbon-containing molecules. The atmospheres of the giant planets Jupiter, Saturn and Uranus are mainly composed of hydrogen and helium, with a noticeable fraction of methane and a lower contribution of ammonia (Pollack & Atreya, 1992). The situation is similar for Neptune but since the temperature is lower, the concentration of ammonia must be much lower (Gautier et al., 1995). The recent discovery of HCN in its atmosphere strongly suggests that nitrogen should be present as a main nitrogen atom source (Lellouch et al., 1994). In all these atmospheres, gas-phase organic chemistry is dominated by methane photochemistry. The main products are saturated and unsaturated hydrocarbons. The coupling of methane and ammonia (or nitrogen) photochemistry can produce N-organics. On Saturn, the coupling of methane and phosphine ( $\text{PH}_3$ ) photochemistry could produce HCP.

Although the exobiological interest of these planets is limited, the study of their atmospheric organic chemistries can provide interesting examples of the wide variety of planetary organic processes.

#### *Venus*

Venus may perhaps have been Earth-like in its past, so its early environmental conditions may have been favourable for the emergence of life. Today, Venus is too hostile on the surface to harbour life. The high surface temperature (464°C) and its permanent thick clouds of aqueous  $\text{H}_2\text{SO}_4$  would impede the search for signatures of primitive life and may even have erased the signatures, if any (Colin & Kasting, 1992).

#### *The Moon*

The data collected in 1996 by radio waves beamed from the Clementine spacecraft

into the Moon's surface polar regions suggest that ice most likely exists at the Sun-deprived South Pole. Generally, the rocks and soil scattered the waves, but in that region the waves were bounced back in a coherent pattern by a smooth surface, most likely water ice. The patch of ice is thought to be about 8 m thick and roughly the size of a lake.

The neutron spectrometer aboard Lunar Prospector in 1998 detected high hydrogen concentrations at both poles, a tell-tale signature of the presence of water ice. Although other explanations for the hydrogen enhancement are possible, the data suggest that significant quantities of water ice are located in permanently shadowed craters in both polar regions. Each polar region could contain as much as roughly  $3 \times 10^9$  t of water ice (Feldman et al., 1998). The ice may have arrived as comets that ploughed into the Moon at or near the South Pole, an area that never sees the Sun. The water may then have migrated south into a deep crater at the Moon's darkest part. The presence of ice does not increase the chances of detecting any lunar microbial life, but rather opens the Moon to cost-effective lunar and planetary exploration by providing water for life support and propellant for rockets.

### Mars

The early histories of Mars and Earth clearly show some similarities, as discussed in Chapter II.2. Geological observations collected from Mars orbiters suggest that liquid water was once stable on the surface, attesting to the presence of an atmosphere capable of decelerating carbonaceous micrometeorites. No organic carbon was found in the Martian soil by the Viking 1 and 2 gas chromatography-mass spectrometers. It was concluded that the most plausible explanation for these results was the presence at the surface of highly reactive oxidants such as  $\text{H}_2\text{O}_2$ , which would have been photochemically produced in the atmosphere (Hartman & McKay, 1995). The Viking lander could not sample soils below 10 cm and therefore the depth of this apparently organic-free and oxidising layer is unknown. Bullock et al. (1994) have calculated that the depth of diffusion for  $\text{H}_2\text{O}_2$  is less than 3 m. As discussed in detail in Chapter II.3, the martian meteorites clearly show the presence of organic molecules (Wright et al., 1989; Grady et al., 1994; Grady et al., 1996; McKay et al., 1996), suggesting that the ingredients required for the emergence of primitive life may have been present on the surface of Mars. Therefore, microorganisms may have developed on Mars until liquid water disappeared from the surface. Since Mars probably had no plate tectonics and since liquid water seems to have disappeared from the surface very early, the martian subsurface perhaps holds a frozen record of the very early forms of an Earth-like life. A detailed study is presented in Part II of this volume.

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# I.3 Limits of Life Under Extreme Conditions

From the view point of exobiology, the problems raised by life under ‘extreme conditions’ (extreme from our human point of view) can be considered from two perspectives: what are the most extreme conditions for life to proliferate, and what can life survive (and for how long)? In both cases, we should consider the most extreme terrestrial organisms, and if there might be similar organisms elsewhere in the Universe. These two aspects are often confused, but they are not necessarily related. The first concerns terrestrial organisms living optimally under extreme conditions, the ‘extremophiles’. The second concerns the problem of survival, which is of utmost importance in the search for fossilised life on other planets or for testing the hypothesis of panspermia.

Life on Earth is based on the chemistry of carbon in water. The temperature limits compatible with the existence of life are thus imposed by the intrinsic properties of chemical bonds involved in this type of chemistry at different temperatures. Two requirements are mandatory. Firstly, the covalent bonds between carbon and other atoms involved in the structure of biological molecules should be sufficiently stable to permit the assembly of large macromolecules with catalytic, informational properties or both. Secondly, non-covalent links (hydrogen and ionic bonds, Van der Waals interactions) should be labile. This is a very important point since only weak bonds can allow fast, specific and reversible interactions of biological molecules and macromolecules. These chemical constraints will mainly define the upper and lower temperatures for life, respectively. As we will see, it is known that terrestrial organisms can live in the temperature range from -12°C to 113°C (Fig. I.3.2/1).

## I.3.2.1 High Temperatures

Presently, the maximum temperature limit known for terrestrial organisms is around 113°C. For a long time, the record was 110°C, following the discovery in 1982 of the

## I.3.1 Introduction

## I.3.2 Extreme Temperature Regimes

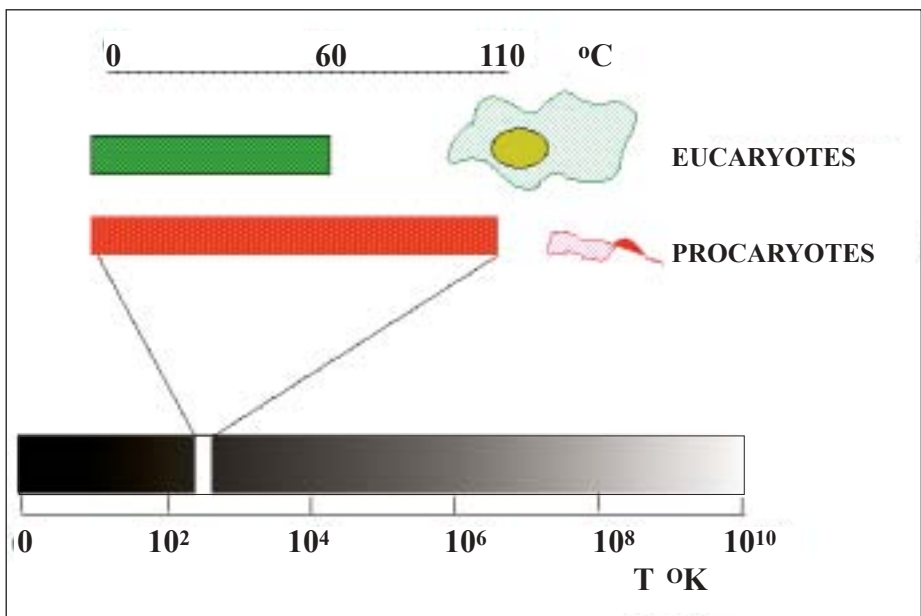
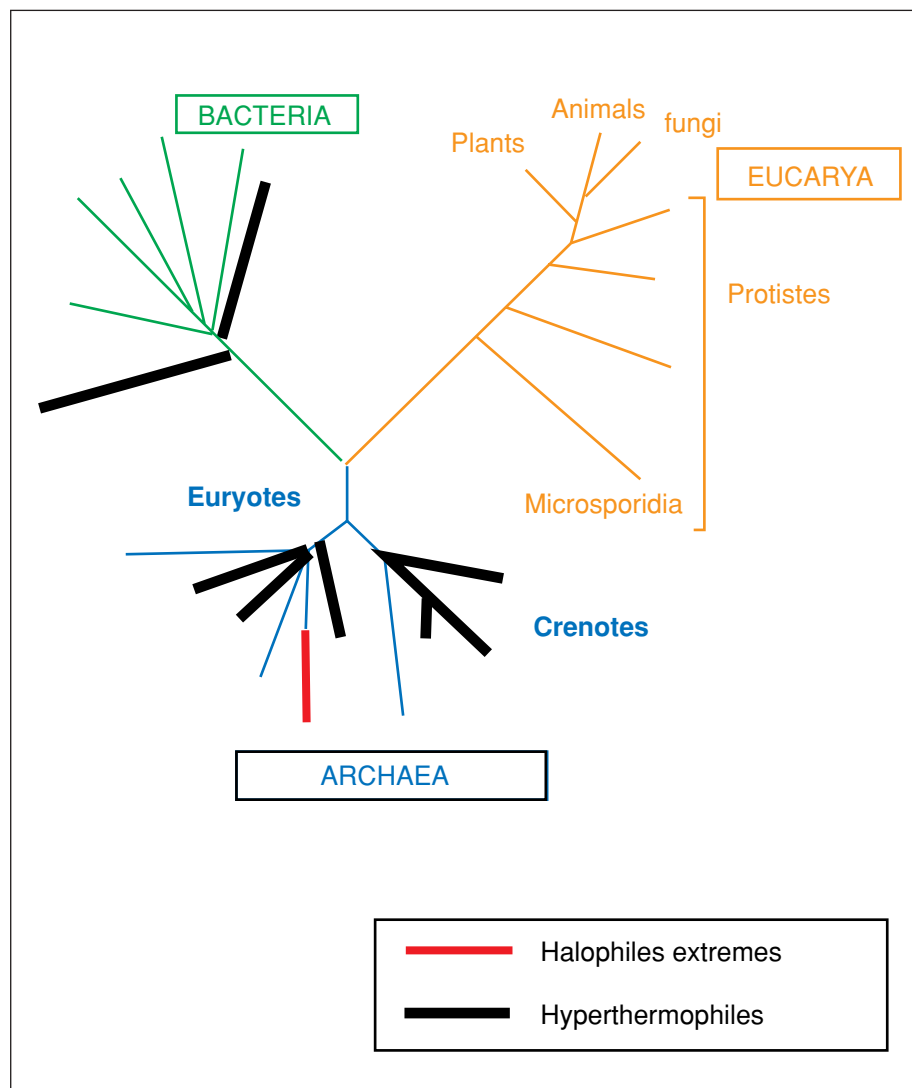


Fig. I.3.2/1. Living organisms thrive within only a small window of temperatures found in the Universe. Eucaryotes, organisms with a nucleus, thrive from around 0°C up to 60°C, while some procaryotes (archaea or bacteria), organisms without a nucleus, can grow at temperatures up to 113°C.

Fig. I.3.2.1/1 Parental relationships between living beings, based on sequence comparison of ribosomal RNA. Extreme halophiles are in red and hyperthermophiles are in black. The short branches of hyperthermophiles indicate that sequences of their rRNA evolve more slowly than similar sequences from organisms living at lower temperatures (mesophiles). This universal tree is usually interpreted as arguing in favour of a thermophilic last universal common ancestor. However, this tree should be regarded with caution. For example, it has been shown recently that microsporidia (represented by *Vairimorpha* and *Encephalitozoon* in the figure) are in fact fungi (yeast). The difference in rate of evolution between the various lineages can indeed produce artifacts.



microbe *Pyrodictium occultum* in shallow water near a beach of Vulcano Island in Italy (Stetter, 1982). This record held for 15 years and was only recently surpassed, although by only 3°C. The new record holder, *Pyrolobus fumarii*, is a deep-sea microbe, again described by Stetter's group (Blöchl et al., 1997). The claim for organisms living at 250°C under a pressure of 350 atm was an artifact, as demonstrated by Trent (1984). It should be stressed that the actual upper limit is, of course, unknown, but the barrier at 110-113°C has so far survived the intensive search in deep-sea vents for organisms growing at higher temperatures.

Many microbes living at temperatures close to or above the boiling point of water, the hyperthermophiles, have been found in all places with volcanic activity, either terrestrial or marine (Stetter, 1996). In particular, there are also large microbial populations at depth within hydrothermal vents, at ocean floor spreading centres, where they exploit the reduced chemicals in the hot vent fluid for energy and growth. They are all procaryotes, i.e. small unicellular microorganisms without a nuclear membrane (chromosomal DNA being directly in contact with the cell cytoplasm). Procaryotes are divided into two phylogenetically distinct domains (or empires): Bacteria and Archaea (Fig. I.3.2.1/1.). Interestingly, the most hyperthermophilic organisms at present all belong to the Archaea, the upper temperature limit for Bacteria being only (!) 95°C (Stetter, 1996).

It should be noted that Archaea living at 110-113°C actually have optimal growth

temperatures of 95-106°C. This suggests that one or several critical factors prevent terrestrial life from proliferating efficiently even at 110°C. This limiting factor cannot be the requirement for liquid water, because there are pressurised environments with liquid water at higher temperatures (the chimneys of hydrothermal vents), but they are sterile (Trent, 1984). An important factor preventing life at temperatures well above 110°C is the thermal instability of some covalent bonds involved in biological molecules.

Proteins can be very stable at high temperatures – some proteins from hyperthermophiles are still active *in vitro* after incubation at 140°C. To achieve such thermostability, evolution has increased the number of non-covalent interactions between amino-acids that maintain the folded structure of the polypeptide chain. They have also managed to get rid of the amino-acid asparagine from their surface – asparagine being rapidly deaminated at such temperature (Hensel et al., 1992). The DNA double-helix is also very stable (at least up to 107°C) as long as the two strands cannot freely rotate around each other, which corresponds to the intracellular situation (Marguet & Forterre, 1994). However, DNA is chemically degraded at high temperature by different mechanisms, the most important being the removal of purine bases (depurination) and the subsequent cleavage of the strands at apurinic sites (Lindahl, 1993). Accordingly, powerful repair mechanisms should exist in hyperthermophiles.

In contrast to proteins and DNA, RNA is highly unstable at high temperatures, because of the presence of a reactive OH group in the 2' position of the ribose moiety that can induce the cleavage of the strands. It has been estimated, for example, that an RNA molecule of 2000 nucleotides (a typical length for a messenger RNA) should be cleaved in two pieces in about 2 s at 110°C (Forterre, 1995). Regions sensitive to heat-destruction appear to be protected in the transfer and ribosomal RNA of hyperthermophiles by methylation of the reactive oxygen. The actual stability of RNA *in vivo* is, however, unclear since thermodegradation is strongly increased by physiological concentrations of magnesium but protected by monovalent salts. This point is under study and is of utmost importance in the problem of life at high temperatures.

Many metabolites are also highly unstable at temperatures near the boiling point of water, in particular the ribonucleotides which contain energy-rich bonds, such as ATP (the energy supplier of the cell) or the substrates for DNA and RNA replication. Some energy-rich covalent bonds are already unstable in the temperature range typical of thermophilic life (80-110°C), but hyperthermophiles have successfully developed original strategies to bypass these limitations. One of them is channelling successive reactions of a given biochemical pathway into a set of physically associated enzymes. Another is to increase considerably the affinity and activity of enzymes dealing with thermolabile substrates.

An important limiting factor preventing life above 110°C could be related to membrane permeability. Biological membranes become leaky at high temperature, allowing the free passage of ions (Driessen et al., 1996). This is not compatible with the formation of ionic gradients across membranes, which is a prerequisite to the building of energy transduction systems in living organisms.

In conclusion, unless future work on subterranean organisms breaks the upper temperature limit (see I.3.6), we are faced with two possibilities: either terrestrial life has been unable in the course of its history to design strategies for living at temperatures above 110°C, or such design is impossible because of the intrinsic limitations of carbon chemistry-based life on Earth.

### **I.3.2.2. Low Temperatures**

Life is extremely diverse in the ocean at temperatures of 2°C. Living organisms, especially microorganisms, are also present in the frozen soils of arctic and alpine environments (Russel, 1992). However, their optimal growth temperatures are usually well above the temperature of the site of isolation. Those organisms with optimal growth temperatures below 15°C and minimal growth temperatures below 0°C are

‘psychrophiles’, while those capable of growth at 0°C but with optimal growth temperatures above 15°C are ‘psychrotrophs’. Psychrotrophs usually outnumber psychrophiles in a given biotope, since they can benefit more efficiently from transient ‘warm’ conditions. For example, bacteria living inside the rocks of dry valleys in Antarctica grow best at temperatures of 10-20°C on rock faces exposed to the Sun in summer (Nienow & Friedman, 1993).

Cold-loving organisms are mainly bacteria (of all types) or eucaryotic micro-organisms. However, putative cold-loving archaea have been detected recently in glacial water from Antarctica by direct amplification of their genetic material (DeLong et al., 1994). Some of the most psychrophilic organisms are algae living in snow-covered areas, where they inhabit the upper 1 cm layer of snow. They often have optimal temperatures below 10°C.

The lower temperature limit for life on Earth is not so clear as the upper limit, because it is very difficult to monitor growth and/or metabolic activity at sub-zero temperatures. As a consequence, very few fundamental studies have been done regarding this problem. In the literature, the lower limit for bacterial growth, -12°C, comes from a 30-year-old paper. It would be valuable to resume such studies. However, -12°C might be correct, because it could correspond to the temperature at which intracellular ice is formed (Russell, 1992). One can speculate that, at lower temperatures, non-covalent bonds become too strong for the kind of reversible reactions required for life to exist.

A major problem faced by cold-loving organisms at very low temperatures is the solidification of their membranes, which risks losing the fluidity required for proper functioning. To bypass this drawback, they usually change their lipid composition in order to increase membrane fluidity at low temperatures. Similarly, the structure of proteins from psychrophilic organisms is modified in order to increase their flexibility such that their activity is optimal at low temperatures compared to mesophilic proteins. The type of modification is somehow a mirror of those occurring in hyperthermophilic proteins, i.e. while proteins from hyperthermophiles are usually more compact than their mesophilic counterparts, homologous proteins from psychrophiles are often less compact with more side-chains loosely connected to the protein core.

Adaptation to low temperatures appears much easier than adaptation to very high temperatures for terrestrial life – whereas hyperthermophily is restricted to certain groups of procaryotes, psychrophily is widespread in all procaryotic and eucaryotic groups. The reason for this difference is unknown, but one can tentatively speculate that it might be due to thermodegradation (breakage of covalent bonds), which is restricted to high temperatures.

### **I.3.3 High-Salt Environments**

A well-studied group of extremophiles are the salt-loving organisms known as extreme halophiles (Galinski & Tyndall, 1992). Monovalent and divalent salts are essential for terrestrial life ( $K^+$ ,  $Na^+$ ,  $Mg^{++}$ ,  $Zn^{++}$ ,  $Mn^{++}$ ,  $Fe^{++}$ ,  $Cl^-$ , etc) because they are required as co-catalysts in many enzymatic activities. In that sense, all organisms are salt-dependent. However, the tolerated salt concentrations are usually quite low (<0.5%) because high salt concentrations disturb the networks of ionic interactions that shape macromolecules and hold together macromolecular complexes. In the case of halophilic microorganisms (both eucaryotes and procaryotes), a wide range of salt concentrations (1-20% NaCl) can be tolerated, and some procaryotes, the extreme halophiles, have managed to thrive in hypersaline biotopes (salines, salted lakes) up to 250-300 g.l<sup>-1</sup> NaCl. They are, in fact, so dependent on such high salt concentrations that they cannot grow (and may even die) at concentrations below 10% NaCl.

Two strategies have been used by halophiles to cope with salty environments (i.e. to prevent the escape of water from the cell). The first strategy, used by eucaryotic algae and most bacteria, is to accumulate in their cytoplasm small organic compounds, known as compatible solutes. In that case, the intracellular machinery of the organism is, in fact, protected from the extreme environment. The second strategy



(mainly found in halophilic archaea) is to accumulate high concentrations of KCl and  $\text{MgCl}_2$  in their cytoplasm (near-saturation). In that case, the intracellular machinery is also in direct contact with the high salt content.

In halophilic archaea, the intracellular machinery is adapted to the high salt concentration of the cytoplasm, corresponding to very low water activity. Proteins from extreme halophiles are not only active at very high salt concentrations, but they are denatured by the removal of salt. A most spectacular experience is to add water to a suspension of halophilic archaea observed under the microscope. The cells vanish as soon as the ionic strength reaches a critical point because membrane proteins dissociate when the salt concentration becomes too low. The mechanism of stabilisation of individual proteins at high salt levels is now beginning to emerge from structural studies. Halophilic proteins exhibit typical networks of negatively-charged amino-acids at their surfaces, allowing them to retain a stabilising network of salt and water molecules. However, the question as to how nucleic acid-protein interaction can occur in 3-4 M KCl remains a major challenge for future work.

The chemistry of life on Earth is optimised for neutral pH. Again, some micro-organisms have been able to adapt to extreme pH conditions, from pH 0 (extremely acidic) to pH 12.5 (extremely alkaline), albeit maintaining their intracellular pH between pH 4 and 9.

Many bacteria and archaea are acidophiles, living at pH values below 4 (for a review see Norris & Ingledew, 1992). The record is held by the archaeon *Picrophilus oshimae*, which grows optimally at pH 0.7 and still grows at pH 0 (Schleper et al., 1995). Like many other acidophiles, this organism is also thermophilic. Among acidophiles, the most thermophilic ones are Sulfolobales (archaea), which can grow at pH 2 and up to 90°C. In many cases, these organisms are actually responsible for the acidity of the biotope in which they live. For example, Sulfolobales are sulphur-respiring organisms and produce sulphuric acid as a by-product of their metabolism.

Many bacteria and a few archaea, the alkaliphiles, live at the other extreme of the pH range, from pH 9 up to pH 12 (for a review see Grant & Horikoshi, 1992). They are present everywhere on Earth. Some of them, which have been discovered in soda lakes rich in carbonates, are also halophiles (haloalkaliphiles). Most alkaliphiles are mesophiles or moderately thermophilic, but Stetter and co-workers have recently described the first hyperthermophilic alkaliphile, *Thermococcus alkaliphilus* (Keller et al., 1995).

Both acidophiles and alkaliphiles rely on sophisticated transport mechanisms to maintain their intracellular pH near neutrality by pumping or excreting protons. As in the case of moderate halophiles, they protect their intracellular environment against the external extreme values of this parameter. They should be also able to maintain adequate gradients of protons or  $\text{Na}^+$  to sustain their energy-producing machinery. Among them, the thermophiles also have to deal with the specific problem of maintaining the correct membrane permeability in an unbalanced ionic environment. This may explain why the upper temperature limits for acidophiles and alkaliphiles is presently 90°C and not 110°C (it might be significant that the upper pH limit of *Thermococcus alkaliphilus* is only pH 9.5).

As with temperature, the intracellular machinery cannot escape the influence of pressure. However, there are organisms in the deepest parts of the ocean (pressure 1100 bar). The extreme pressure limit for life on Earth is unknown – environments of above 1100 bar have not been explored. However, it might be quite high, because macromolecules and cellular constituents apparently only begin to denature at 4-5000 bar.

Some microorganisms living in the deep ocean can be obligate barophiles, but most of them are only barotolerant. In fact, it is not yet clear if barophiles have had

### I.3.4 Acidic and Alkaline Environments

### I.3.5 High-Pressure Environments



to invent specific devices to adapt to high pressure (for review, see Prieur, 1992). High pressure seems to increase the growth rate of some hyperthermophiles, but this phenomenon is not general and not very spectacular. Although some proteins from hyperthermophiles are more active at high pressure, high pressure does not increase the thermal stability of macromolecules (Bernhardt et al., 1984).

### I.3.6 Subterranean Life

For a long time, it was believed that deep subterranean environments were sterile. It has now been recognised that bacteria (and probably archaea, too) actually thrive in the terrestrial crust. Subterranean microorganisms are usually detected in subterranean oil-fields or in the course of drilling experiments (Parkes & Maxwell, 1993; Parkes et al., 1994; Stevens & McKinley, 1995). For example, recent research conducted within the International Ocean Drilling Program (ODP) has demonstrated that procaryotes are present much deeper in marine sediments than was previously thought possible, extending to at least 750 m below the sea floor, and probably much deeper (Parkes et al., 1994). These microbial populations are substantial (e.g.  $10^7$  cells  $\text{cm}^{-3}$  at 500 m below sea floor) and likely to be widespread. To depths of at least 432 m, microbes have been identified as altering volcanic glass, which comprises a substantial volume of the volcanic component of the ocean crust (Furnes et al., 1996), and may have significance for chemical exchange between the oceanic crust and ocean water. These data provide a preliminary, and probably conservative, estimate of the biomass in this important new ecosystem: about 10% of the surface biosphere. These discoveries have radically changed our perception of marine sediments and indicate the presence of a largely unexplored deep bacterial biosphere that may even rival the Earth's surface biosphere in size and diversity!

Elevated procaryotic populations and activities are also associated with gas hydrates. This, together with the presence of large microbial populations in oil reservoir fluids (Stetter et al., 1993; Haridon et al., 1995; Bernard, et al., 1992; Rueter et al., 1994), suggests that deep bacterial processes may even be involved in oil and gas formation by comparison with the key catalytic role of near-surface procaryotic communities. Procaryotes are also likely to drive deep geochemical reactions such as mineral formation and dissolution, be responsible for the magnetic record via production of magnetic minerals, produce deep methane gas essential for hydrate formation.

Procaryotes in the deep biosphere must be uniquely adapted to live in this extreme environment, and populations have even been shown to increase at depth (>200 m) as they exploit deep geochemical fluxes such as geothermal methane and brine incursions.

Heterotrophic subterranean communities use either remnant organic carbon deposited with sediments or dissolved oxygen as a metabolite terminal electron acceptor. They are thus actually indirectly dependent on photosynthetic activity. Hence they can become extinct when their nutrients are exhausted. However, evidence has also been obtained for the existence of anaerobic subsurface lithoautotrophic microbial systems in basalt aquifers (Stevens & McKinley, 1995). These communities apparently derived their energy from geochemically-produced hydrogen. Because the energy sources and inorganic nutrients are both supplied by geochemical means, such a subsurface lithoautotrophic microbial ecosystem (SLiME) could *a priori* persist indefinitely even if the conditions on the Earth's surface became alien to life.

Since the temperature increases with depth, it has been suggested that hyperthermophiles, in particular chemiolithoautotrophs, are abundant in subterranean environments, forming a deep hot biosphere (Gold, 1992). Indeed, procaryotic activity has even been reported up to 120°C and possibly even higher (Cragg & Parkes, 1994), i.e. above the upper temperature limit defined in the laboratory. The challenge now is to cultivate these putative novel hyperthermophiles. Up to now, all microbes sampled from subsurface environments have turned out to be close relatives of well-known bacteria, but these studies have been rather limited and one

cannot exclude finding really novel microbes in these relatively unexplored biotopes.

These deep communities clearly suggest that life may also exist deep below the surfaces of other planets. Hence, we need to build this into any assessment of extraterrestrial life, as conditions for life may actually improve below the surface (e.g. Mars). The absence of surface life, therefore, may not necessarily indicate the absence of all life. Indeed, it seems rather naïve to limit the search for life to a planet's surface when we already know that the conditions are far beyond the possible ranges for life on Earth.

The revival of microorganisms from ancient rocks, salt and coal has been reported many times. There are several claims of microorganisms being revived from rocks more than 100 millions years old, some even from Precambrian (650 millions years old) (for an exhaustive review see Kennedy et al., 1994). These claims have usually been disputed on various grounds (contamination, theoretical impossibility) but Kennedy et al. concluded that the observations are too many to be dismissed en bloc. In particular, the US Department of Energy has established a collection of 5000 revived microorganisms (bacteria and fungi) from various subsurface sites about 200 million years old. For Kennedy et al. the best alternative to revival is *in situ* reproduction, which is as interesting as real revival from the perspective of recovering ancient living forms on Mars or other planets.

Apart from the availability of resources, the balance between latent life and *in situ* reproduction in subsurface biotopes should be dependent on temperature effects: *in situ* reproduction being favoured at high temperatures and latency at low temperatures. Microorganisms in general, and even some macroorganisms, are extremely resistant to freezing temperatures. It is well known that microorganisms are routinely kept alive for years in liquid nitrogen. Indeed, at extremely low temperatures, most deleterious processes linked to metabolic activities that can impair survival are slowed down.

Many (but not all) of the microorganisms revived from ancient material are spore-formers. Bacteria of the Gram positive kingdom (one out of the roughly 12 bacterial kingdoms), have indeed developed the ability to produce extraordinarily resistant spores that can survive extremely harsh conditions (high temperature, absence of nutrients, high doses of radiation). Spores are also produced by fungi and plant minute seeds, and protozoan cysts can be considered as kinds of spores.

Concerning the problem of latency, a critical point would be the stability of DNA molecules under very long-term storage, and the possibility for the organism to repair the lesions introduced during the dormant stage, once the conditions again become favourable. Indeed, even in the absence of UV or ionising radiation, DNA is subjected to spontaneous chemical modifications such as depurination, cytosine deamidation and hydrolysis of the phosphodiester bond (Lindahl, 1993). These reactions are very slow at low temperatures but can still produce significant damage in the very long-term. This point has been raised against the possibility of revival for organisms several millions year old.

DNA in spores is protected by specific DNA binding proteins that prevent depurination and probably other damage (see below), but it is not clear how effective this protection can be over very long periods. It is interesting to note that high salt concentrations protect DNA against chemical modifications (Marguet & Forterre, 1994). Thus, halophilic organisms might have more chance to survive, and/or for their DNA to remain intact (for analyses), than non-halophilic ones. The best conservation effect would thus be achieved for frozen extreme halophiles.

Natural radioactivity in rock might be also a problem for long-term DNA stability. However, some microorganisms are extremely radio-resistant. This can be a secondary adaptation to dessication that produce DNA lesions leading to double-stranded breaks when the cells are again in contact with water. The radio-resistant bacterium *Deinococcus radiodurans* exhibits a very efficient repair-recombination mechanism that allows the cell to reconstruct intact chromosomes from deficient ones (Smith et al., 1992).

### I.3.7 Survival of Lifeforms in Space

In order to study the survival of resistant microbial forms in the upper atmosphere and free space, microbial samples have been exposed *in situ* aboard balloons, rockets and spacecraft, such as Gemini, Apollo, Spacelab, Long-Duration Exposure Facility (LDEF), Foton and Eureka, and their responses investigated after recovery (reviewed in Horneck, 1993). *A priori*, the environment in space seems to be very hostile to life. This is due to the high vacuum, an intense radiation of galactic and solar origin, and extreme temperatures. In the endeavour to disentangle the network of potential interactions of the parameters of space, methods have been applied to separate each parameter and to investigate its impact on biological integrity, applied singly or in controlled combinations.

#### *Responses to Vacuum*

In free interplanetary space, pressures down to  $10^{-14}$  Pa prevail. The pressure increases in the vicinity of a body because of outgassing. In low Earth orbit, where most of the experiments were done, pressure reaches  $10^{-4}$ - $10^{-6}$  Pa. Experiments in space have demonstrated that certain microorganisms survive exposure in space vacuum for extended periods of time, provided they are shielded against the intense solar UV radiation. Most results are available from spores of the bacterium *Bacillus subtilis*. If shielded against UV, spores survive at least 6 years in space (Horneck et al., 1994). The survival time is substantially increased if the spores are exposed in multiple layers and/or in the presence of glucose as a protective. In the surviving spores, the genetic material is affected, as indicated by an increased mutation rate, delayed germination, crosslinking of DNA and protein, DNA strand breaking and the requirement of cellular repair processes to restore viability. This cellular damages is probably caused by the dehydration of the spores in vacuum. With increasing exposure time, free water is removed first, followed by the hydrated water and, finally, even chemically bound water and other volatile molecules may be removed. This has severe consequences on the stability and functionality of the membranes and macromolecules.

#### *Responses to Solar UV Radiation*

The spectrum of solar electromagnetic radiation spans several orders of magnitude, from short-wavelength X-rays to radio frequencies. At the distance of the Earth (1 AU), solar irradiance amounts to  $1360 \text{ Wm}^{-2}$  (the solar constant). Of this radiation, 45% is attributed to IR, 48% to visible and only 7% to the UV range. Solar UV radiation has been found to be the most damaging factor of space as tested with dried preparations of viruses, bacterial and fungal spores (Horneck, 1992, 1993; Horneck et al., 1994). Action spectra of the solar photons at 160-320 nm for killing bacteriophage T1 or *B. subtilis* spores closely correlate with the absorption spectrum of DNA, indicating DNA as the critical chromophore for lethality. The incidence of the full spectrum of solar UV light ( $>170 \text{ nm}$ ) kills 99% of *B. subtilis* spores within seconds. To have the same effect on Earth, the exposure to sunlight takes approximately 1000 times longer. This difference is attributed to the ozone layer that protects the biosphere from the most harmful fraction of solar UV radiation ( $<295 \text{ nm}$ ). Using a UV-monitoring system in space, consisting of dry monolayers of immobilised spores of *B. subtilis* and an optical filtering set to simulate ozone columns of different thickness, the sensitivity of the biologically-effective irradiances to ozone from a normal stratospheric ozone column down to its complete loss, the real 'ozone hole' was experimentally demonstrated (Horneck et al., 1996). It showed a strong increase in biologically-effective solar UV irradiance with decreasing (simulated) ozone concentrations. A complete loss in ozone (full spectrum of extraterrestrial sunlight) leads to an increment of the biologically effective irradiance by nearly three orders of magnitude compared to standard stratospheric ozone values on the surface of the Earth. If spores of *B. subtilis* are simultaneously exposed to solar UV radiation and space vacuum, they respond with increased sensitivity to a broad spectrum of solar UV ( $>170 \text{ nm}$ ), as well as to selected wavelengths. This is due to the generation of specific photoproducts in the DNA of microorganisms exposed to UV light in vacuum (Horneck, 1993).

### *Responses to Cosmic Radiation*

The radiation field of our Solar System is governed by components of galactic and solar origin. The galactic cosmic radiation entering our Solar System is composed of protons (85%), electrons, alpha-particles (14%) and heavy ions (1%) of charge  $Z > 2$  (HZE particles). The solar particle radiation, emitted as the solar wind and during solar flares, comprises 90-95% protons, 5-10% alpha-particles and a relatively small number of heavier ions. In the vicinity of the Earth, in the radiation belts, protons and electrons are trapped by the geomagnetic field. Among the ionising components of radiation in space, the heavy primaries (HZE particles) are the most effective species. To understand how cosmic particle radiation interacts with biological systems, methods have been developed to localise precisely the trajectory of an HZE particle relative to the biological object and to correlate the physical data of the particle relative to the observed biological effects along its path. By use of visual track detectors sandwiched between layers of biological objects in a resting state – a concept known as the Biostack (Buecker & Horneck, 1975) – in a variety of test systems including viruses, bacterial spores, plant seeds or shrimp cysts, injuries such as somatic mutations in plant seeds, development disturbances and malformations in insect and salt shrimp embryos, or inactivation in bacterial spores were traced back to the traversal of a single HZE particle (reviewed in Horneck, 1992). Such HZE particles of cosmic radiation are conjectured as setting the ultimate limit on the survival of spores in space because they penetrate even heavy shielding. The maximum time for a spore to escape a hit by an HZE particle (e.g. iron of LET  $> 100$  keV/m) has been estimated to be  $10^5$ - $10^6$  years.

### *Responses to Temperature Extremes*

The temperature of a body in space – which is determined by the absorption and emission of energy – depends on its position relative to the Sun and its surface type, size and mass. In Earth orbit, the energy sources are solar radiation ( $1360 \text{ Wm}^{-2}$ ), Earth albedo ( $480 \text{ Wm}^{-2}$ ) and terrestrial radiation ( $230 \text{ Wm}^{-2}$ ). In Earth orbit, the temperature of a body can reach extreme values. During the major part of a hypothetical journey through deep space, if shielded from solar thermal radiation, microorganisms are confronted with the 4K cold emptiness of space. Under these very cold conditions, thermodynamic and chemical reactions are nearly frozen. The photobiological response to solar UV radiation may then be completely different from room-temperature conditions. Only the latter response (at room temperature) so far has been tested in space. Laboratory experiments under simulated interstellar medium conditions point to a remarkably less damaging effect of UV radiation at these low temperatures. Treating *B. subtilis* spores with three simulated factors simultaneously (UV  $> 110$  nm, vacuum and low temperature of 10K), produces an unexpectedly high survival rate, even at very high UV fluxes. In this low temperature regime, the inactivation cross-sections obtained are up to 2-3 orders of magnitude lower than at room temperature (Weber & Greenberg, 1985). The temperature profile of bacterial spore UV-sensitivity shows a maximum at 190K. From these data, it has been estimated that, in the most general environment in space, spores may survive for hundreds of years (Weber & Greenberg, 1985).

### *Chances and Limits of Interplanetary Transfer of Life*

The recent analyses of the martian meteorite ALH 84001, with its putative indications of enclosed fossils (see Chapter II.3), has initiated increased interest in the question of whether endolithic microorganisms can survive an interplanetary journey. Although it will be difficult to prove that life can be transported through our Solar System, the chances for the different steps of the process to occur can be estimated. These include (1) the escape process, i.e. the removal to space of biological material that has survived being lifted from the surface to high altitudes; (2) the interim state in space, i.e. the survival of the biological material over timescales comparable with the interplanetary passage (3) the entry process, i.e. the non-destructive deposition of the biological material on another planet. Following the identification of some

meteorites of lunar and probably some of martian origin, the escape of material ranging from small particles up to boulder-size from a planet after the impact of large meteorites is evidently a feasible process. It is interesting to note that bacterial spores can survive with low frequency (approximately  $10^{-4}$ ) shockwaves produced by a simulated meteorite impact of 42.5 GPa. Concerning the survival during the interim state in space, 6 years is so far the maximum observed exposure time of bacterial spores to space. The high survival rate of these spores and the high UV-resistance of microorganisms at the low temperatures of deep space are interesting results. However, travelling from one planet to another, e.g. from Mars to Earth, by chance requires an estimated mean time of several  $10^5$ - $10^6$  years for boulder-sized rocks; periods of only a few months have been calculated for microscopic particles. Evidently, more data on the long-term effects in space are required to allow meaningful extrapolation to the time spans required for the interplanetary transport of life.

### **I.3.8 Implications for Exobiology in Future Searches**

When asking what lessons extremophiles and survival in the extreme teach us about possible extraterrestrial biotopes and our chances of identifying relic or active extraterrestrial life in the Solar System, we should make the distinction between the conditions required for the emergence of life from those for its evolution and maintenance.

In some theoretical scenarios, life appeared at very high temperatures. That means today's hyperthermophiles are viewed as relics of the last common universal ancestor of all living beings (Stetter, 1996). In that case, the origin of life would have required the presence of stable warm biotopes at an early stage of planetary evolution. However, this hot origin of life hypothesis has been challenged, based on the fact that early life on Earth was probably based on RNA instead of DNA, and RNA is very unstable at high temperatures (Forte, 1995).

The hot-origin-of-life scenario is mainly based on the grouping of hyperthermophiles at the base of the universal tree of life deduced from rRNA analysis, on each side of its root (Stetter, 1996). However, the rooting of the universal tree has been disputed (Forte, 1997) and recent data indicate that rRNA phylogenies can be very misleading. In particular, it is now clear that microsporidia (eucaryotes without mitochondria), which were supposed to be the most primitive eucaryotes according to rRNA phylogeny, are in fact fungi (see reference in Palmer, 1997).

Even if life did not originate at very high temperatures, the production of efficient catalysts at freezing temperatures might have been an impossible task at an early stage of evolution. The most attractive hypothesis might be that life appeared in a moderately thermophilic environment: hot enough to boost catalytic reactions, but cold enough to avoid the problem of macromolecule thermodegradation.

It is important to obtain new knowledge about hyperthermophiles to help us define the optimal conditions for the origin of life and estimate the plausibility of such conditions in the histories of Solar System planets. In particular, it should be kept in mind that our knowledge of the mechanisms required for life at high temperatures are far from exhaustive. It might be important to understand thoroughly why there is apparently an upper limit of 113°C for life on Earth. Is it possible to imagine alternative biochemistry active at higher temperatures?

Another possibility is that life did not invent strategies to proliferate above 110°C, where the temperature gradient in a hydrothermal chimney is very steep, reducing the size of the potential biotopes with a constant 110-130°C. The systematic search for subterranean hyperthermophiles in the deep terrestrial crust could be most interesting in this respect, since one expects a gradual transition of temperature on a large geographic scale.

The study of microorganisms living in subterranean environments can also teach us much about the effect of pressure on living organisms. This is of great importance as many possible biotopes in the Solar system experience either very low or very high pressures. Fundamental studies about the effects of pressure on enzymes and nucleic



acid to identify the theoretical limits of pressure for life are thus an important line for future research.

Finally, the study of subterranean biotopes may be relevant to the problem of the origin of life in general. It may have been that life originally developed on Earth below the surface and then grew upwards when conditions became less extreme in the early Archean period. If this is correct, life might have appeared on several other Solar System bodies in the appropriate temperature zone at the boundary between external cold and internal heat (see Chapter I.3.2).

What can we learn from the biology of terrestrial extremophiles? An important observation is that most terrestrial extremophiles are procaryotes. This is especially striking in the case of thermophiles and hyperthermophiles, since all microorganisms living at 60-110°C are procaryotes. The reason for this discrimination is unknown. It could be related to the requirement for long-lived messenger RNA in eucaryotes (Forterre, 1995), but this is a hypothesis. Why only archaea thrive above 95°C is also still a mystery. More work is needed to understand these different frontiers. On the other hand, we have seen that all terrestrial present-day extremophiles, although procaryotes, are complex microorganisms, the products of long evolution, which exhibit elaborate mechanisms for coping with their extreme environments. For example, terrestrial hyperthermophiles have developed strategies to protect their macromolecules (proteins, DNA and RNA) against the damaging effects of very high temperatures (Forterre, 1996). Similarly, the two strategies used by halophiles to cope with salty environments (production of compatible solutes and accumulation of high intracellular salt concentrations) require the presence of very effective transport systems across membranes and/or complex metabolic pathways to synthesise compatible solutes, two hallmarks of highly evolved organisms. This again suggests *a priori* that life, as we know it, probably evolved first in a mild environment before invading extreme ones. However, would it be possible to imagine simpler and even more robust extremophiles?

The terrestrial dichotomy between procaryotes and eucaryotes raises other interesting questions. For example, do all life forms on other planets automatically extend at least up to 110°C or is this expansion dependent on the appearance of a procaryotic lifestyle? The answer to this question requires us to define *a priori* what is the nature of procaryotes. Traditionally, procaryotes are considered to be ancient and primitive forms of life on Earth. In such a scenario, the procaryotic type of cellular organisation is often viewed as an early stage of life evolution, which should have been common to all life, consequently making it the most widespread life form in the Universe (hence the search for martian bacteria). However, some authors consider that procaryotes are very evolved organisms that originated by reduction from more complex forms of life (Reaney, 1974; Forterre, 1995). In that case, the presence of life in many extremophilic environments could be dependent on the historical appearance of 'procaryotes'. It might be important to determine which is correct in order to define more precisely what we are looking for in other planets. In any case, we should not consider the procaryote/eucaryote dichotomy as valid for all possible life in the Universe. Simpler, different or more complex forms could exist. Finally, we are probably ignoring some of the capabilities of terrestrial extremophiles. It might be worth challenging them with some environmental conditions deduced from our knowledge of planetary atmospheres, hydrospheres and lithospheres. Surprises could emerge from such studies.

We have suggested that life might have appeared on planets only within a narrow spectrum of physical parameters and that, once life existed, these requirements could be relaxed (but not too much) for evolved life forms to proliferate. However, if these conditions changed drastically in some direction (freezing, desiccation), living organisms could survive (waiting for better times) for very long periods, possibly on an astronomical scale. Question marks predominate in this area of research. How long can extremophiles or resistant forms survive in hostile conditions? Many aspects of this problem need further investigations. Some of them have been reviewed by Kennedy et al. (1994). What is the migration rate of microorganisms through a rock



structure? For how long can nucleic acids survive long-term storage? How do we design new methods to assess the antiquity of 'revived' terrestrial microorganisms? How do we study bacterial reproduction in very old samples?

We should keep very open minds in this area. In terms of survival, one immediately think of spores, but we should remember that sporulation is not a general phenomenon of the living, but an historical invention that may or may not have occurred in the history of life on other planets. Furthermore, we keep our microorganisms frozen for years and, though they are not spores, they survive. We also tend to think of procaryotes, but some eucaryotes (acaria) could exhibit surprising (and scarcely explored) resistant properties.

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# I.4 Morphological and Biochemical Signatures of Extraterrestrial Life: Utility of Terrestrial Analogues

Any veneer of life covering the surface of a planet will likely interact with the solid and fluid phases with which it is in contact. Specifically, it is bound to impose a thermodynamic gradient on all planetary near-surface environments (inclusive of the atmosphere and hydrosphere), which ultimately stems from the accumulation of negative entropy by living systems. Consequently, life acts as a driving force for a number of globally-relevant chemical transformations. On Earth, typical examples of such life-induced chemical inequilibria are the glaring redox imbalance at the terrestrial surface caused by photosynthetic oxygen, or the release of large quantities of hydrogen sulphide by sulphate-reducing bacteria in the marine realm. Also, the dynamic persistence of metastable atmospheric gas mixtures (such as  $O_2$ ,  $N_2$  and  $CH_4$  in the terrestrial atmosphere), and of isotopic disequilibria (e.g. between water-bound oxygen of the hydrosphere and atmospheric  $O_2$ ), is ultimately sustained by the thermodynamic imbalance imposed by the biosphere on its environment. Conspicuous thermodynamic inequilibria within the gaseous and liquid envelopes of a planet may, therefore, be taken as *a priori* evidence of the presence of life (cf. Hitchcock & Lovelock, 1967; Lovelock, 1979). Applying this criterion to the present composition of the martian atmosphere (Owen et al., 1977), the latter gives little, if any, indication of contemporary biological activity.

Moreover, a planetary biosphere is apt to leave discrete vestiges in the surrounding inorganic habitat. Relying on terrestrial analogues, it is safe to say that organisms commonly generate a morphological and biochemical record of their former existence in sedimentary rocks. Though in part highly selective, this record may survive, under favourable circumstances, over billions of years before being annealed during the metamorphic and anatexis reconstitution of the host rock. This is particularly true for relics of multicellular life (Metaphyta and Metazoa), but also – albeit with restrictions – for microorganisms that held dominion over the Earth during the first 3000 million years of recorded geological history.

In the following, an overview is presented of the principal morphological and biogeochemical evidence indicating the presence of life on a planetary surface. Section 4.2 summarises this evidence for an extant biosphere, while Section 4.3 reviews the respective signatures of fossil life (with special reference to the oldest terrestrial record because Early Martian scenarios should have largely resembled those on the Archaean Earth).

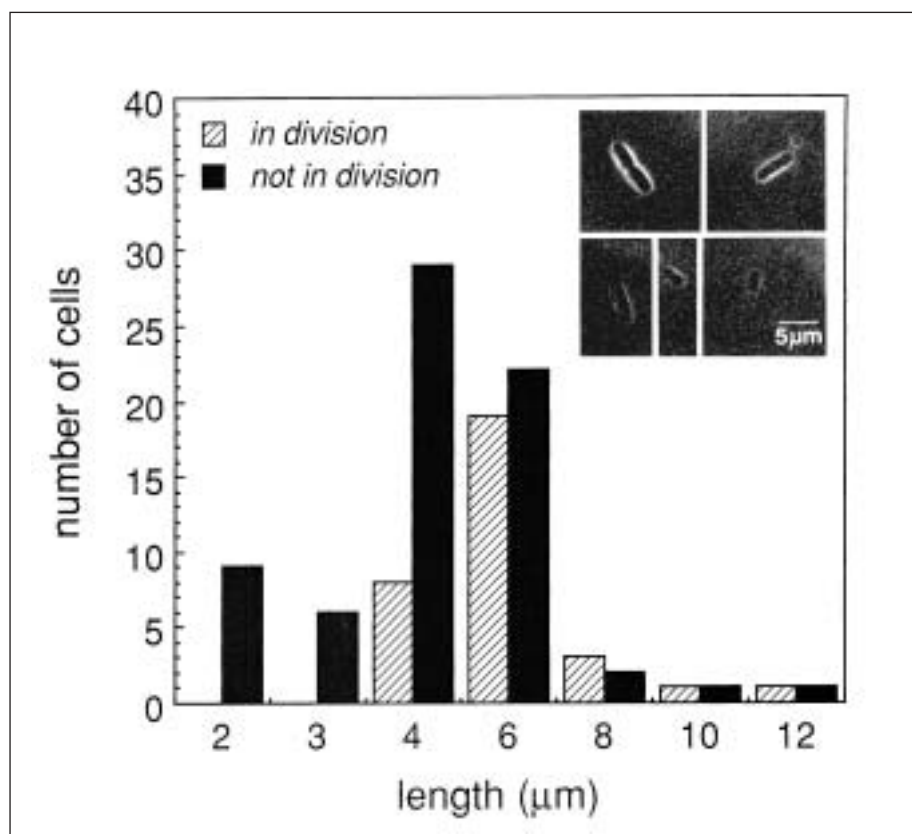
## I.4.2.1 The Microbial World

Microbial prokaryotes have flourished on Earth for more than 3.5 Gyr. They dominated the Earth's biosphere during the first 2 Gyr of its history before the first unicellular mitotic eukaryotes appeared. Therefore, in a search for extant life beyond the Earth, microorganisms are the most likely candidates for the biota of an extra-terrestrial habitat. Structural, functional and chemical characteristics that have been frequently used to identify terrestrial microbial communities are discussed below. This includes signatures for actively metabolising or even proliferating lifeforms (active state) as well as for resting lifeforms, such as dehydrated or frozen organisms or bacterial spores (dormant state). Special attention is given to methods to detect microbial biota of extreme environments, such as hot vents, permafrost, permanent ice, subsurface regions, high atmosphere, rocks and salt crystals. These environmental extremes may be considered as terrestrial analogues of possible niches on Mars and on other selected planets and moons of our Solar System.

## I.4.1 Introduction

## I.4.2 Evidence of Extant Life

Fig. I.4.2.2/1. Size distribution of unicellular cyanobacteria observed by phase-contrast microscopy in an evaporite (from Rothschild et al., 1994).



The most commonly used direct methods to detect and analyse microbial communities in extreme environments include:

- Direct observations of structural characteristics in the micro- and macroscale;
- Culture techniques for isolating microorganisms in pure culture and then analysing these cultures for their biochemical properties;
- Activity measurements in microcosms focusing on the net effects of microbial processes in the community;
- Chemical marker techniques to record characteristic biochemical primary substances or chemical secondary products.

Based on the fact that, during its >3.5 Gyr history, life on Earth has substantially modified the terrestrial lithosphere, hydrosphere and atmosphere, indirect proofs of life will also be considered. The applicability of these methods in a search for life at extraterrestrial sites are discussed later.

#### I.4.2.2 Structural Indications of Life

Cells and subcellular structures can be detected directly by microscopy. Microbial cells have been observed in samples collected from natural environments, such as smears of soil or subsurface aquifer sediments, droplets taken from hydrothermal vents or brine, airborne microorganisms trapped on a sticky surface, or thin fractures or slices of rocks or salt crystals (evaporites). Most of them are sphere-, ovoid- or rod-shaped objects of one or a few microns in size. Fig. I.4.2.2/1 shows the size distribution of a unicellular cyanobacterium observed by phase-contrast microscopy of the coloured layers of an evaporite (Rothschild et al., 1994). The direct microscopical observation also allows us to distinguish between dividing and non-dividing cells (Fig. I.4.2.2/1). The observation of dividing cells, i.e. paired cells or cells in chains, would indicate that the cells were actively growing *in situ* immediately before or during the sampling procedure; this is direct evidence of how microbes interact with their environment.



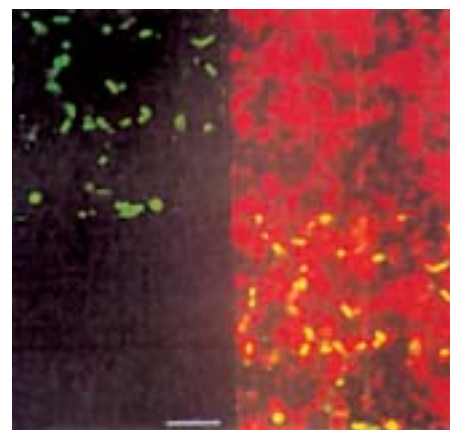
To differentiate between cellular structures and similar structures of abiotic origin, e.g. soil particles or aerosols, dyes have been used that specifically bind to subcellular components (e.g. acridine orange binds with cellular nucleic acids to form a fluorescent dye). When excited by UV light, the nucleic acid-acridine orange complex emits visible light which can be observed by fluorescence microscopy (Chapelle, 1993). However, it should be pointed out that acridine orange may also bind with mineral or organic particles in the sample and that a clear-cut identification of cellular structures requires experience and skill.

Recently, confocal laser scanning microscopy (CLSM) has been introduced to stone ecology to obtain a 3D visualisation of stone-inhabiting microbial communities (Quader & Bock, 1996; Bartosch et al., 1996). Signals from the plane of focus of an object contribute to the information of an image. By sequentially moving the microscopic stage, series of optical sections can be obtained and calculated into a 3D image. This CLSM method allows to visualise microorganisms in transparent stones (e.g. quartzite) over a depth of approximately 200  $\mu\text{m}$ . Fig. I.4.2.2/2 shows CLSM images of sandstone after staining with acridine orange (Quader & Bock, 1996). The green fluorescent bacteria are easily distinguishable from the red fluorescent clays.

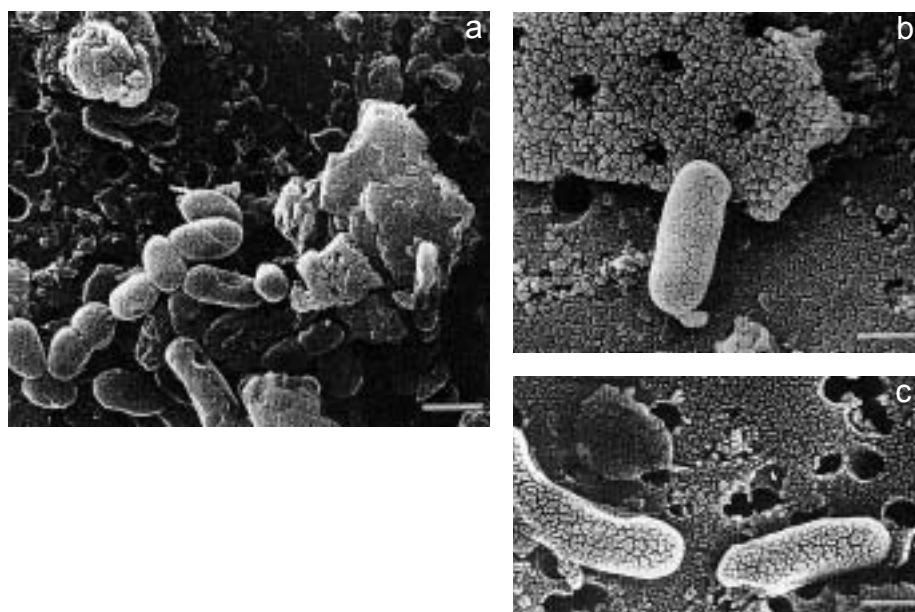
CLSM gives a snapshot inside the stone matrix of the resident microbial community which consists of microorganisms of different sizes and shapes occurring also in micro-aggregates or microcolonies composed of from several up to hundreds of cells.

To observe further minute details of the cells, which are not visible in light microscopy, such as cell walls, membranes and vacuoles, scanning electron microscopy (SEM) and transmission electron microscopy (TEM) have been applied. These methods provide further details on the structure of subcellular components, e.g. gram-positive or gram-negative cell walls, and the occurrence of inclusions typical for a metabolic pathway, such as the energy-storing compound polybeta-hydroxybutyrate (Chapelle, 1993). Fig. I.4.2.2/3 shows a series of scanning electron micrographs of microorganisms within ancient layers of the Antarctica ice sheet at depths of 1.6-2.4 m (Abyzov, 1993).

Large microbial communities are also macroscopically visible. Examples are found in evaporites of up to 4 cm in thickness which contain one or two horizontal coloured bands several mm in thickness well below the surface (Rothschild et al., 1994). These layers appear in different colours, from tan to pink-brown to green or green-grey within a basically white salt matrix (Fig. I.4.2.2/4). Similar signatures have been observed for cryptoendolithic microbial communities colonising sandstone



**Fig. I.4.2.2/2.** Confocal laser scanning microscopy (CLSM) of microorganisms in green sandstone after staining with acridine orange. Upper left: green fluorescent bacteria; upper right: red fluorescent clay; lower right: combination of both pictures, now the bacteria appear yellow. Bar = 5  $\mu\text{m}$  (from Quader & Bock, 1996).



**Fig. I.4.2.2/3.** Scanning of ice-core samples from ancient layers of the Antarctic ice sheet at depths of 1.6 m (a) and 2.4 m (b and c). The bar represents 1  $\mu\text{m}$ . (From Abyzov, 1993).



**Fig. I.4.2.2/4. Evaporite with horizontal bands of endoevaporitic microbial communities (from Rothshild et al., 1994).**

in cold and hot deserts (Friedmann, 1982; Nienow & Friedmann, 1993). The colonised zone below the rock crust reaches up to 10 mm deep and is composed of distinct parallel bands of black, white, green and blue-green. Throughout the colonised zone, the brown iron oxyhydroxide stain is leached out, probably by biogenic oxalic acid, and the quartz appears colourless. It is interesting to note that both the endoevaporitic and endolithic communities are found living embedded within a few mm of the surface mineral matrix. Solar irradiance is attenuated by almost one order of magnitude by the overlying mineral layers. Hence, endoevaporitic and endolithic communities obviously have conquered an ecological niche that allows microorganisms to live in an otherwise hostile environment.

Desert crusts, formed by microorganisms, may cover even larger areas of hundreds of square metres with a thickness of a few cm (Campbell, 1979). These mats, dominated by cyanobacteria, show stromatolitic features, such as sediment trapping and accretion, a convoluted surface and polygonal cracking. Sand and clay particles are trapped within a network of filamentous cyanobacteria which secrete mucous sheets to which the particles adhere. Such desert crusts are observed on rocks or soil in areas of flat topography. Comparable large areas of stratified mats of microbial communities are also observed in evaporite flats of hypersaline lagoons, such as the Laguna Figueroa on the Pacific coast of the Baja California (Stolz & Margulis, 1984; Stolz, 1985) and a host of related habits.

#### **I.4.2.3. Evidence of Microbial Activity as a Functional Characteristic of Life**

The most unequivocal indication of microbial life is obtained by the isolation of microorganisms in pure culture from a sample under investigation, followed by a detailed analysis for their biochemical properties. Microbial isolates have been obtained from diverse extreme habitats. Examples are deep crystalline rock aquifers several hundreds of metres below the surface (Stevens & McKinley, 1995), the interiors of ice-cores from drillings in the Antarctic ice down to a depth of several thousand metres (Abyzov, 1993) and cores from drillings in permafrost regions in



Siberia at similar depths (Gilichinsky et al., 1993). It was found that the interiors of rocks in cold and hot deserts provide ecological niches for endolithic microbial communities (Siebert & Hirsch, 1988; Friedmann, 1993) just as do crystalline salts from evaporite deposits (Rothschild et al., 1994). Microorganisms have been isolated from extremely cold environments, such as Antarctic soils (Vishniac, 1993), as well as from hot environments at temperatures of 80–115°C, which are usually associated with active volcanism as hot springs, solfataric fields, shallow submarine hydrothermal vents, abyssal hot vent systems ('black smokers,') as well as oil-bearing deep geothermally heated soils (Stetter, 1996). Microbial communities are found buried in river and lake sediments (Kolbel-Boekel et al., 1988), and in the upper regions of the atmosphere up to an altitude of about 70 km (Imshenetsky et al., 1978).

The successful isolation and revival of bacterial spores from the abdomen tissue of 25–40 million-year-old bees that had been preserved in amber was recently reported (Cano & Borucki, 1995). For the enrichment of the indigenous microorganisms, the culturing conditions must correspond to the original environment as closely as possible. Furthermore, special care has to be taken to avoid contamination by non-indigenous organisms during the process of sampling, transportation, isolation and enrichment in the laboratory. Successful cultivation of microorganisms from extreme environments provides a unique tool for understanding the interaction in microbial communities, e.g. between primary producers and consumers of organic matter. After successful isolation of the indigenous microbial species, technologies in microbiology and molecular biology can be applied in order to determine the phylogenetic and physiological properties.

Nucleic acid sequencing technology has provided a powerful tool for establishing the evolutionary relationships of organisms and for grouping microorganisms according to their genotype, independent of any phenotypic observation. This is based on the fact that basic biochemical features, such as the genetic code, the set of amino acids in the proteins and the machinery of transcription of the genetic code and its translation into the proteins are common to all forms of life on Earth. During the evolution of life on Earth, at the level of the genotype, changes constantly occur randomly in time. By comparing the genotypic information stored in the nucleic acids and proteins, all organisms are grouped within three domains of the phylogenetic tree: Bacteria, Archaea and Eukarya (Woese, 1987). As biological macromolecules, 16S rRNA, DNA-dependent polymerases and proton-pumping ATPases have been most commonly used. Different methods are applied to determine the percent sequence similarity of the macromolecules of an unknown organism with that of a well-established representative of a domain.

However, it should be borne in mind that, even under terrestrial conditions, a large number of microorganisms are not amenable to cultivation. Included are those from hot springs, where the presence of a large number of hitherto unknown species has been identified by the DNA extracted from samples, which was then amplified and compared with known 16S rRNA sequences (Barns et al., 1994).

The viability of microorganisms collected from extreme environments and their biomass can also be determined directly from biochemical analyses without cultivation of the cells. An example is the determination of the concentration of adenosine 5'-triphosphate (ATP) or of total adenylates in the cells (Karl et al., 1980). A high concentration of ATP or total adenylates indicates a substantial population of viable microorganisms. The ATP concentration can also be converted to living biomass carbon by using a conversion factor of microbial C/ATP of 200–500 (Jannasch & Mottl, 1985). If the guanosine 5'-triphosphate (GTP) is simultaneously determined, the ratio GTP/ATP (which is positively related with the microbial growth rate) can be used as an index of the rate of cellular biosynthesis (Karl et al., 1980).

As an alternative to the isolation of microorganisms from sites under investigation, their *in situ* examination in the undisturbed environment allows an adequate determination of the extent and distribution of microbial colonisation and interactions among the different representatives of the microbial community. This has been achieved in the Antarctic fellfield soils by a combination of epifluorescence

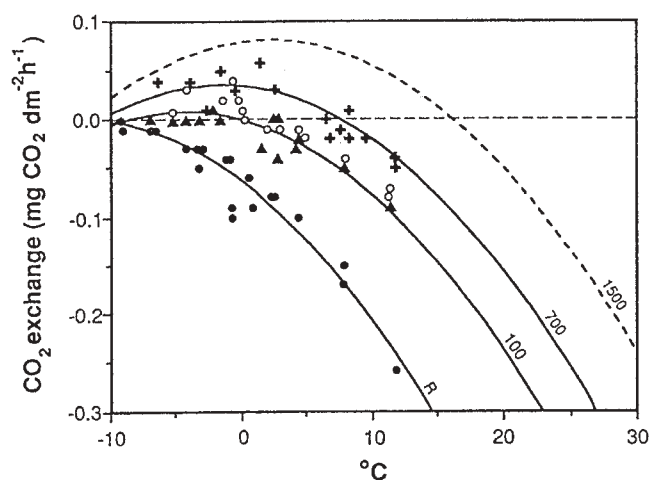
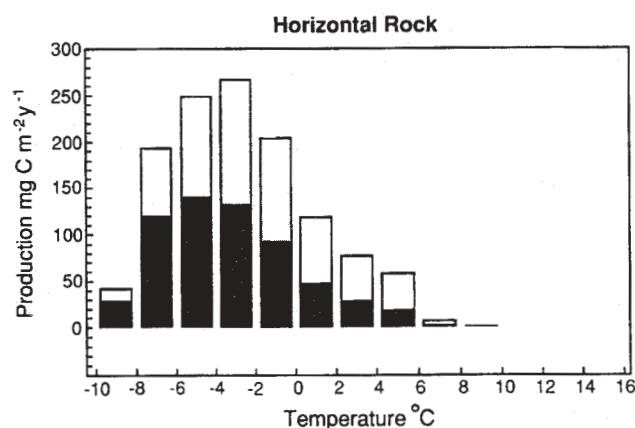


Fig. I.4.2.3/1A (left). Net photosynthetic response and dark respiration of a cryptoendolithic community in Antarctica at different levels of solar irradiance (100, 700, 1500 mol photons  $\text{m}^{-2}\text{s}^{-1}$ ). Fig. I.4.2.3/1B. Annual gross productivity (whole bars) and net photosynthetic gain (dark bars) by the same cryptoendolithic community. (From Nienow & Friedmann, 1993)



microscopy and television image analysis (Wynn-Williams, 1988). It uses the autofluorescence of primary producers, e.g. the primary photosynthetic pigment chlorophyll and phycocyanine or phycoerythrin as accessory pigments of cyanobacteria, and detects the presence of heterotrophs by means of fluorochromes, e.g. fluorescein, isothiocyanate or acridine orange. This technique makes cells visible relative to their opaque substrate. By using stains that selectively stain viable cells better than non-viable ones, the degree of viability of the population can be determined (Wynn-Williams, 1988). Using this technique, the microalgal colonisation of the Antarctic soil surface was successfully recorded over a period of 6 years (Wynn-Williams, 1996). To detect microorganisms that are not amenable to cultivation, fluorescently labelled rRNA-targeted oligonucleotide probes have been used to identify individual prokaryotic cells (Amann et al., 1992). Such rRNA-targeted nucleic acid probes are either highly specific at the species/subspecies level or rather unspecific reacting with all living organisms. They have been widely used to identify *in situ* the phylogeny of uncultured microorganisms and to analyse complex microbial communities (Amann et al., 1992).

Direct tests of carbon and nitrogen metabolism can be performed *in situ* in the environment under investigation. The productivity of primary producers is generally determined by the rate of total carbon uptake, e.g. in the form of radioactively labelled bicarbonate, in crushed rock samples containing endolithic communities or in intact endolithic communities by the  $\text{CO}_2$  gas exchange at the rock surface (Fig. I.4.2.3/1A). From such data, the annual gross productivity and net photosynthetic gain can be assessed, as shown in Fig. I.4.2.3/1B (Nienow & Friedmann, 1993). Similarly, carbon fixation was determined in Sun-exposed evaporites containing microorganisms (Rothschild et al., 1994). In deep sea hydrothermal vents, the presence of primary producers was proven by *in situ* measurements of the uptake of  $^{14}\text{CO}_2$  over a period of several days (Karl et al., 1980). The presence and activity of nitrogen fixation was determined in evaporites by measuring acetylene reduction (Rothschild et al., 1994), denitrification activity was determined essentially by the same method, with acetylene used to block nitrous oxide reductase. The activity is determined by gas chromatography from periodically collected gas samples (Rothschild et al., 1994). All *in situ* analyses described above require carefully planned control experiments.

It is worth mentioning that the life-detection instrument package of the Viking missions which, among other scientific issues, addressed the question of extant life on Mars, comprised three experiments to detect metabolic activity of potential microbial soil communities:

1. the pyrolytic release experiment tested carbon assimilation, i.e. photoautotrophy, as a method of incorporating radioactively labelled carbon dioxide in the presence of sunlight (Horowitz et al., 1977);

2. the labelled release experiment tested catabolic activity, e.g. digestion of food, as a metabolic capability to release radioactively labelled carbon from organic nutrient compounds (Levin & Straat, 1977);
3. the gas-exchange experiment tested respiration as metabolic production of gaseous byproducts in the presence of water and nutrients (Oyama et al., 1977).

In the first instance, all three Viking biology experiments gave results indicative of active chemical or even biological processes when samples of martian soil were subjected to incubation under the conditions that were imposed on them. However, when taking the data together with all other Viking results, non-biological processes have generally been interpreted to be responsible for the observed reactions (Klein, 1979). The strongest argument against extant life was the complete lack of any organic residues in the martian soil samples investigated by gas chromatography-mass spectrometry (GCMS). The detection limit was in the range of ppb for compounds containing three carbon atoms or more, and in the range of ppm for molecules with one or two carbon atoms. The conclusion is that extant life is absent at the two Viking sites. So far, the mechanisms underlying the reactions of the biology experiments are not known. The most likely explanation suggests the presence of highly reactive peroxides produced in the soil by the intense unfiltered solar UV radiation, which is also responsible for the lack of organics in the samples (reviewed in Horneck, 1995).

#### I.4.2.4. Chemical Signatures and Biomarkers

Microorganisms that are isolated and enriched from their primary habitats can be subjected to a thorough biochemical analysis, such as determination of the total biomass and the content of proteins, nucleic acids, lipids, sugars etc. The detailed characterisation of the pattern of biomarker mixtures from a sample permits the assessment of the major contributing species and their metabolic state, e.g. actively metabolising, dormant or extinct. Phospholipids, which are part of every bacterial membrane, have been considered as valuable indicators of the living biomass that is present because they maintain a relatively constant portion of the cell mass and disintegrate fairly rapidly after cellular death (Chapelle, 1993). The pattern of lipids is also diagnostic for different species of a microbial community, such as phototrophic cyanobacteria, photosynthetic bacteria, bacterial heterotrophs and archaea (Summons et al., 1996).

Isotopic ratios are another valuable set of chemical biomarkers. For instance, the enzymatic uptake of inorganic carbon during photosynthesis is typically associated with a discrimination against  $^{13}\text{C}$  relative to  $^{12}\text{C}$  (e.g. Schidlowski, 1993a). It is interesting to note that the  $\delta^{13}\text{C}$  values of average biomass usually range about 20–30‰ more negative than those of inorganic carbon. These low  $\delta^{13}\text{C}$  values are maintained rather conservatively over the whole history of life on Earth (see Fig. I.4.3.2.2/1 and relevant discussion in section I.4.3.2.2.). By the combination of compound-specific isotopic information with diagnostic molecular structure a powerful tool is available for characterising the constituents of a microbial community (Summons et al., 1996).

Optical handedness of monomers, e.g. amino acids in proteins and sugars in nucleic acids, is a *sine qua non* for maintaining the secondary, tertiary and quaternary structure of the polymers and is therefore considered as a basic requirement for terrestrial life. Therefore, evidence for optical activity in a class of compounds that may make up polymers represents an interesting biomarker. Measurements of optical activity can be done in extracts by gas chromatography (Pollock et al., 1970) or directly with the sample collected using polarised light and a light detector (MacDermott et al., 1996).

A non-intrusive *in situ* high-precision analysis of the distribution of organic and inorganic components of samples containing living microorganisms is obtained by Fourier transform Raman spectroscopy (Edwards et al., 1995). From the vibrational bands, the presence of organic compounds, such as chlorophyll and calcium oxalate di- and monohydrate, was detected in different zones of endolithic communities.

A detailed chemical analysis of martian surface soil was performed during the Viking missions. The elemental composition was determined by X-ray fluorescence, which analysed the composition for elements heavier than Mg. It was shown that the most essential major elements that make up the biological matter, such as C, H, O, N, P, K, Ca, Mg and S, are present on the surface of Mars. However, organic compounds were not detected by GCMS, which was capable of detecting organic residues in martian soil down to ppb for compounds containing three or more C and to ppm for compounds with one or two C (Biemann et al., 1977).

#### **1.4.2.5 Indirect Fingerprints of Life**

During its >3.5 Gyr history, life on Earth has substantially modified the terrestrial lithosphere, hydrosphere and atmosphere. Examples are the fossil deposits of petroleum and coal, the sediments of shell limestone, the coral reefs and the deposits of banded iron formation, biomineralisation and bioweathering, which all bear witness to biological activity in the geological past. Stromatolites and related biosedimentary build-ups are examples of microbial biomineralisation, which are widespread in the geological record (Walter, 1976).

Composition and dynamic cycles of the terrestrial hydrosphere and atmosphere are decisively influenced by the terrestrial biosphere. Examples are the water, CO<sub>2</sub> and nitrogen cycles. The albedo of our planet is also modified by the surface vegetation. Concerning the water cycle, evapotranspiration, especially of the tropic rain forest, is an important biogenic effect contributing to the release of water. The atmospheric O<sub>2</sub> is largely a product of photosynthetic activity that began in the early history of life with cyanobacteria as the main primary producers. Photodissociation of O<sub>2</sub> in the upper layers of our atmosphere has led to the build-up of the UV-protective ozone layer in the stratosphere. Concerning the CO<sub>2</sub> cycle, the marine phytoplankton constitutes a large CO<sub>2</sub> sink, which is essential for maintaining the steady-state.

According to the Gaia hypothesis, proposed by Lovelock, the composition, oxidative-reductive state and temperature of the atmosphere are actively regulated by life activities (Lovelock, 1979). However, it is also possible that lifeforms have conquered ecological microniches, where a symbiosis of photosynthetic primary producers and heterotrophic consumers may produce a steady-state where the influx and efflux of carbon are equal (Morita, 1975). These ‘hidden’ econiches would not necessarily contribute to global cycling.

There exist several biogenic minerals, which have distinctive crystallographies, morphologies and isotopic ratios that make them distinguishable from their abiotically-produced counterparts of the same chemical composition. Those minerals that result from genetically-controlled mineralisation processes and that are formed within a preformed organic framework have non-interchangeable characteristics, such as orientation of the crystallographic axes and the microarchitecture. Examples are the skeletons of the unicellular marine Acantharia (composed of strontium sulphate), the shells of amorphous silicate of diatoms, and the biogenic magnetite formed by bacteria (Schwartz et al., 1992). It is important to note that minerals produced under biologically-controlled processes are not necessarily in equilibrium with the extracellular environment. In contrast, those minerals that are formed extracellularly by biologically-induced mineralisation processes can be less easily distinguished from their abiotically-formed counterparts. They are formed in an open environment and are in equilibrium with this environment.

#### **1.4.2.6 Conclusions**

The search for signatures indicative of extant life on Mars or on any other celestial body of exobiological interest in our Solar System can only be one of the final steps in the quest for extraterrestrial life. For future exobiology exploration of Mars, a stepwise approach might be the most promising investigative strategy (Chicarro et al., 1989). In particular, before any search-for-extant-life experiment, more data are required on martian geology (paleolakes, volcanism, hydrothermal vents, carbonates), climate (hydrosphere, duration of phases that allow liquid water), the past and present

and radiation environment, as well as organic molecules in sediments. The search for possible biological oases will be connected with the detection of areas where liquid water still exists under the current conditions on that planet. By analogy with terrestrial ecosystems, potential protected niches have been postulated for Mars, such as sulphur-rich subsurface areas for chemoautotrophic communities, rocks for endolithic communities, permafrost regions, hydrothermal vents, soil and evaporite crystals.

Much information can be obtained from remote-sensing global measurements, such as the seasonal atmospheric and surface water distribution, the mineralogical inventory and distribution, geomorphologic features obtained with high spatial resolution, thermal mapping of potential volcanic regions to determine possible geothermally active sites, and trace gases such as  $H_2$ ,  $H_2S$ ,  $CH_4$ ,  $SO_x$ , and  $NO_x$ .

It is only after identifying sites that are suitable environments for a potential martian biota that landers are required for more detailed characterisations. Then analyses of the elemental and isotopic compositions, of mineral composition and structure, and of organic compounds can follow. Concerning Mars, it is also very important to explore and understand the strong oxidative processes on the surface.

If the *in situ* investigations confirm that the sites constitute potential biological oases, detailed searches for direct and indirect biomarkers should follow. The search protocol, i.e. what methods to apply, depend largely on the environmental conditions offered by the sites. Alternatively, the analysis of samples collected and returned to Earth should be considered. This would allow much more crucial tests for extant biology in ground-based laboratories. However, in the latter case, a stringent planetary protection programme is required.

#### I.4.3.1 Paleontological Evidence

Given the validity of terrestrial analogues, the stratigraphic (sedimentary) record of any planet should serve as a potential store for both bodily remnants of former organisms and other possible traces of their life activities. This should also apply to Mars during the early stages of its history, when the planet may have been bathed in abundant water and environmental conditions on the surface did not differ much from those on the early Earth (Carr & Wänke, 1992; Carr, 1996). If all terrestrial planets – and notably Mars and Earth – had occupied comparable starting positions in terms of solar distance, condensation history and primary endowment with matter from the parent solar nebula (Moroz & Mukhin, 1978), then the surface conditions on both planets should have been very similar in their juvenile states.

Specifically, with evidence at hand for a denser atmosphere and extensive aqueous activity on the martian surface during the planet's early history, a convincing case can be made that the primitive martian environment was no less conducive to the initiation of life processes and the subsequent emplacement of prolific microbial ecosystems, than the surface of the ancient Earth (McKay, 1991; McKay & Stoker, 1989). Even if the evolutionary pathways of both planets diverged during their later histories, so that life became extinct on Mars as the gradual deterioration of surface conditions rendered the planet inhospitable to protein chemistry in the widest sense, Mars could still have started off with a veneer of microbial (prokaryotic) life comparable to that existing on the Archaean Earth (Schopf, 1983; Schidlowski, 1993a). Given the apparent failure of the Viking life-detection experiment for the present martian regolith (Biemann et al., 1977), the prime objective of a search for life on Mars should be to seek evidence of extinct (fossil) life. The oldest Martian sediments would be appropriate targets for such efforts.

In any search for extinct life on Mars, the basic problem would not rest with the cognitive aspects of the identification of the fossil evidence, but rather with the serendipity inevitably involved in the selection and recovery of suitable sampling material from the vast stretches of potential host rocks exposed on the planetary surface. Among the two-thirds of the martian surface deemed to be covered by rocks older than 3.8 Gyr (McKay, 1986), there are several occurrences of well-bedded

### I.4.3 Evidence of Extinct (Fossil) Extraterrestrial Life



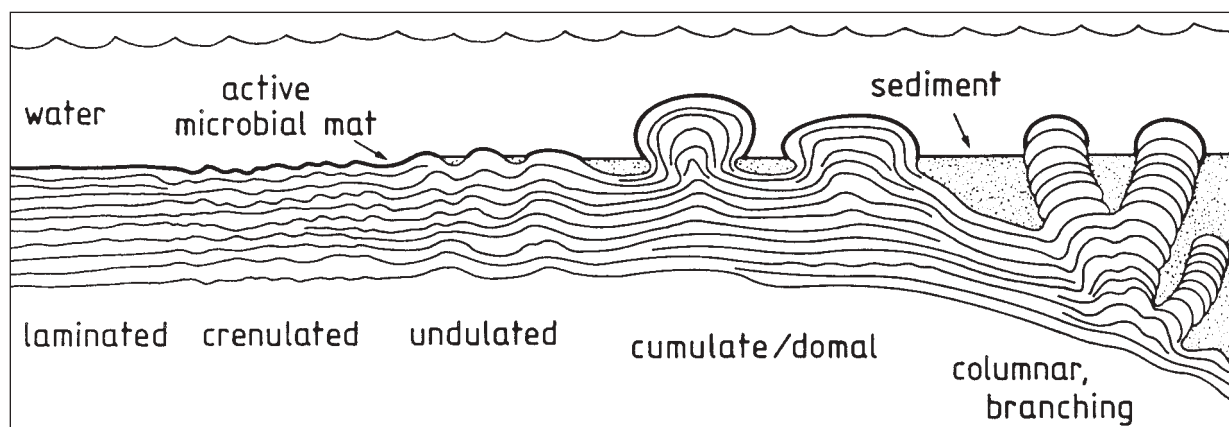


Fig. I.4.3.1.1/1. Scheme of principal morphologies of laminated microbial ecosystems that thrive at the sediment-water interface. These structures subsequently lend themselves to lithification in the form of 'stromatolites' (see Fig. I.4.3.1.1/2). The mat-forming is mostly made up of cyanobacteria.



Fig. I.4.3.1.1/2. Typical microbialite (or 'stromatolite') from the Precambrian Transvaal Dolomite (~2.3 Gyr) that is made up of a succession of superimposed fossil microbial mats. The bun-shaped and partially interfering laminae represent consecutive growth stages of the primary microbial community.



sediments (notably in the Tharsis region and the associated Valles Marineris canyon system, see II.2.7) that are interpreted as lake deposits and believed to include thick sequences of carbonates (McKay & Nedell, 1988). If present at all in these early martian formations, morphological relics of fossil life should lend themselves to as ready a detection as do their terrestrial counterparts in Archaean sediments, provided the host rock is accessible to either robotic sensing or direct investigation following a sample return mission.

In that regard, it seems to be a reasonable conjecture to resort to the paleontological inventory of the oldest terrestrial sediments (3.50–3.85 Gyr old) for guidance concerning potential fossil evidence from coeval martian rocks. On Earth, the earliest prokaryotic (bacterial and archaeobacterial) microbial ecosystems have basically left two categories of morphological evidence, namely (i) stromatolite-type biosedimentary structures ('microbialites') and (ii) cellular relics of individual microorganisms ('microfossils').

#### **I.4.3.1.1 Microbialites**

Microbialites or 'stromatolites' (cf. Burne & Moore, 1987) are laminated biosedimentary structures that preserve the matting behaviour of bacterial and algal (primarily prokaryotic) microbenthos. Microbial buildups of this type represent stacks of finely laminated lithified microbial communities that originally thrived as organic films at the sediment-water interface, with younger mat generations successively superimposed on the older ones (cf. Figs. I.4.3.1.1/1 and I.4.3.1.1/2). The structures derive from the interaction of the primary biologically active microbial layer with the ambient sedimentary environment, the fossilisation of the laminae resulting from either trapping, binding or biologically mediated precipitation of selected mineral constituents.

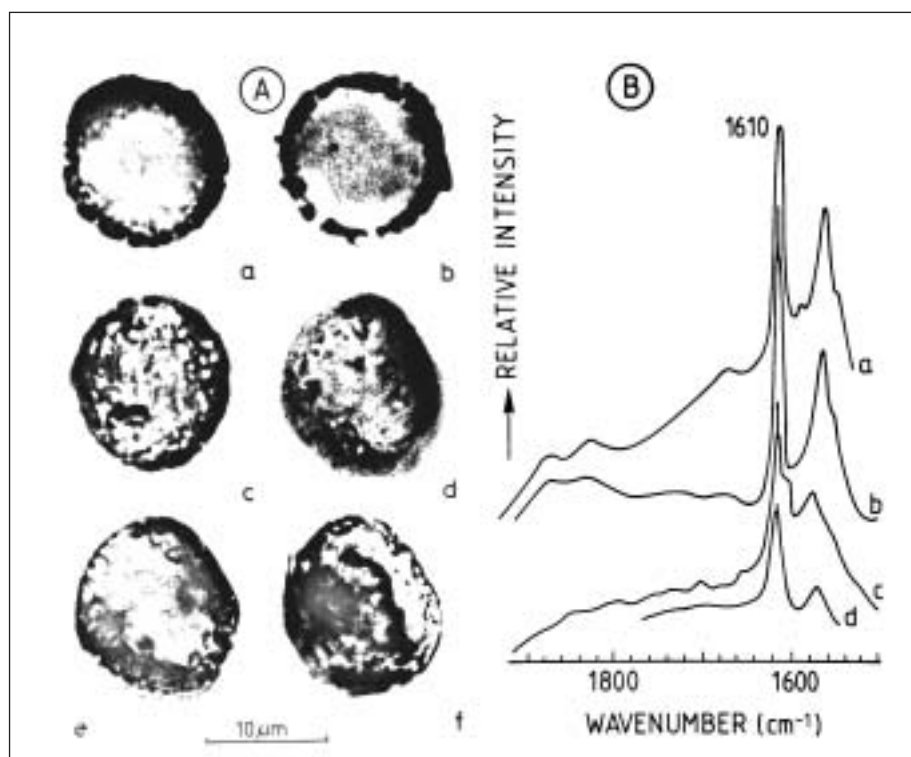
In terrestrial sediments, microbialites represent the most obvious (macroscopic) expression of fossil microbial life, with a record extending back to the Early Archaean (~3.5 Gyr ago). This constitutes *prima facie* evidence that benthic prokaryotes were already widespread in suitable aquatic habitats of the Archaean Earth. Both the morphological inventory of the oldest stromatolites and the observed microfossil content of the ambient rock or coeval sequences (see below) allow a fairly elaborate reconstruction of the Earth's earliest microbial ecosystems, indicating that Archaean stromatolite builders were not markedly different from their geologically younger counterparts (inclusive of contemporary species). It appears well established that the principal microbial mat builders were filamentous and unicellular prokaryotes capable of phototactic responses and photoautotrophic carbon fixation (Walter, 1983). The unbroken stromatolite record from Archaean to present times attests, furthermore, to an astounding degree of conservatism and uniformity in the physiological performance and communal organisation of prokaryotic microbenthos over 3.5 Gyr of geological history.

#### **I.4.3.1.2 Cellular Microfossils**

Apart from the macroscopic vestiges of past microbial activity in the form of stromatolites and related biosedimentary structures, there is a second (microscopic) category of paleontological evidence stored in the sedimentary record. This microscopic or cellular evidence is currently supposed to go back to at least ~3.5 Gyr (as in the case of microbialites), with the biogenicity of microfossil-like morphologies reported from the ~3.8 Gyr-old Isua metasedimentary suite from West Greenland (Pflug, 1978) still being debated.

While a wealth of authentic microbial communities has been reported from Early and Middle Precambrian (Proterozoic) formations, the unequivocal identification of cellular microfossils becomes notoriously difficult with increasing age of the host rock. In Early Precambrian (Archaean) sediments, both the progressive diagenetic alteration and the metamorphic reconstitution of the enclosing mineral matrix tend to blur the primary morphologies of delicate organic microstructures. This results in a large-scale loss of contours and other critical morphological detail. At the extreme of

Fig. I.4.3.1.2/1. A: Comparison of *Huroniospora* sp. from the ~2.0 Gyr-old Gunflint iron formation, Ontario (a-c) with *Isuapphaera* sp. from the ~3.8 Gyr-old Isua metasedimentary suite, West Greenland (d-f). The optically distinctive marginal rim may be explained as a relic of the original cell wall. B: Laser Raman spectra obtained from *Huroniospora* sp. as an isolated particle (a) and in thin sections (b) compared with those from *Isuapphaera* sp. (c, d) obtained under the same conditions. The close resemblance of the spectra suggests similarities in the composition of the residual organic component of the two types of microstructures (the prominent peak close to 1610  $\text{cm}^{-1}$  is indicative of aromatic double bonds among the carbon atoms of the molecular structure). Adapted from Pflug (1987).



such alteration series lie the so-called ‘dubiofossils’, with variable and sometimes questionable confidence levels. To ascertain the biogenicity of possible cellular morphotypes in Archaean rocks, a hierarchical set of selection criteria has been proposed, postulating that genuine microfossils (i) be authentic constituents of the rock as testified by their exposure in petrographic thin sections, (ii) occur in vast multiples, (iii) be associated with residual carbonaceous matter, (iv) equal or exceed the minimum size of viable cells and display a central cavity plus structural detail in excess of that resulting from inorganic processes. Moreover, it has been proposed that, as a matter of principle, putative evidence from metamorphosed sediments should not be considered.

In spite of the evident impoverishment of the Archaean record, there are, however, single reports of well-preserved microfossils that, for the most part, comply with the above criteria. Most prominent among these assemblages are chert-embedded microfloras from the Warrawoona Group of Western Australia, which closely approach the 3.5 Gyr age mark. After an initial controversy about the authenticity of these fossil communities on grounds of imprecisely constrained petrographic background parameters for the host lithologies (Awramik et al., 1983; Buick, 1991), Schopf & Packer (1987) and notably Schopf (1993) have forwarded evidence prompting an acceptance of the observed morphotypes as *bona fide* microfossils. Conspicuous within the Warrawoona microbial community are both the coccoidal and filamentous (trichomic) micromorphologies that have been found to abound in the cyanobacterial precursor floras of Proterozoic formations. While the septate filaments stand for fossil trichomes that could be attributed to either filamentous cyanobacteria or more primitive prokaryotes (such as flexibacteria), the coccoidal aggregates described by Schopf & Packer (1987) have been claimed to strictly exclude other than cyanobacterial affinities.

Given the remarkable degree of diversification of the Warrawoona microflora, we must, of necessity, infer that the genetic lineages of the principal microbial species had emerged well before Warrawoona times. We may, therefore, reasonably assume

that precursor floras had been extant prior to ~3.5 Gyr when the preserved rock record becomes scant and increasingly metamorphosed. In this context, the observation of cell-like carbonaceous structures in the 3.8 Gyr-old metasediments from Isua, West-Greenland, attracted considerable attention. Described as *Isuasphaera isua* (Pflug, 1978), the biogenicity of this morphotype (Fig. I.4.3.1.2/1) has been violently disputed, specifically on grounds of the improbability of survival of delicate cell structures during the amphibolite-grade metamorphism of the host rock.

Meanwhile, however, there is ample evidence that fossils in general and microfossils in particular may – in variable degree – withstand obliteration in rocks subjected to medium-grade metamorphism. Therefore, we cannot *a priori* exclude microbial affinities for selected cell-like microstructures from the Isua metasediments, and notably for *Isuasphaera*-type micromorphs that show a striking resemblance to a possible counterpart of recognised biogenicity in the younger (Proterozoic) record described as *Huroniospora sp.* In spite of the uncertainty surrounding a large number of morphotypes described from Isua, and of occasional convergences with purely mineralogical features, there is a reasonable chance that the microstructure inventory as a whole includes at least some elements of a structurally degenerated microfossil assemblage such as might result from metamorphic impairment of a Warrawoona-type microflora. As detailed in Section I.4.3.2, the existence in Isua times of microbial ecosystems would not only be consistent with, but also conditional for, the actually observed carbon content and carbon isotope geochemistry of the Isua suite.

#### I.4.3.2 Biogeochemical Evidence

Apart from morphological relics, organisms also leave a chemical record of their former existence. While the bulk of the body material degrades after the death of the organism (with the dominant carbon component remineralised as CO<sub>2</sub>), a miniscule fraction of the organic substance (between 10<sup>-3</sup> and 10<sup>-4</sup>) usually escapes decomposition by burial in newly-formed sediments. Here, the primary biopolymers undergo a large-scale reconstitution ('humification' with subsequent transformation into inorganic carbon polymers), resulting finally in the formation of kerogen, a chemically inert (acid-insoluble) polycondensed aggregate of aliphatic and aromatic hydrocarbons that represents the end-product of the diagenetic alteration of primary biogenic matter in sediments (Durand, 1980). Representing the residuum of living substances, kerogenous materials and their graphitic derivatives constitute *per se* first-order proxies of past biological activity.

Moreover, the isotopic composition of the kerogenous carbon constituents gives further testimony to their biogenicity as it reflects <sup>13</sup>C/<sup>12</sup>C fractionations of a magnitude and direction that are typically obtained in the common assimilatory (photosynthetic) pathways, notably the ribulose biphosphate (RuBP) carboxylase reaction of the Calvin Cycle that operates in C3 photosynthesis. Particularly if seen in conjunction with the isotope record of coexisting carbonate, the <sup>13</sup>C/<sup>12</sup>C compositions of sedimentary organic carbon convey a remarkably consistent signal of biological carbon fixation that derives, for the most part, from a kinetic isotope effect imposed on the first CO<sub>2</sub>-fixing enzymatic carboxylation reaction of the photosynthetic pathway.

A third category of biochemical evidence of ancient life is represented by quasi-pristine organic molecules (mostly pigments or single discrete hydrocarbon chains) that have preserved their identities over the entire pathway of kerogen evolution from dead organic matter to the polymerised aggregates of solid hydrocarbons that mark the end of the maturation series of primary biogenic substances. The record of such 'biomarker' molecules or 'chemofossils', respectively (Eglinton & Calvin, 1967), goes far back into Precambrian times, gaining special significance in connection with the geologically oldest (Proterozoic) petroleum occurrences (Summons & Powell, 1992). Current work on molecular fossils from the kerogen fraction of Precambrian rocks (Hoering & Navale, 1987) holds the promise of considerable potential for the further elucidation of the early history of life.

In the following, the above categories of evidence that document the biochemical record of former life are characterised in more detail.

#### ***1.4.3.2.1 Sedimentary Organic Carbon as a Recorder of Former Life Processes***

As set out above, reduced or organic carbon ( $C_{org}$ ) stored in sedimentary rocks constitutes the fossil residue of biological matter, the bulk of which is made up of highly polymerised aliphatic and aromatic hydrocarbons ('kerogen') that figure as end-products of the diagenetic alteration of primary biogenic substances in the sediment. The  $C_{org}$ -content of the average sedimentary rock normally lies between 0.5% and 0.6% (Ronov et al., 1990). With a total mass of the Earth's sedimentary envelope of about  $2.4 \times 10^{24}$ g, this would imply that, over geologic time,  $1.2$ – $1.4 \times 10^{22}$ g  $C_{org}$  had leaked out from the surficial carbon cycle and come to be stored in the crust, thus making kerogen the most abundant form of organic matter on this planet. The fossil relics of biogenic matter thus accumulated can be traced back to the very beginning of the sedimentary record some 3.8 Gyr ago (Schidlowski et al., 1979), with shales representing the  $C_{org}$ -richest (up to 12–16%) and sandstones the  $C_{org}$ -poorest lithologies. The mobile (volatile) fraction of the sedimentary  $C_{org}$ -burden has commonly oozed out from the source sediment during the maturation process of the kerogenous substances, assembling as oil and gaseous hydrocarbons in suitable host rocks.

Systematic assays for  $C_{org}$  carried out on Phanerozoic (<0.54 Gyr) rocks indicate that the deposition rates of organic matter have, for the most part, moderately oscillated around a mean of about 0.5%  $C_{org}$  for the average sediment over this time span. Although both the preserved rock record and the available data base are progressively attenuated as we proceed into the older geological past, the evidence suggests that the scatter of  $C_{org}$  in Precambrian sediments is basically the same as in the Phanerozoic record (Schidlowski, 1982). It is worth noting that the (largely graphitised) organic carbon load of the 3.8 Gyr-old Isua metasediments of West Greenland that mark the very beginning of the record may considerably surpass the local average of 0.67% in the case of carbon-rich members of the suite.

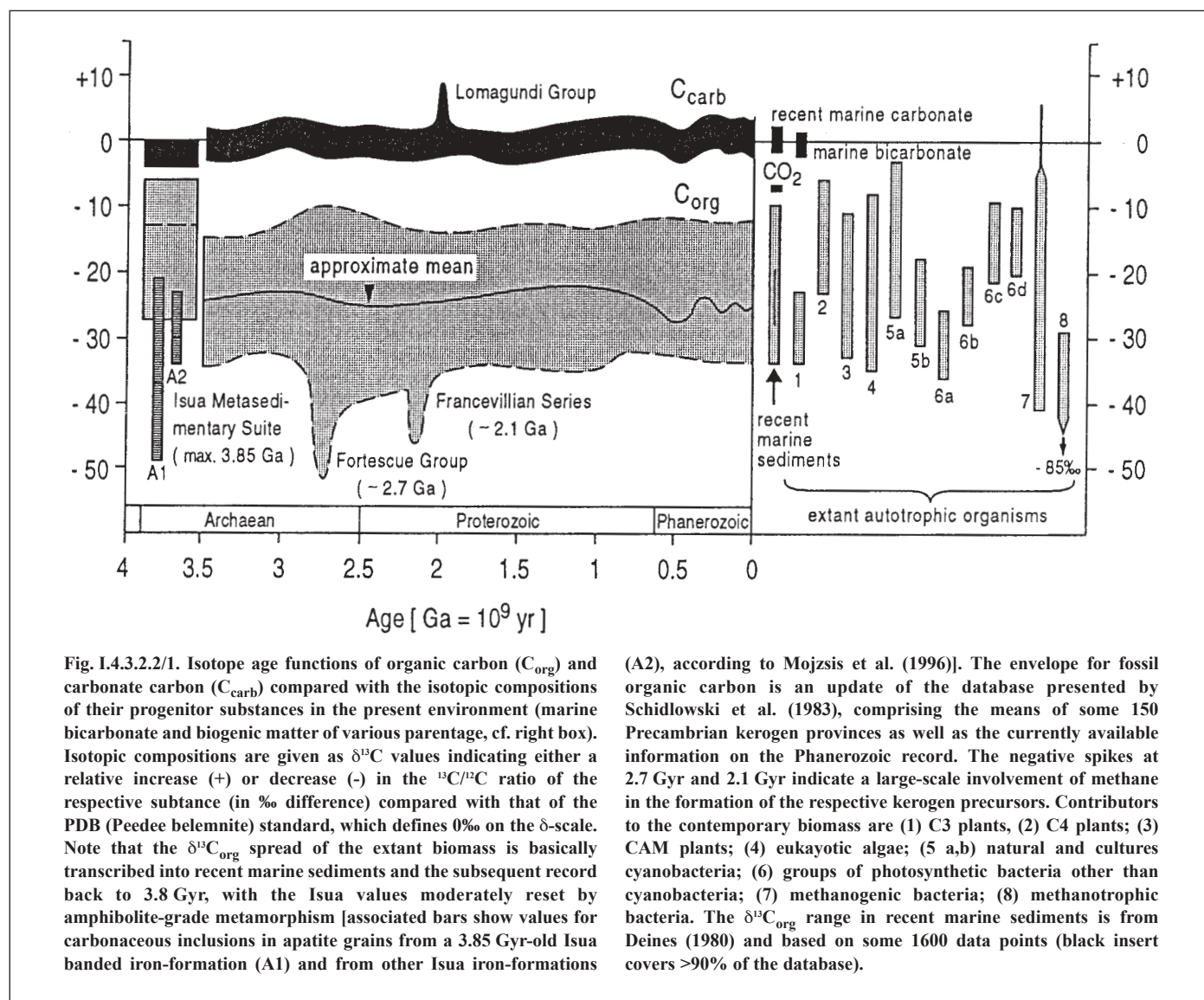
Apart from constituting materialised residues of primary living substances in the widest sense, the elemental composition (H, C, O, N, S) of kerogens may offer additional information on the source organisms and their habitat. Plots of H/C vs O/C ratios have permitted differentiation between sapropelic kerogens of microbial and algal pedigree (characterised by high H/C and low O/C) and a humic moiety derived from higher plants (with low H/C and high O/C). When plotted against each other in a 'Van Krevelen diagram', both ratios decrease with increasing maturity of the respective kerogen species, their evolution paths finally converging in the overlap of H/C = 0.5–0.8 and O/C = 0.0–0.1. The degree of dehydrogenation as quantified by the H/C ratio serves, furthermore, as an index of aromaticity, reflecting the progressive replacement of aliphatic hydrocarbon chains by cyclic (aromatic) hydrocarbons during the polymerisation process. At the very end of the kerogen evolution series stands graphite as the H- and O-free variant of reduced sedimentary carbon.

In sum, it can be stated that organic carbon in the form of kerogen and its graphitic derivatives is a common constituent of sedimentary rocks over the whole of the presently known record. Based on hitherto available data, a good case can be built that the  $C_{org}$ -content of the average sediment has stayed remarkably uniform from Archaean to present times, ranging broadly between 0.4% and 0.7%. The abundance of highly graphitised kerogenous materials in the 3.8 Gyr-old Isua metasediments is likely to give eloquent testimony to the operation of life processes already during the time of formation of the oldest terrestrial sediments.

#### ***1.4.3.2.2 $^{13}C/^{12}C$ in Sedimentary Organic Matter: Index of Autotrophic Carbon Fixation***

Carbon basically consists of a mixture of two stable isotopes,  $^{12}C$  and  $^{13}C$ ; a third, short-lived radioactive nuclide,  $^{14}C$ , occurs only in trace amounts. All transformations of the element in the geochemical cycle have been shown to entail thermodynamic and/or kinetic isotope effects that cause newly-formed phases to be isotopically distinctive from their precursor substances. The largest isotope effects are commonly brought about during the conversion of inorganic to organic carbon in autotrophic





carbon fixation. This is primarily the assimilation of  $CO_2$  and the bicarbonate ion ( $HCO_3^-$ ) by plants and microorganisms that proceeds by a limited number of pathways which invariably discriminate against the heavy carbon isotope ( $^{13}C$ ), mostly as a result of a kinetic isotope effect inherent in the first irreversible enzymatic  $CO_2$ -fixing carboxylation reaction (cf. O'Leary, 1981; Schidlowski et al., 1983). This latter reaction promotes the incorporation of  $CO_2$  into the carboxyl ( $COOH$ ) group of an organic acid which, in turn, lends itself to further processing in subsequent metabolic pathways. As biochemical carbon-fixing reactions are largely enzyme-controlled, and living systems constitute dynamic states undergoing rapid cycles of anabolism and catabolism, it is generally accepted that most biological isotope fractionations are due to kinetic rather than equilibrium effects.

Because of the discrimination against 'heavy' carbon in the common carbon-fixing pathways, we observe a marked enrichment of  $^{12}C$  in all forms of biogenic (reduced) carbon as compared with the inorganic feeder pool of oxidised carbon consisting mainly of atmospheric  $CO_2$  and dissolved marine bicarbonate ion ( $HCO_3^-$ ). In terms of the conventional notation,  $\delta^{13}C$  values of average biomass usually turn out to be 20-30‰ more negative than those of marine bicarbonate, the most abundant inorganic carbon species in the environment.

The isotopic difference thus established between organic (biogenic) carbon and the surficial bicarbonate-carbonate pool is largely retained when organic and carbonate carbon enter newly-formed sediments. As is obvious from Fig. I.4.3.2.2/1, both the carbon isotope spreads of extant primary producers and those of marine carbonate and bicarbonate are basically transcribed into the sedimentary record back to 3.5 Gyr or even 3.8 Gyr ago. This would imply that organic carbon and carbonate carbon had always been transferred from the surficial environment to the crust with relatively little change in their isotopic compositions. For example, the  $\delta^{13}\text{C}_{\text{org}}$  spread in recent marine sediments (cf. Fig. I.4.3.2.2/1) faithfully integrates over the spread of the contemporary living biomass with just the extremes eliminated, indicating that the effect of a later diagenetic overprint on the primary isotope values is rather limited (usually below 3‰) and, for the most part, gets lost within the broad scatter of the original values. Consequently, the kinetic isotope effect inherent in photosynthetic carbon fixation is propagated from the biosphere into the rock section of the carbon cycle almost unaltered, which opens up the possibility of tracing the isotopic signature of this process back into the geologic past.

With these relationships established, decoding of the vast body of isotopic information stored in the sedimentary record is fairly straightforward. There is little doubt that the conspicuous  $^{12}\text{C}$ -enrichment displayed by the data envelope for fossil organic carbon (Fig. I.4.3.2.2/1) constitutes a coherent signal of autotrophic carbon fixation over almost 4 Gyr of recorded Earth history as it ultimately rests with the process that gave rise to the biological precursor materials. Moreover, the long-term uniformity of the signal attests to an extreme degree of conservatism of the basic biochemical mechanisms of carbon fixation. In fact, the mainstream of the envelope for  $\delta^{13}\text{C}_{\text{org}}$  depicted in Fig. I.4.3.2.2/1 can be most readily explained as the geochemical manifestation of the isotope-discriminating properties of one single enzyme, namely, ribulose-1,5-bisphosphate (RuBP) carboxylase, the key enzyme of the Calvin Cycle.

It is well known today that the carbon transfer from the inorganic to the organic world largely proceeds via the RuBP carboxylase reaction that feeds  $\text{CO}_2$  directly into the Calvin Cycle as a 3-carbon compound (phosphoglycerate). Most autotrophic microorganisms and all green plants operate along this pathway of carbon assimilation; higher plants relying on it entirely are termed C3 plants. As a result, the bulk of the Earth's biomass (both extant and fossil) bears the isotopic signature of C3 (or Calvin Cycle) photosynthesis characterised by the sizeable fractionations of the RuBP carboxylase reaction that assigns a mean  $\delta^{13}\text{C}_{\text{org}}$  range of  $-26 \pm 7\text{‰}$  to most biogenic matter.

Occasional negative offshoots from this long-term average (Fig. I.4.3.2.2/1) are commonly restricted to the Precambrian (Hayes et al., 1983; Schidlowski et al., 1983) and suggest the involvement of methanotrophic pathways in the formation of the respective kerogen precursors. Basically, these excursions appear to be oddities confined to side stages of the carbon cycle that have never affected the economy of the global cycle as a whole.

The only apparent discontinuity in the  $\delta^{13}\text{C}_{\text{org}}$  record depicted in Fig. I.4.3.2.2/1 is the break between the  $\sim 3.8$  Gyr-old Isua metasediments from West Greenland and the whole of the post-Isua record. The observed isotope shift is, however, fully consistent with the predictable effects of an isotopic re-equilibration between coexisting organic carbon and carbonate in response to the amphibolite-grade metamorphism experienced by the Isua suite. Both currently available thermodynamic data on  $^{13}\text{C}/^{12}\text{C}$  exchange between  $\text{C}_{\text{org}}$  and carbonate carbon ( $\text{C}_{\text{carb}}$ ) as a function of increasing metamorphic temperatures and observational evidence from a host of geologically younger metamorphic terranes make it virtually certain that the 'normal' sedimentary  $\delta^{13}\text{C}_{\text{org}}$  and  $\delta^{13}\text{C}_{\text{carb}}$  records had originally extended back to 3.8 Gyr ago, and that the Isua anomaly is clearly due to a metamorphic overprint (Schidlowski et al., 1979; 1983). Apparently pristine  $\delta^{13}\text{C}_{\text{org}}$  values in the range  $-21\text{‰}$  to  $-49\text{‰}$  (average  $-7 \pm 3\text{‰}$ ) have been recently reported for minor carbon inclusions in apatite grains from 3.85 Gyr-old Isua banded iron-formation (Mojzsis et al., 1996). Here, carbonaceous



material sealed in a mineral matrix and hosted by a carbonate-free lithology had obviously escaped high-temperature isotope exchange, thereby preserving its original biogenic  $\delta^{13}\text{C}_{\text{org}}$  spread with probably little alteration.

#### ***1.4.3.2.3 Molecular Biomarkers ('Chemical Fossils') in Sediments***

As mentioned earlier, biological markers, or 'biomarkers', are terms used for the wide variety of organic compounds that are derived from living organisms and can be found in sediments. Such compounds have also been termed 'chemical fossils' but the most commonly-used term is that of biomarker (Peters & Moldowan, 1993; Engel & Macko, 1983; Killops & Killops, 1993). Those biomarkers showing little or no change in structure from those of the original biochemical compounds are easily recognised in extracts of sediments, but compounds with strongly diagenetically or catagenetically altered structures, wherein the carbon skeletons differ from those of the original biological compounds yet carry sufficient residual portions of the original structures, may still be useful biomarkers.

Biomarkers are extractable from sediments and fossils by organic solvents, the most studied being of the lipid type, although certain pigments, e.g. metal porphyrins, are also referred to as biomarkers. The key to the use of biomarkers is frequently their complexity of structure, since this represents a major inheritance of original biologically-derived information. In addition, the molecular distributions in terms of their relative amounts can be highly informative, as the original biosynthesis imparts characteristic distributions of the different compounds. However, subsequent diagenetic alterations and mineralisation modify those patterns. Hence, the biochemist generally prefers to work with unaltered, original molecular biomarkers, because these compounds carry the full original biochemical information and exhibit an extremely high degree of order both in structure and in relative abundances.

#### ***Molecular Characteristics of the Biosphere***

What is the recognisable record of the past biosphere in the surface of the Earth? In general terms, the biosynthetic imprint of a living organism is seen in the carbon skeletons, the functionalities and the stereochemistries of the compounds it manufactures under enzymic, molecular machinery. The fundamental scheme of life's universal, but extremely precise, molecular machinery is simply:



DNA and RNA consist of genetically determined sequences of five different nucleotide bases, with the DNA sequence determining the RNA sequence. It, in turn, controls the synthesis of proteins from some 20 homochiral alpha amino acids. The proteins consist of short ( $10^2$ - $10^3$  amino acids) polypeptide chains and normally generate their own specific intramolecular crosslinks, thereby defining the precise shape and location of reactive functionalities required for enzymatic activity. Proteins in turn catalyse the synthesis of specific metabolites and homologous metabolic series. Enzymatic synthesis (metabolism) and breakdown (catabolism) of molecules provides for all the needs of the organisms. The metabolites comprise a large range of molecules that include, *inter alia*, the building blocks to synthesize a variety of cellular biochemicals, such as lipids, polysaccharides, and co-enzymes.

Chiral centres such as those at a singlet tetrahedrally substituted carbon atom are almost always synthesised with a single chirality. Very unusually, an organism may make both, while, in other rare instances, one enantiomer may be made by one species and the other by a different species. Cholesterol, a sterol widely distributed in eukaryotes, is an example of a highly ordered carbon skeleton in which the overall shape is dependent upon the stereochemistry of the ring fusions and of the methyl and hydroxyl substituents of the rings. It has eight chiral centres, as indicated in Fig. 1.4.3.2.3/1. The structure is highly specific, both in the gross skeleton and in the stereochemistry, so that it makes an excellent biomarker, one which has been traced back hundreds of millions of years in immature sediments. It is also a carrier of

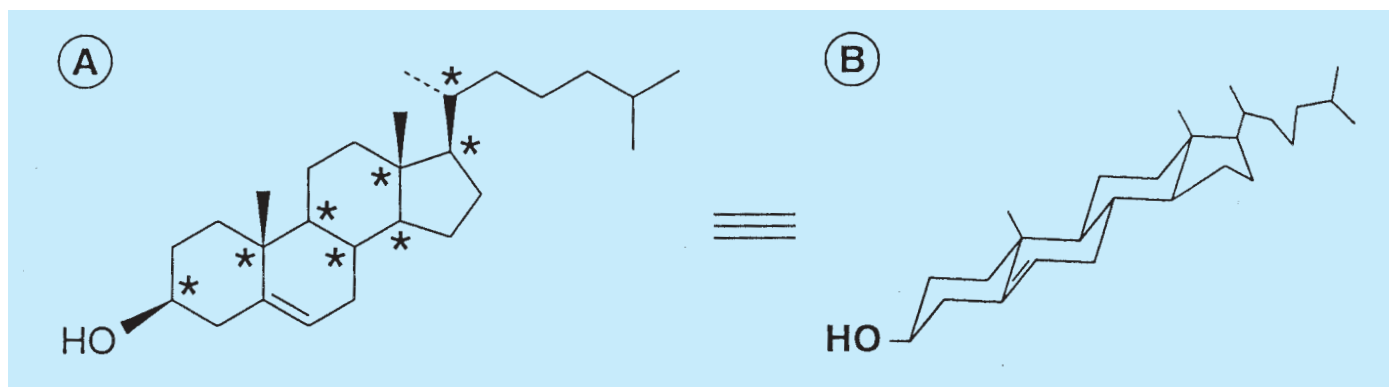


Fig. I.4.3.2.3/1. Cholesterol as an example of a biomarker molecule. (A) is a conventional line drawing of the molecular structure in which the stereochemistry of the ring junctions and C-atom substitutions is indicated, in part, by the thick and the dotted lines at the chiral carbon centres denoted by an asterisk. (B) is a simple 3D representation of the structure, showing the flat tabular shape created from the four rings.

abundant isotopic information, due to the biosynthesis pathway. Thus carbon isotope measurements can help to define the origin of the cholesterol found in such sediments, since its  $\delta^{13}\text{C}$  value will reflect that of the carbon pool from which the individual C5 units were assembled. Normally, of course, the gross averaged isotopic composition for the whole compound is measured, but there is considerable scope for the study of the intramolecular variations in isotopic composition.

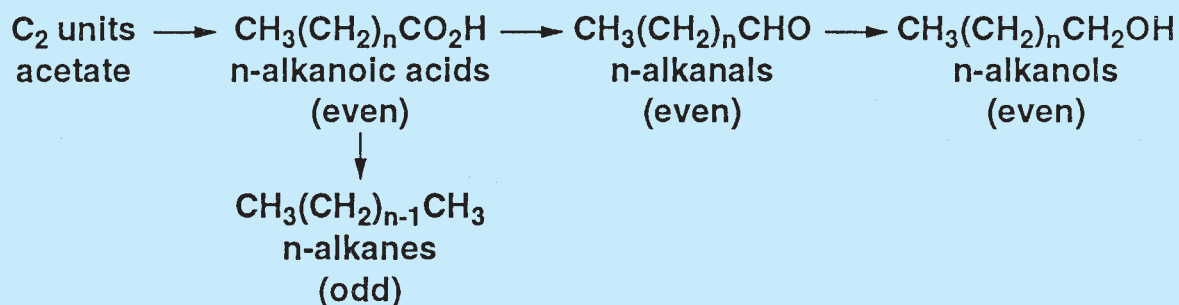
The biosynthetic reaction sequences will generate intramolecular order by processes that involve :

1. The use of pre-synthesised units;
2. The sequential coupling of such units;
3. The control of functionality and of any cyclisation steps introduced;
4. Stereochemical controls of all steps, including the chirality of any asymmetrically substituted carbon or other centres;
5. The isotopic fractionations introduced during the fixation of inorganic carbon, the coupling of units, and in functionality changes.

Intermolecular order is also introduced by the control of the amounts of the different compounds biosynthesised. For a homologous series of compounds, this frequently results in characteristic molecular distribution patterns whereby homologues maximise around a particular carbon number, with the amounts of the other homologues decreasing rapidly to lower and higher carbon number.

Molecular distributions of this type are characteristic indications of the action of living organisms. This is true of individual species, whole groups of families, orders and, in fact, throughout the kingdoms of living organisms. Thus, straight-chain carbon compounds are synthesised almost universally by the acetate pathway, operated by an evolutionary highly conserved polyketide enzyme system. The result is an assemblage of long-chain compounds distributed over a range of carbon numbers that often maximises at a single carbon number and displays a marked odd-over-even or even-over-odd dominancy. These distributions and accompanying carbon isotope characteristics are seen in extracts of sediments from the present time right back to the Archean period. Thus, the contemporary biota use enzyme systems inherited more or less unchanged from the Archean. Similar carbon number distribution systematics apply to other biosynthetic molecular assemblages such as isoprenoids and oxygenated aromatics. Hence, a number of characteristic biosynthetic patterns of carbon compound abundances are obvious in the extracts of both recent and ancient sediments. Abiotic reaction systems are not capable of producing such highly ordered distribution patterns. Furthermore, there are within each organic compound further imprints of their biosynthesis in the isotopic values of each carbon atom. Such imprints must also reside in any atoms other than carbon in the structures, notably hydrogen, oxygen and sulphur, but this area of knowledge remains almost entirely unexplored.

Fig. I.4.3.2.3/2 is an example of such a homologous series. The four different groups of compounds (acids, aldehydes, alcohols and hydrocarbons) are all biosynthetically related through reduction and decarboxylation steps. The alkanolic acids are



typically synthesised by almost all organisms from C2 units as a homologous series reaching to C30 (with a marked dominance of even-over-odd carbon number). Correspondingly, the derived series of alkanals and alkanols display a similar even-over-odd predominance. In contrast, the alkanes (formed by decarboxylation of the acids) show a predominance of odd carbon numbers. These biological patterns of biomarker distribution carry through into sediments. However, the odd-over-even dominance in the n-alkanes is gradually lost when these compounds are subjected to geothermal alteration.

In general, these straight-chained homologues do not carry such organism-specific information, since they are of such universal biosynthesis. Only their  $^{13}\text{C}/^{12}\text{C}$  compositions remain significantly informative, because these are controlled by the respective ratios of the C2 precursor units which are, in turn, dependent upon the photosynthetic pathway of carbon dioxide fixation, as discussed in I.4.3.

#### *Imprint of the Biosphere in the Geosphere*

Standard methods for examining living biota, when applied to young immature sediments, reveal morphological evidence of decayed biota as microscopically recognisable organic debris and molecular evidence as detectable concentrations of simple and complex biochemicals of both original and diagenetically modified structures. For example, amino acid analyses of teeth, bones and shells in young sediments display distributions characteristic of proteins which reveal increased racemisation with related temperature and time of burial, a measure that can even be used for dating and chronostratigraphy. Similar considerations apply to other types of molecules, e.g. steroids, triterpenoids, alkaloids and the whole suite of secondary metabolites synthesised by the enzymes.

The initial record is very close to that of contemporary biochemistry, with small amounts of fairly well-preserved DNA, protein and carbohydrates still detectable. Most of this initial record is rapidly removed within a few tens of years, principally by microbial action. However, removal and destruction is selective, patchy and incomplete. Thus, the end result is that relatively easily biodegraded compounds and biopolymers survive in small to trace amounts for thousands to millions of years in aquatic sediments that have not been deeply buried and subjected to geothermal heating. Some depositional microenvironments e.g. frozen sediments, anoxic sediments, organic resins, copal and amber, result in an exceptional preservation of biochemicals. Certainly, low temperature, the restriction of access afforded by the close packing of clay particles, absence of reactive species such as free radicals, lack of nutrients (e.g. electron donors such as nitrate, sulphate), and lack of liquid water all have a preservative role. At the molecular level, the fundamental factors facilitating preservation seem to be restrictions on the mobility of molecules and increased steric hindrance at reactive centres, both intra- and intermolecularly. All molecules are ultimately degradable, whether by microbiota, reagents and heat, operating through time, either singly or together. But differences in intramolecular bond strengths are fundamental controls on molecular persistence, especially in

**Fig. I.4.3.2.3/2. A homologous series of long, straight-chained, aliphatic lipid biomarkers, which are biosynthetically related through reduction and decarboxylation steps.**

regard to non-biologically mediated reactions. Thus, novel aliphatic type and aromatic type biopolymers, whose skeletons are based essentially on carbon carbon bonds, have been detected recently in several species of aquatic unicellular algae, and have been shown to persist in significant amounts in both recently deposited and ancient sediments. These biomaterials, or rather their alteration products, may make up a significant portion of sedimentary organic matter.

#### *Bulk Terrestrial Organic Matter*

The bulk (circa 98%) of the organic matter held in the sedimentary rocks of the Earth's crust is the insoluble, amorphous, heteropolymeric organic 'kerogen'. In young, immature sediments it is rich in hydrogen and contains other elements such as oxygen, sulphur and nitrogen in lesser amounts, while in old, mature sediments it is highly carbonaceous, even graphitic, and is very low in hydrogen and elements other than carbon. Whatever its state, kerogen is almost entirely derived ultimately from biological debris which, when immature, contains component pieces of biomacromolecules and smaller metabolites crosslinked into the kerogen structure by carbon-carbon, carbon-sulphur and other bonds. Some components may be trapped in the molecular interstices, while others may have suffered additional modification through oxidation and other processes. Experiments specifically designed to release molecular moieties from this heteropolymeric material have shown that the kerogen matrix has protected some components from diagenetic and other changes experienced by free unbonded biomarkers in the same sediment. However, the original biosynthetic order incorporated as molecular components of the kerogen is gradually obscured and destroyed by the diagenetic processes in the sediment. Indeed, deep burial with accompanying rise in geothermal temperature, pressure and the passage of time eventually erases almost all of the original biosynthetic order as the carbonisation proceeds, e.g. the biosynthetic dominance of odd-over-even carbon numbers in the C30 region of plant wax alkanes is gradually replaced by a smooth distribution at lower carbon numbers, as the molecules are broken down through thermally-induced carbon-carbon bond breakage.

Improved analytical methodologies are now needed to maximise the retrieval of biomarker information from kerogens. We need to release the bound and trapped biochemical moieties with minimal loss of order, both intramolecular, in the form of detailed molecular structures, and intermolecular in their relative abundances. Pyrolysis techniques are good candidates, especially where the duration of the heating step is extremely brief and recombination of the pyrolysates is minimised. The analysis of the pyrolysates by gas chromatography/mass spectrometry (GCMS) provides information on the nature of the original organic material contributed to the kerogen. The pyrolysis provides a reflection of the original biosynthetic order in the structures of the compounds released, their abundance patterns and their isotopic distributions.

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# I.5 Potential Non-Martian Sites for Extraterrestrial Life

The outer regions of the icy satellites have water (albeit frozen) in abundance. One of the necessary conditions for life is thus met. We can conceive of life existing on or in an icy satellite provided there are sites where nutrients and an energy source are available.

Hot vents on Earth's deep ocean floors (see I.3.2.1) are sites where bacteria and bacteria-like organisms (the hyperthermophilic archaea) feed off the supply of chemical energy, and are entirely independent of energy from the Sun. Life on Earth may have begun in such a setting, perhaps at bubble membranes between alkaline spring-water and acidic sea-water (Russell & Hall, 1997). Many of the icy satellites probably once hosted comparable environments, and a few may still do so today. Given an adequate source of heat within an icy satellite, we would expect water to be drawn down into the rocky core, to emerge elsewhere as a hot saline fluid bearing many constituents dissolved from the rock. These could act both as nutrients and as an energy source to sustain life, in the same way as the effluent at hydrothermal vents on the Earth's deep ocean floors. There is a growing body of evidence that life was established on Earth no more than 700 million years after the planet's formation (Mojzsis et al., 1996; Schopf, 1993), and so the limited duration of comparable conditions on most icy satellites cannot be used as an objection to the development of life in those settings.

Photosynthesis is a less credible energy source of life in icy satellites, for two reasons: 1) the distance from the Sun means that each square metre of Jupiter's satellites receives only 4% the amount of solar power incident on Earth; 2) it would be viable only in the uppermost few metres of ice, a long way from any easily replenishable source of nutrients (unless these could be supplied by cometary infall). The latter drawback is least serious in those bodies undergoing (or have recently undergone) resurfacing by processes capable of bringing fresh nutrients from below (e.g. in the form of minerals dissolved in brines), so whether searching for hydrothermally-sustained or photosynthetic life it is the active or recently active icy satellites that are the primary targets.

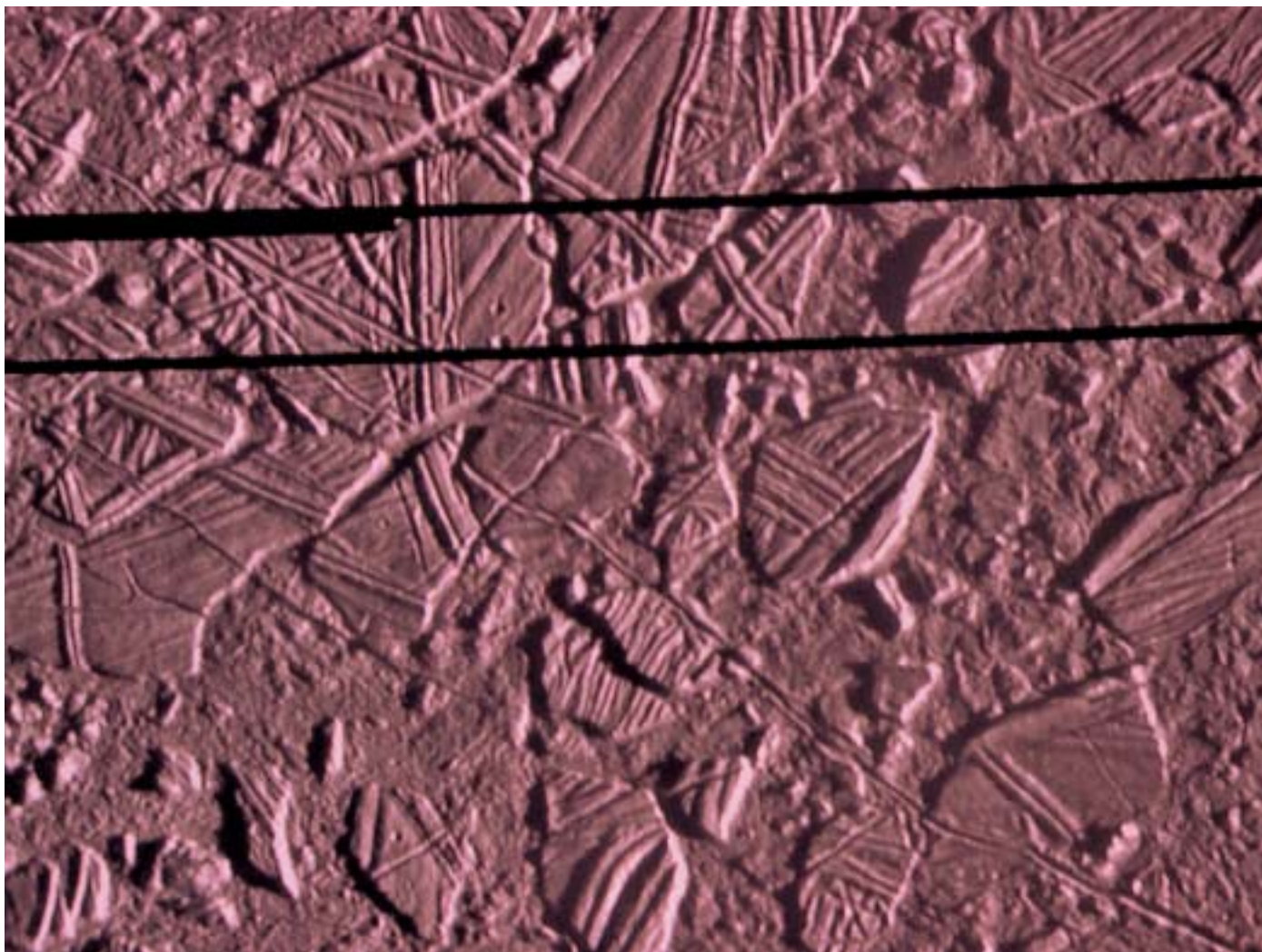
## I.5.1.1 Europa

It is very likely that Europa is heated by tidal forces at present. Europa's 2:1 orbital resonance with Io is the cause of the tidal heating that makes Io the most volcanically active body in the Solar System, and we expect a smaller, but far from negligible, rate of heating within Europa. Dissipation of tidal energy within Europa's rocky layer would require heat to be transported to the surface through its ice/water shell. The Voyager images showed very few impact craters on Europa's surface, confirming recent (probably continuing) resurfacing by cryovolcanic and tectonic processes. Galileo images have revealed more small impact craters, but too few to demonstrate that resurfacing has ceased (Belton et al., 1996).

Europa has the thinnest icy carapace of any large icy satellite. In the absence of seismic data, its thickness cannot be determined with certainty. Density arguments depend on the size of Europa's metallic core and the extent of hydration of the outer part of the rocky layer. An estimate based on the first two Galileo encounters with Europa has an ice (or ice plus water) shell 100-200 km (Anderson et al., 1997).

The existence of a global water ocean between Europa's ice and the rocky interior is controversial, but Galileo's high-resolution images (Figs I.5.1.1/1 and I.5.1.1/2) make it very hard to deny at least the temporary occurrence of localised melts within or below the icy layer. The final scale of the texture of some of the ridge and groove patterns can be used to argue in favour of thin-skinned tectonics (with a deforming

## I.5.1 The Icy Satellites



**Fig. I.5.1.1/1.** A 34×42 km area of Europa imaged by Galileo, where ice floes have apparently drifted apart, either floating on a liquid sea or lubricated at their bases by a mushy icy layer (P-48526, NASA).

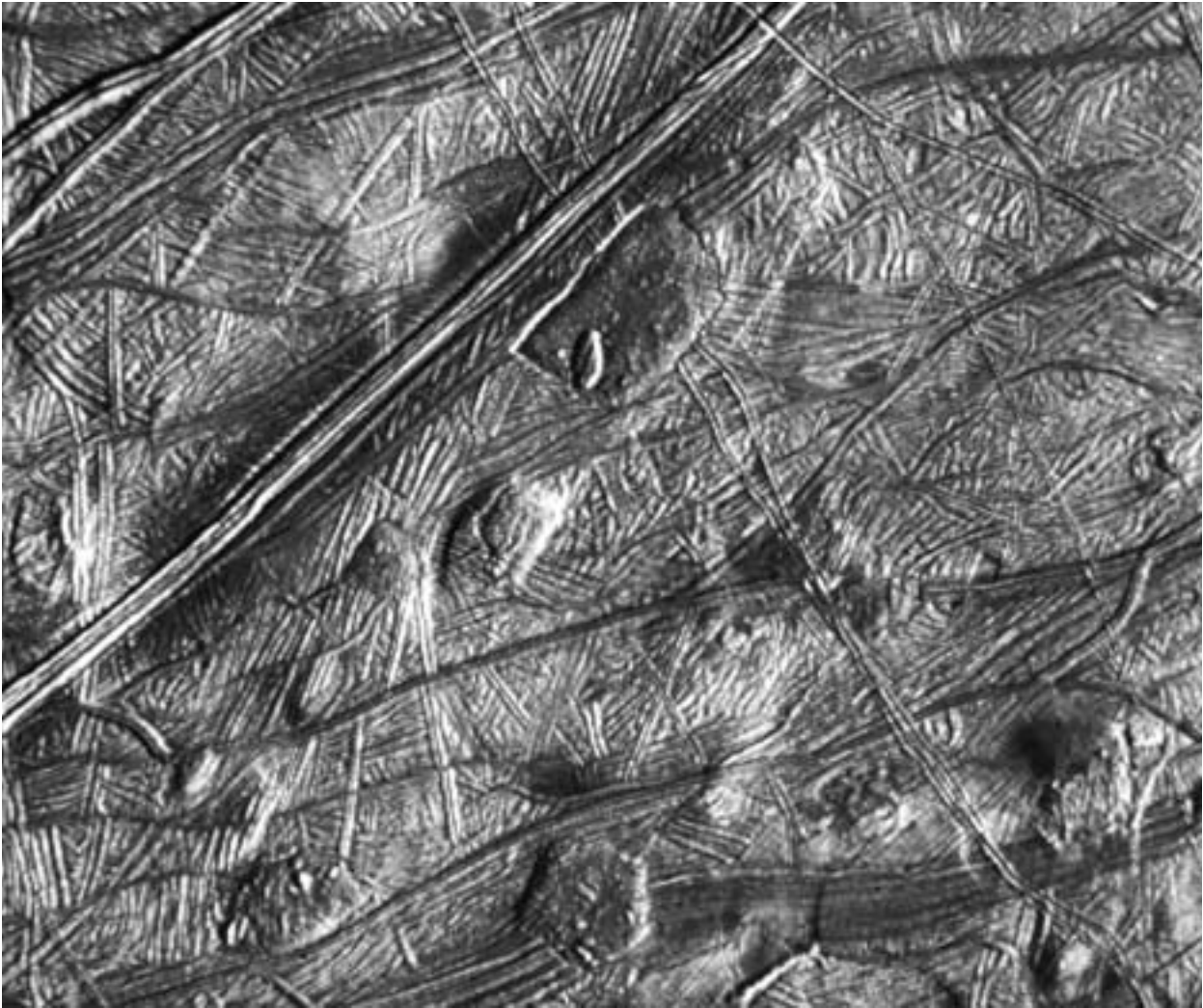
layer of the order of 1 km thick), but clearly the ice is generally much thicker than this.

The most likely sites for extant life would be at hydrothermal vents below the most recently resurfaced areas. To study these directly would require making a borehole through the ice in order to deploy a robotic submersible. The technology for such submersibles already exists, but the means of penetrating ice of unknown thickness poses a greater challenge. However, biological processes in and around hydrothermal vents could produce biomarkers that would appear as traces in cryovolcanic eruptions and thereby be available at the surface for *in situ* analysis or sample return. Mineral nutrients delivered through cryovolcanic eruption would make the same locations the best candidates for photosynthetic life.

#### **I.5.1.2 Ganymede**

Large tracts of Ganymede have been resurfaced by some kind of tectonovolcanic process. Galileo images reveal that the ridge and groove patterns are repeated on a scale an order of magnitude smaller than that of those discovered by Voyager (Belton et al, 1996). However, the density of superposed impact structures even of Ganymede's youngest terrains indicates that this activity ceased long ago (probably more than 1 Gyr). Moreover, Ganymede's ice is probably more than 1000 km thick, so mineral nutrients are likely to be very deeply buried. Thus, although hydrothermally sustained life at the ice-rock interface could have been viable in the distant past, the prospects of finding biomarkers in the present surface ice are much poorer than for Europa.





**Fig I.5.1.1/2.** An 80×95 km area of Europa where the youngest features are domes that are probably viscous cryovolcanic flows or sites of shallow intrusion. Either explanation would make these features good sites to search for traces of life. (Galileo image released 18 April 1997, NASA.)

### **I.5.1.3 Other Icy Satellites**

Several other icy bodies have experienced episodes of tidal heating, and moreover they would have had hotter interiors in the distant past when radioactive decay was a more potent heat source. Saturn's small satellite Enceladus has terrains where the impact crater record has been erased by recent resurfacing. This indicates an episode of enhanced tidal heating that could have supported hydrothermal life. Indirect evidence that hydrothermal reactions have occurred in icy satellites comes from the apparent absence of carbon monoxide and the preponderance of methane in outer Solar System ices (Simonelli et al., 1989). This could have been caused by the oxidation of rock, and may have been organically mediated. Neptune's large satellite Triton has abundant methane and a degree of relatively recent cryovolcanic resurfacing and ongoing geyser activity (Soderblom et al., 1990) that make it the most enticing target beyond Saturn, as discussed below.

### **I.5.2.1 The Titan Atmosphere**

Titan's atmosphere was revealed mainly by the Voyager-1 mission in 1980, which yielded the bulk composition (90% molecular nitrogen and about 1-8% methane). Also, a great number of trace constituents were observed in the form of hydrocarbons (acetylene, ethane, ethylene, etc) nitriles (HCN, C<sub>2</sub>N<sub>2</sub>, HC<sub>3</sub>N) and oxygen compounds (CO and CO<sub>2</sub>). Recent analysis of the Voyager-1 spectroscopic data provided precise

## **I.5.2 Titan**



Fig. I.5.2.1/1. Titan's atmospheric longitudinal composition variations. (From Coustenis & Bézard, 1995.)

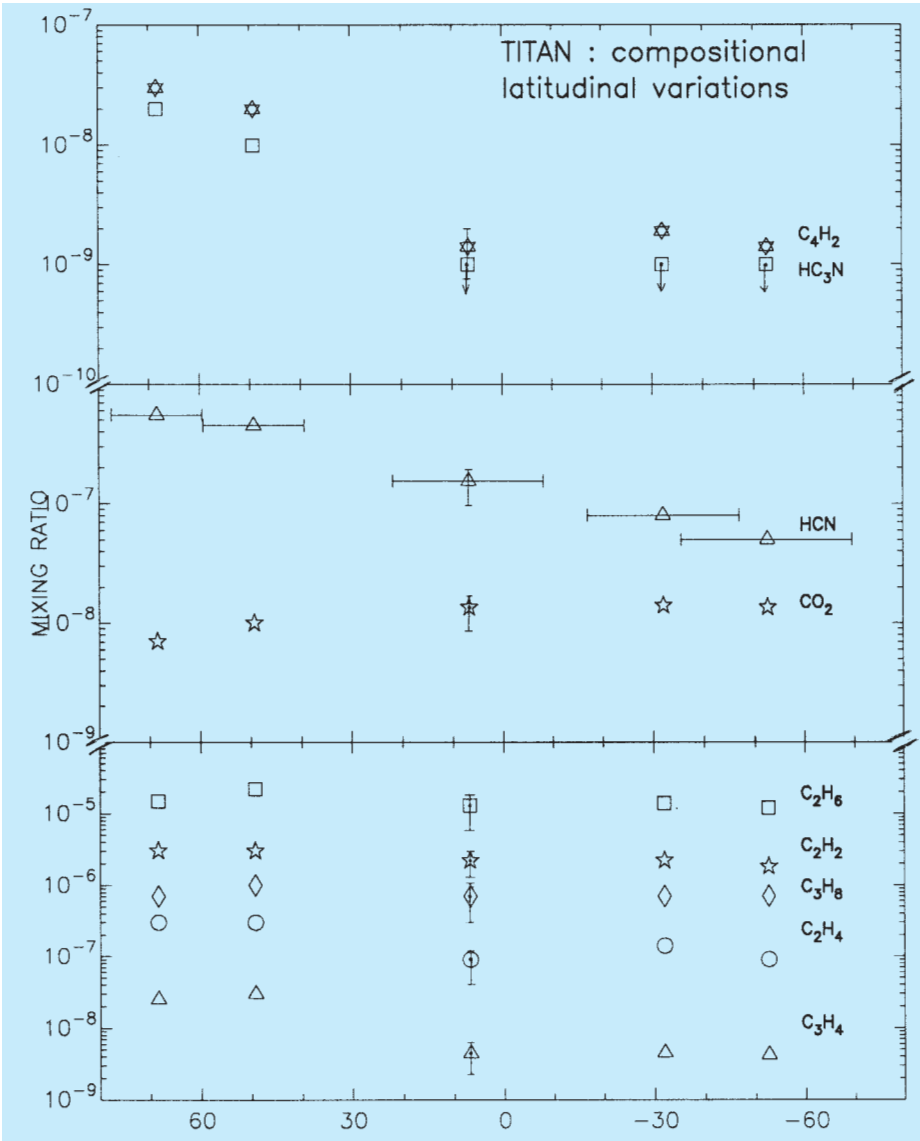
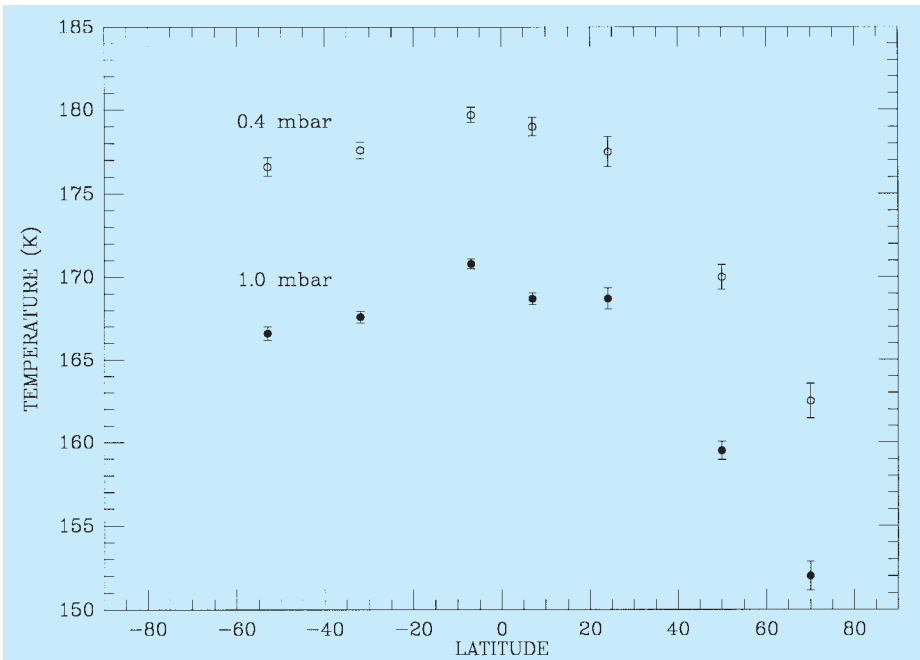


Fig. I.5.2.1/2. Variation with latitude of the temperature of Titan's upper atmosphere. (From Coustenis & Bézard, 1995.)



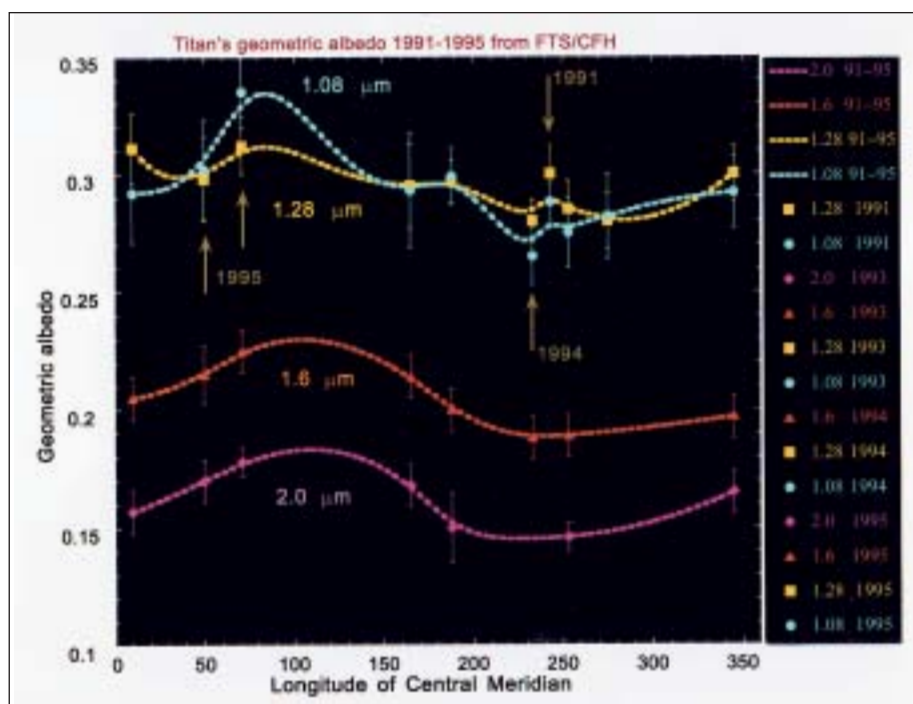


Fig. I.5.2.2/1 Titan's geometric albedo, 1991-1995 (Coustenis, private communication).

abundances at various locations over Titan's disc (Coustenis & Bézard, 1995). Seasonal effects caused an enhancement of some of the less abundant minor components over Titan's North Pole at the time of the Voyager encounter (Fig. I.5.2.1/1).

The exact degree of complexity of the organic chemistry active in Titan's atmosphere is not well known. More complex organic compounds are still being discovered in gaseous or solid form in the atmosphere.

Titan is the only other object in our Solar System to bear a strong resemblance to our own planet in terms of atmospheric composition and temperature structure. The latter shows an inversion near the tropopause and increases towards the surface ( $T=94\text{K}$ ) owing to a small greenhouse effect. The temperature profile in the stratosphere also varies as a function of latitude (Fig. I.5.2.1/2).

Measurements by ESA's Infrared Space Observatory (ISO) in January 1997 have confirmed models based on the Voyager data (Coustenis et al., 1993), and are still under analysis. They provide more accurate abundance measurements in the grating mode (although on a disc-averaged basis) and even provide vertical distributions for some of the minor components by use of the Fabry-Perot mode.

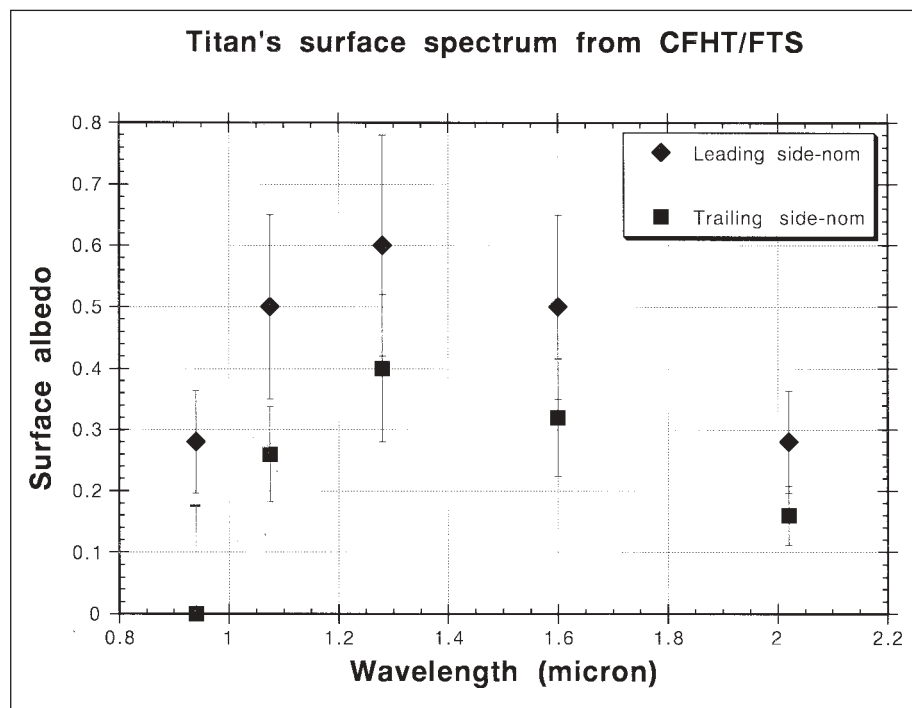
Water vapour was discovered in Titan's atmosphere by ISO/SWS (Coustenis et al., 1998).

### I.5.2.2 The Titan Surface

The surface of Titan has never been viewed directly because of the thick cloud deck surrounding the satellite. Models have predicted the existence of oceans or large lakes of hydrocarbon material on the ground. These models have recently been disputed by ground-based and Hubble Space Telescope (HST) spectroscopic observations.

Observations of Titan have been performed with the Fourier Transform Spectrometer at the CFH Telescope in Hawaii. The spectra were recorded over a period of 5 years (1991-1996) and cover most of Titan's orbit (16 days) in the 1-2.5  $\mu\text{m}$  region. The observations show that, within an orbit, Titan's geometric albedo exhibits significant variations indicative of a brighter leading hemisphere (facing the Earth) and a darker trailing one (Fig. I.5.2.2/1). The maximum albedo appears near  $120 \pm 20^\circ$  LCM (Longitude of Central Meridian) and the minimum near  $230 \pm 20^\circ$  LCM. The variations (25-35%) have been observed independently by various groups since 1989 and they are recurrent. They must therefore be correlated with Titan's

Fig. I.5.2.2/2. Modelled Titan surface spectrum (Coustenis et al., 1999, in preparation).



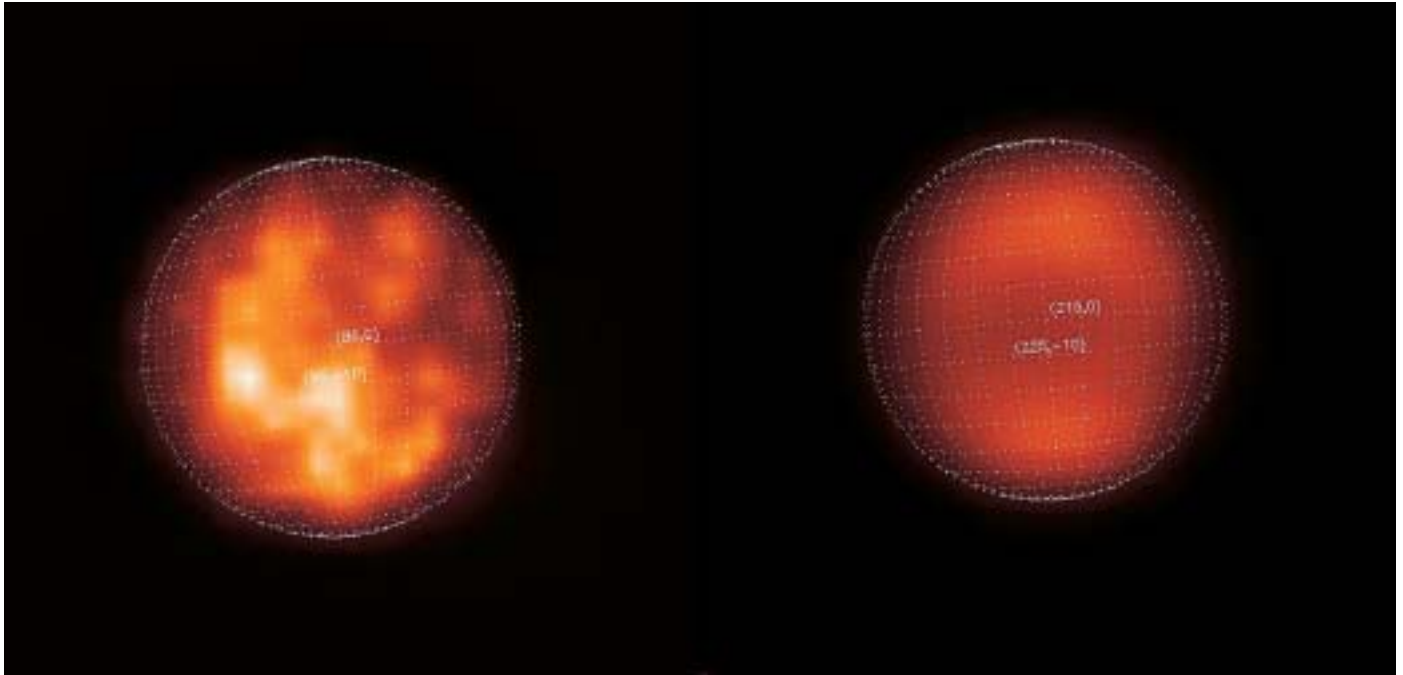
surface morphology, rather than atmospheric cloud structures.

The Titan surface spectrum, as modelled from the observations (Fig. I.5.2.2/2), is remarkably consistent with a mostly solid surface dominated by water ice and it therefore resembles that of other satellites, such as Hyperion or Callisto (Coustenis et al., 1995).

Spatially-resolved images of Titan, using the European Southern Observatory (ESO) 3.6 m. telescope and ADONIS optics, were obtained in October 1993, September 1994, October 1995 and November 1996 (Coustenis et al., 1997). The images were recorded through a set of narrowband filters (K1, H1, J1), defined in order to maximise the contributions of the light reflected by Titan's surface in the total signal recorded at the centre of the 2.0, 1.6 and 1.28  $\mu\text{m}$  transparency windows of Titan's atmosphere. At those three wavelengths, Titan's atmosphere is transparent because of very low molecular methane absorption. In order to allow the subtraction of the stratospheric contribution and hence improve the visibility of ground features, images were recorded in adjacent narrowband filters (K2, H2, J2) in the wings of the strong methane bands.

The 2.2  $\mu\text{m}$  ADONIS images show the expected north-south asymmetry in the stratosphere, the South Pole brighter than the North Pole, reversed with respect to Voyager and due to seasonal effects. After deconvolution, correction of centre-limb effects and subtraction of the atmospheric component, the 2.0  $\mu\text{m}$  surface images show a bright well-defined equatorial spot at around 120° LCM (leading hemisphere) extending over 60° in longitude and 30° in latitude, and some smaller and less contrasted features near the poles, all rotating over six consecutive nights at the expected rotation rate of Titan's solid body. The area attributed to ground features is in agreement with HST images (Smith et al., 1996) taken near 1  $\mu\text{m}$ , both as to the location and the shape. The spatial resolution is at the limit of diffraction (0.13 arcsec at 2.0  $\mu\text{m}$ ), very similar to the HST images. Moreover, the ADONIS images of Titan's trailing hemisphere show high-latitude bright zones with a north-south asymmetry; the northern latitudes appear brighter by about a factor two with respect to the south (Fig. I.5.2.2/3). These bright zones might exist over Titan's entire disc.

The entire surface of Titan appears quite dark in the water ice bands, in agreement with spectroscopic measurements by Coustenis et al., 1997. The model of a global ocean covering the surface must be ruled out. It is inferred that large cloud structures



are not present in the troposphere. It is suggested that the observed quite complex bright zones may be related to local portions covered by icy methane (or ethane), which is bright at  $2\text{ }\mu\text{m}$  and is plausible at temperatures below 90K, expected for example at the poles, or on top of a mountain near the equatorial regions. Another possibility for the polar brightness would be a permanent polar cloud deck (or mist) in the low troposphere. Patchy methane clouds have been suggested for the tropopause.

**Fig. I.5.2.2/3.** Images of Titan's surface in the near-IR. At left is the leading hemisphere; the trailing hemisphere is at right. (From Combes *et al.*, 1997.)

### I.5.2.3 The Cassini/Huygens Mission

Launched in October 1997, the spacecraft will arrive in the vicinity of Saturn in 2004 and perform several flybys of Titan, making spectroscopic, imaging, radar and other measurements. The Huygens Probe will penetrate the atmosphere to reveal its true nature.

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## I.6 Science and Experiment Strategy

*‘Dans le champs de l’observation le hasard ne favorise que les esprits préparés.’ – Louis Pasteur, 1854.  
(‘Where observation is concerned, chance favours only the prepared mind.’)*

Pasteur’s observation is particularly appropriate in the context of the search for life beyond the Earth. There is inevitably a large measure of chance involved in finding evidence of extinct microbial life, and especially so when searching for existing life. But the chances of success can be improved by taking note of the lessons to be learned from the Viking mission, from the relevant studies on Earth, as discussed in Sections I.3 and I.4, and from the information derived from studies of the Martian meteorites, discussed in Section II.3.

To maximise the chances of clear and unambiguous conclusions on the existence of extinct or extant life in any analysis, it is absolutely essential that the coverage of search instrumentation be broad enough to reduce the opportunities for alternative interpretations. At the same time, each component instrument must be carefully designed so that it does not form a serious weak link in the chain of observations across the instrument array.

Consequently, the search science strategy is to provide, as far as possible, a self-consistent set of instruments that will give concrete evidence, for or against, the existence of a range of biosignatures and associated images at a given search site. In order to do that, an integrated package of analysis instruments is required.

What must not be attempted, under any circumstances, is a search for biosignatures with inadequate instrumentation. It would be foolhardy to attempt to establish the existence, or otherwise, of life signs on Mars for example, by the use of a small narrowly-based measurement unit located at one inadequately characterised landing site. The inevitable resulting confusion would simply discredit the subject of exobiology.

Clearly, a strategy relying on the careful collection of samples from the planet and their return to Earth is highly desirable. The experience from the lunar programme confirms this. Indeed, this is the route preferred by NASA in the search for life on Mars. The returned samples can then be subjected to investigations using the full spectrum of instrumentation available on Earth by a diverse range of experts.

The problem with such a strategy is that, quite apart from the cost and technical difficulties, it would be leaving too much to chance to mount such a project before carrying out a careful programme of exploration and *in situ* analysis at several selected locations on Mars. We do not yet know, for example, from what depth samples must be obtained in order to be entirely free from the effects of the surface oxidant. Nor do we know which of the currently listed sites on Mars are actually the most interesting. There is much to be done, and done well, before a sample return mission should be attempted.

In the meantime, a sophisticated automated analysis system of the type considered here can pave the way with essential observations, as well as including a large amount of information on geochemistry and mineralogy. It can do this with a reasonable chance of succeeding in the first detection of the signatures of life.

In the preceding chapters we have seen that life as we know it relies upon the existence of water, above all else. Without that, life ceases or goes into a quiescent mode. Hence, a search for extant life translates in the first place into a search for liquid water. This may lie at some considerable depth below the sterile surface of a planet, as could be the case in certain areas of Mars. An orbital survey to define the water-ice boundary across Mars is of paramount importance. In the case of Europa, the water-ice boundary is likely to be many kilometres below the icy carapace, except in localised regions subjected to cryovolcanic flows.

### I.6.1 Where to Search?

TABLE I.6.2/1

## EVIDENCE OF EXTANT LIFE

**1. Structural Indications:**

*Microscopic Observation:* of cells and subcellular structures. Size distributions, dividing cells, and selective dyes to identify specific cellular components, e.g. acridine orange can indicate nucleic acid.

*Confocal Laser Scanning Microscopy:* provides very high-resolution 3D imagery, including observation of communities inside transparent rocks.

*Electron Microscope:* to provide nm-resolution of cell walls, membranes, vacuoles, etc.

*Macroscopic Observation:* of large (10 mm) microbial communities in, e.g. sandstone and evaporites. Desert crusts of cyanobacteria and mats on hypersaline lagoons may be cm thick.

**2. Culture Indicators:** isolation and successful culturing, with subsequent biochemical analysis, for example, of nucleic acid sequencing, protein, lipid and sugar content, provides the most unequivocal evidence of extant life.

**3. Metabolic Indicators:** the chemical products of metabolism, in particular the gaseous products, can be observed in culture or *in situ* colonies, using isotopic tracers or direct analysis, e.g. gas chromatography. The Viking experiments were based on testing for carbon assimilation, catabolic activity and respiration.

**4. Isotopic Indicators:** the discrimination against  $^{13}\text{C}$  relative to  $^{12}\text{C}$  during enzymatic uptake of carbon in photosynthesis is a valuable biomarker, whose record extends back 3.5 Gyr. Combining compound-specific isotopic data with diagnostic structural information permits specific characterisation.

**5. Chirality Indicator:** homochirality is a characteristic of life; without it, polymerisation and template replication do not proceed effectively. Determination can be by observing optical activity.

**6. Spectral Observations:** vibrational spectroscopy, e.g. Raman spectroscopy, can detect a variety of organic compounds involved in living systems.

To sustain a water environment requires a heat source. In the case of Mars, that is from the internal outflow. In the case of Europa, it is likely to be due to the tidal forces, as discussed in Section I.5.1.1.

Even in the case of extinct life, the search strategy will inevitably focus upon sites originally occupied by bodies of water over extended periods of time. Hence, martian search sites will be sedimentary deposits in ancient lake beds, outflow points of past water channels and hydrothermal vents, as discussed in Section II.2.7.

Given that water is now unstable on the martian surface, solar energy is unlikely to be a determining factor as regards any existing life, although it may well have provided a significant energy source to surface microbial life in earlier aeons. Consequently, a search for extant life on Mars will likely focus on a search of hydrothermal vents and the subsurface regions, if evidence is obtained of the existence of a water table at accessible depths.

Current research on subterranean life on Earth (Section I.3.6) points to a thriving biosphere at depths several hundred metres below the surface. It would not be surprising therefore to find evidence of similar microbial activity on Mars, and possibly on other bodies, where there are active subterranean water supplies heated by internal energy sources and carrying the necessary nutrients to support life.

In answering the question ‘Where to search?’ in the general sense, the strategy must therefore be to look to a planet where the above conditions seem to exist and about which there is a solid body of survey information available. That is clearly the case with Mars and it is therefore recommended as the prime location to search for life.

Several further Mars survey missions are already underway, including one from ESA for 2003. This Mars Express mission will probably include a Lander and with that perhaps the possibility of a serious attempt to search for evidence of extinct life on Mars.

*Given that opportunity, Europe has the possibility to be among the first to discover evidence of life beyond the Earth.*

TABLE I.6.2/2

## EVIDENCE OF EXTINCT LIFE

**1. Structural Indicators:**

*Microscopic Observations:* of groups of possible microfossil structures in sedimentary deposits, including petrological analysis of the associated minerals and the study of any residual carbonaceous matter.

*Electron Microscopy:* to provide nm-resolution study of the structure of possible microfossils detected by optical microscopy and to search for 'nanofossils'.

*Atomic Force Microscopy:* to obtain 3D images of the structure, at nm-resolution, of possible microfossils and associated material.

*Macroscopic Observation:* of stromatolite-type biosedimentary structures in freshly exposed scarps or in rock sections, using a telemicroscope or telescope with IR or Raman spectroscopic capability to confirm the mineral and carbonaceous constituents.

**2. Biogeochemical Indicators:** from the determination of the elemental abundances in reduced (organic) carbon remnants using a Gas Chromatograph/Mass Spectrometer (GCMS) and Alpha-Proton-X-ray Spectrometer (APX), to give e.g. H/C ratio as index of aromaticity by assay of the 'organic' to 'carbonate' carbon content of fossil-containing sediments from an analysis of the volatile (hydrocarbon) component of the reduced carbon constituents in associated sedimentary material, using pyrolysis/gas chromatography/mass spectroscopy.

**3. Isotopic Indicators:** the enhancement of the  $^{12}\text{C}$  isotope to  $^{13}\text{C}$  during conversion of inorganic carbon to organic in autotrophic fixation is retained in biogenic carbon residues. It provides a distinct biomark when compared with the inorganic carbon pool. The Pyrolyser/GCMS is used. An Ion Microprobe is used for microstructures. Similarly, D/H fractionation and  $^{34}\text{S}/^{32}\text{S}$  isotope composition change between sulphides and sulphates can be indicators of earlier biological activity.

**4. Molecular Indicators:** certain distinct biochemical compounds, e.g. lipid type and pigments, may withstand degradation over very long periods as part of the kerogen residue. They may be extracted by organic solvents from residues and fossils for analysis (including chiral and isotopic) to obtain an indication of the nature of the original body.

**5. Chirality Indicator:** homochirality is characteristic of life and this structural feature is retained on death of the organism. Racemisation proceeds very slowly under cold dry conditions such as now exist on Mars. An optical rotation detector is used.

**6. Spectral Observations:** vibrational spectra of organic compounds provide a valuable analytical tool which can be applied to help unravel the nature of sedimentary carbonaceous samples of biological and abiotic origin. It also is able to identify the mineralogical content of the associated sediment. IR and Raman spectroscopy predominate and can be included in the microscopy.

The chances of finding evidence of existing life on a planet at this point are remote. We cannot reach deep below the surface with any equipment transportable on current lander missions. Nor would we know just now where to look. Perhaps a future detailed survey will identify an active surface hydrothermal system by means of water vapour emission and a thermal signature. If so, a specialised Lander to investigate that region would be warranted. At the moment, however, we must concentrate the search on evidence of extinct life. To that end, it will be necessary to provide the following:

**I.6.2 What to Search For?**

- i. clear images of groups of fossil 'organisms' and high-resolution studies of individual structural features;
- ii. accompanying geochemical and mineralogical evidence, in the form of biosignatures, which are related both spatially and temporally (in geological terms) with the imaged structures;
- iii. organic residuals, shown to be of biological origin.

Other than these small-scale features, we may also seek evidence of extinct microbial matting communities through the observation of macroscopically visible laminations, the so-called stromatolite structures. However, confirmation of a biological origin for any such observed structures will still require, as on Earth, accompanying information of the type indicated in (ii) and (iii) above.

The main points are summarised in Tables I.6.2/1 and I.6.2/2.

### I.6.3 What to Search With?

The preceding Tables indicate the types of instrumentation that would normally be required in order to search for the evidence of extant and extinct life on Mars. Focusing upon the evidence for extinct life, we have the following requirements:

1. Optical Microscope, with a range from medium- to high-power to cover sample selection through to high-resolution observation of larger 'fossil'-like structures and associated minerals.
2. IR or Raman Spectrometer, designed to work in conjunction with the microscope system for analysis of individual grains and any associated organic material.
3. Atomic Force Microscope, allied to the stage of the optical microscope, to examine in 3D in the nm-range selected elements of the microscope field containing possible fossils.
4. Alpha-Proton-X-ray Spectrometer, to carry out elemental analysis (excluding H), on minerals associated with 'fossil' sediments and on any associated carbonaceous material.
5. Gas Chromatograph/Mass Spectrometer, to analyse the volatiles from carbon residues, to provide elemental, molecular, and isotopic abundances, and compositions. Various types of vaporisers can be used.
6. Ion Microprobe, could be valuable, in conjunction with the microscope, to analyse specific regions of the samples that are associated with 'fossils' and to give structurally defined  $^{13}\text{C}/^{12}\text{C}$  ratios for organic traces.
7. Homochirality detector, to search for optical activity. This may be a dedicated optical instrument using a liquid sample or it may be simply a chiral column included in the gas chromatograph.
8. Telescope, to search for macrostructures of possible biological origin in cleaved rocks or at outcrops. It would need to be associated with a spectroscopic capability.

Clearly, it will not be feasible to carry all of these instruments on a single small Lander. In making a selection of the instruments to form a unified package we have been conscious of the need to use instruments that have already flown or are under development for flight. In that context, we have looked to the European instruments that are already associated with a Mars programme or are being produced for a relevant ESA mission, such as Rosetta.

From that analysis, the following instruments were identified for further study at this point (others, especially a spectrometer, may be considered later if circumstances change):

Optical Telemicroscope  
Alpha, Proton, X-ray Spectrometer  
Atomic Force Microscope  
Gas Chromatograph/Mass Spectrometer  
Homochirality Detector

# I.7 Summary of the Science Team Recommendations

MARS is the prime target. The search for life on Mars is presented in Part II of this volume.

EUROPA and other bodies possibly having subsurface water, with the accompanying internal heat sources, are also candidates for both extant and extinct life searches in the future. NASA has an outline plan for a Europa Orbiter mission in 2004 and a Lander in 2011.

TITAN has an atmosphere of nitrogen (90%) and methane, together with a great number of trace hydrocarbons, nitriles and oxygen compounds (CO, CO<sub>2</sub>). Surface deposits of hydrocarbons have been predicted, although water ice is now considered to be dominant. Interest lies in the study of fundamental physical and chemical interactions driving a planetary organic chemistry and in the possible development of a life system in the absence of liquid water but with other liquids. The NASA/ESA Cassini/Huygens mission will deliver an atmosphere/surface probe to Titan in 2004. A NASA Titan Aerobot mission is indicated for 2013.

METEORITES provide a sample of various types of solar 'debris' and a sample of the material ejected from other Solar System bodies – Mars and the Moon being the sources of a small number of meteorites of identified origin. The Mars meteorites are being closely studied for evidence of extinct life. Efforts should be made to reduce the bias of the meteorite-collection process in the field, which tends to favour the more readily distinguishable meteorites of igneous origin. The acquisition of a sample derived from martian sedimentary material would be a major breakthrough and one that would likely yield important information concerning the possibility of life on Mars as well as the associated geochemistry.

COMETS were probably an important source of organics for the primitive Earth and other planetary bodies at that early epoch. Hence ESA's Rosetta mission, including a lander, to Comet Wirtanen, launched in 2003 and culminating in 2013. Rosetta will be a major step forward in determining the full nature of the range of cometary materials and establishing their likely role as precursors of life.

METEORITES and MICROMETEORITES have also been responsible for the import of major amounts of extraterrestrial organic material to the Earth. It has been estimated that they may have brought in about 10<sup>20</sup>g of carbon over the 300 million years of the late bombardment phase. This exceeds the carbon contained in the current biomass (10<sup>18</sup>g). Continuing studies on the organic components of the carbonaceous chondrites and improved collection of micrometeorites to allow unambiguous analysis of the organic components are needed. The latter will involve the further development of in-orbit collection techniques to be used on the International Space Station and other vehicles.

## I.7.1 The Search for Extant and Extinct Life

## I.7.2 The Study of the Precursors of Life



### **I.7.3 Organic Chemistry Processes and Microorganisms in Space**

ORGANIC MOLECULES, representing potential building blocks of pre-biological materials, may suffer degradation and racemisation under space conditions. It is therefore necessary to study the effects of space conditions on organics such as amino acids, sugars, lipids and nucleic acid bases. The potential protective effects of association with mineral dust particles will indicate the influences of the micrometeorite and cometary environments. Similar studies are also needed on the polycyclic aromatic hydrocarbons (PAHs). Experiments of this type will require access to the external environment of a space station or other vehicle with dedicated facilities.

MICROORGANISMS subjected to the space environment, especially the UV radiation and high-Z particle flux, will tend to be damaged progressively and die. It is possible, however, for certain types of organisms to survive in space over long periods, especially if screened within meteorite-like structures. Continuing experiments using space vehicles and eventually the International Space Station will improve the understanding of the underlying damage processes, the survivability of a range of organisms, and the possibility of life being distributed among the Solar System by meteorite delivery.

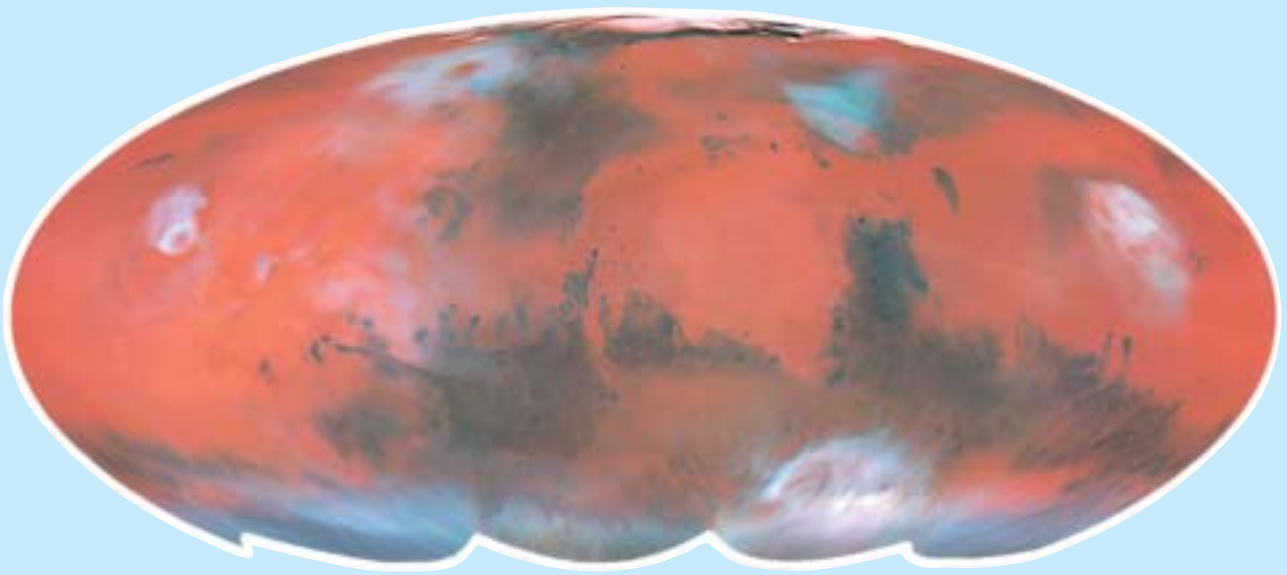
### **I.7.4 Laboratory-based Studies**

LABORATORY SIMULATIONS can provide valuable information on the organic chemistry processes in comets, on interstellar grains and in meteorites. They can lead to a better understanding of the processes occurring during the transformation of organic residues in sediments. Simulation of planetary environments and the study of the effects on microorganisms will have both fundamental and practical value.

LABORATORY STUDIES will continue to provide the essential fundamental experiments in the search to understand the earliest steps in the emergence of life. Careful studies of the most ancient sedimentary rocks may yet yield further information on that process. In conjunction with *in situ* experiments in the field, laboratory observations will continue to make important contributions to the knowledge of how life develops and survives under extreme conditions, including deep subterranean life and its application to the search for life elsewhere in the Solar System.

# II

## THE SEARCH FOR LIFE ON MARS



## II.1 Introduction

The Viking Lander experiments failed to detect any organic matter in the martian soil, either at the surface or from samples collected a few centimetres below the surface. The indications were that strong oxidation processes were at work at the surface. Subsequent theoretical studies have shown that photochemical processes, as well as the effects of oxidants such as hydrogen peroxide, are likely to be responsible for the destruction of all such material in the surface region. That will be true whether the organics are of an intrinsic martian origin or have been imported via carbonaceous meteorite and micrometeorite bombardment over geological time.

In order, therefore, to have any chance of detecting and analysing intrinsic organic material on Mars, it will be necessary to obtain samples from several centimetres below the chemically-affected 'rind' that covers the surface rocks. Similarly, if we are to explore the true regolith, it will be necessary to drill deep below the surface in order to escape the likely effects of the oxidant diffusion reactions. How deep is unknown and probably cannot be accurately modelled, because the effects of regolith 'churning' are highly uncertain. The approach adopted in this study was to aim for subsurface drilling to the maximum depth that is technically feasible on a given mission. Typically, this will be at least 1 m.

The chances of a successful detection of organics are greatly improved if the landing site is selected to be a major sedimentary basin, composed of highly compacted dense material. Not only will the oxidant penetration be more limited but also, by analogy with the situation on Earth, such sites are more likely to have hosted life at some earlier epoch.

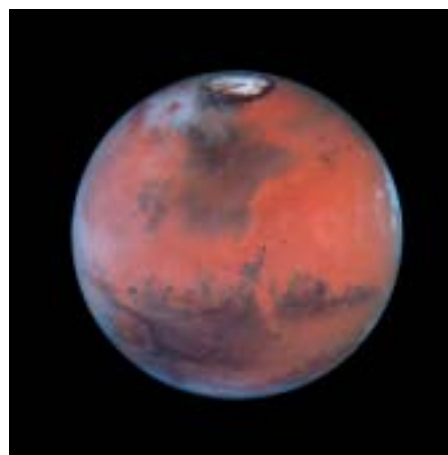
These combined aspects (oxidant-free samples collected from sedimentary domains) must be satisfied if there is to be any reasonable chance of finding organics on Mars and of increasing the likelihood of observing fossils of extinct life. To improve those chances further, mobility is necessary to extend the locations for such sampling. Together, these three requirements are fundamental to any future search for evidence of life on Mars.

While exciting in itself, simply detecting some basic organic compounds or observing some morphology set that looks like bacteria are unlikely on their own to confirm unambiguously that life once existed on Mars. Much of the discussion in Part I, which is extended here, is concerned with establishing a minimum set of interlocking measurements and observations that, *taken together*, are likely to provide convincing evidence for or against the existence of extinct, or extant, life in these samples.

The core of these experiments concerns the search for indicators or requirements for extant or extinct life. These analyses will include not only the search for carbonaceous residues and organics, but also particular inorganic minerals such as carbonates and phosphates, which can serve as biomarkers. The detection of water and its depth profile is obviously important. So, too, is the accurate determination of the isotopic ratios for hydrogen, carbon, oxygen, sulphur and nitrogen, which can give clear evidence of biologically mediated fractionations.

Besides the chemical and spectroscopic equipment needed for those analyses, microscopes are required to study mineral structures and to search for evidence of fossils, on scales ranging from about 1  $\mu\text{m}$  up to 1 mm. Observations and visual selection is also a prerequisite for subsequent sophisticated chemical and spectroscopic analysis, especially of localised areas.

It can be argued that *in situ* analysis could never match the results using samples returned from Mars, so why not wait until that opportunity arises? Apart from the



*S. Lee (Univ. Colorado), J. Bell (Cornell Univ.),  
M. Wolff (Space Science Inst.) and NASA.*

obvious delay in awaiting such an event and the uncertainties attending that endeavour, there are good reasons for performing a soundly based *in situ* programme of the type set out here. First, there is the question of terrestrial carbon contamination, at the levels detectable in these experiments. We have seen how the Apollo lunar samples became contaminated, despite all of the precautions. *In situ* experiments will minimise that problem. Apart from that factor, the combination of on-site analysis and the collection of samples by a mobile system is potentially a very powerful technique, if used correctly. It becomes possible to target specific types of samples, if required, or to expand the range and diversity. Coupled with a sample return mission, such a system provides major gains and can also reduce, by careful selection after analysis, the total amount of samples returned to cover a specified mineralogical range.

## II.2 The Planet Mars

Although the two Viking Landers touched down about 6500 km apart (Viking-1: Chryse Planitia, 22°N/48°W; Viking-2: Utopia Planitia, 44°N/110°W; both in northern lowlands), the chemical composition of the surface soil was found to be almost identical. Large areas of the martian surface and especially the lowlands are obviously covered with a soil thoroughly mixed by repeated dust storms. On the other hand, both the IR and gamma-ray spectrometers of Phobos-2 found considerable regional variations in the surface chemistry. The data indicate that the martian crust has a mafic nature (Mg- and Fe-rich), with only a low degree of fractionation.

The geology of Mars is highly diverse. The heavily cratered southern highlands represent the oldest surface, remaining topographically almost unaltered since the planet's accretion. They extend to almost two-thirds of the surface at elevations of 2-5 km above the northern plains. A large impact at the end of accretion has been discussed as the cause of this striking asymmetry. Compared to the lunar highlands, the craters of the martian highlands are highly degraded, indicating high erosion rates on the early Mars, perhaps owing to the warm and wet climate at that time. The lobed ejecta patterns of many of the craters there have been attributed to the pervasive presence of ground ice (Fig. II.2.1/1). There are also numerous branching valley networks that superficially resemble terrestrial river valleys (Fig. II.2.1/2).

The northern plains or lowlands cover most of that hemisphere. Their varying crater densities indicate that they formed throughout martian history. The origins of the plains are diverse. On some, numerous flow fronts can be seen, indicating formation by lava flows superimposed on one another. Evidence for lava flows is most common around the volcanic centres of Tharsis and Elysium. Volcanic features, but no flows, have been recognised on other plains. Wrinkly ridges in Lunae Planum have also been observed.

However, the vast majority of the low-lying northern plains lack obvious volcanic

### II.2.1 The Geology of Mars

**Fig. II.2.1/1.** Fluidised ejecta deposits around the crater Yuty. This 18 km-diameter crater is surrounded by ejecta flows created when the energy of impact melted the subsurface ice. This type of crater is common at equatorial and mid-latitudes, suggesting a subsurface cryosphere. (NASA)



**Fig. II.2.1/2.** The source area of the Ravi Vallis outflow channel (1°S/42°W), another indication of subsurface ice/water. This 300 km-long portion of the channel begins abruptly, without tributaries. It suggests that water was released under great pressure from below a layer of frozen ground, causing the surface to collapse. Fluidised ejecta can also be seen around the crater at bottom left. (NASA)





**Fig. II.2.2/1.** The central portion of the Valles Marineris (12°S/64°W). These two parallel troughs were created by tectonic activity and are about 500 km long in this section. (NASA/Lunar & Planetary Institute)



**Fig. II.2.2/2.** A landslide in the Valles Marineris trough. This collapse extends across about 20 km and reveals distinct layering in the subsurface region. Observation of such layers by a Lander telescope/spectrometer could be rewarding in the search for extinct life and for mineralogy/geochemistry. (NASA)



features. Many of their characteristics have been attributed to the action of ground ice or to their location at the end of large flood features where lakes must have been formed and sediments deposited. In some areas, particularly around the North Pole, dune fields are visible.

In general, the plains are complex in origin, having been formed by volcanism and sedimentation. They were then modified to varying extents by tectonism and wind, water and ice.

## II.2.2 Volcanism and Tectonism

One of the most prominent geophysical features of Mars is the large Tharsis bulge, a deformation greater than 4000 km across and up to 10 km high. Three large volcanoes (Arsia Mons, Pavonis Mons, Ascraeus Mons) are close to summit of Tharsis and Olympus Mons, the largest volcano on the planet and in the Solar System (550 km across, 27 km high) is located on its north-west flank.

The small number of impact craters on the flanks of the huge Tharsis volcanoes indicate that the uppermost flows are young. The large size of the volcanoes suggests that they have developed over a long time by a series of Hawaiian-style fluid lava eruptions in the absence of plate tectonics. Apart from this quiet effusion of fluid lava, there are also examples of more violent pyroclastic eruptions resulting in extensive ash deposits.

The crystallisation ages of the SNC (shergottite, nakhlite, chassignite) meteorites

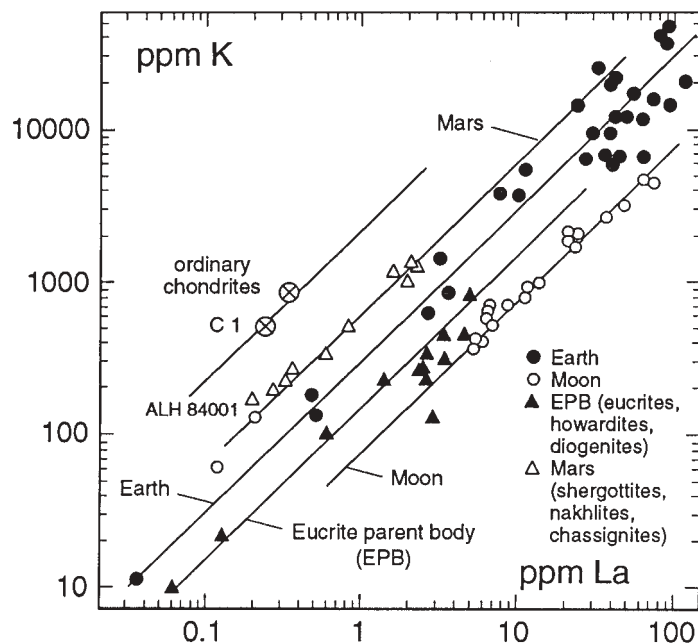


Fig. II.2.3/1. Correlation of K vs La in SNC meteorites from Mars, the Eucrite Parent Body (EPB), the Earth and Moon. Assuming a C1 abundance of the refractory element La (= 0.48 ppm) in the martian mantle, the abundance of the moderately volatile element K (= 315 ppm) can be obtained from this correlation.

cover the range 4.5-0.16 Gyr. Hence, Mars had active volcanism throughout its history and it is likely that there is some volcanic activity even today.

There is widespread evidence on Mars of surface deformation caused both by extension as well as by compression. The most obvious of these features are associated with Tharsis. Around this bulge there is a vast system of radial grabens affecting about one third of the planet. Fractures also occur on other locations around large impact basins and around large volcanoes.

The most prominent result of crustal deformation is the spectacular Valles Marineris (Figs. II.2.2/1 and II.2.2/2), a large canyon extending from Tharsis about 4000 km to the east. In its central section, where several canyons merge, the valley has a width of 600 km and a depth of several kilometres.

All SNC meteorites (see Section II.3), assumed to be of Mars origin, are igneous rocks of quite variable composition. The high FeO contents of the SNCs reflect a high FeO content of the martian mantle, while their high MnO and Cr<sub>2</sub>O<sub>3</sub> concentrations indicate that, contrary to the Earth's mantle, the martian mantle is not depleted in MnO and Cr<sub>2</sub>O<sub>3</sub>.

Dreibus & Wänke (1984) used element correlations observed in SNC meteorites (see example Fig. II.2.3/1) and certain cosmochemical constraints to estimate the bulk composition of Mars.

The ratio FeO/MnO is extraordinarily constant in all shergottites, with an MnO abundance equal 1.00 (normalised to C1 and Si; where C 1 = carbonaceous chondrites type 1, the most primitive meteorites, containing all 'condensable' elements in solar abundances). An abundance of FeO of 0.399, corresponding to 17.9% FeO, was derived for the martian mantle. For the other major elements (MgO, Al<sub>2</sub>O<sub>3</sub>, SiO<sub>2</sub>, CaO), Dreibus & Wänke assumed strictly C1 abundances. In the case of iron, a C1 Fe/Si ratio was assumed to be valid for the whole planet. Hence, the fraction of iron not present in form of FeO in the martian mantle is assumed to have segregated to the core in the form of iron metal or FeS. It was further assumed that all refractory lithophile elements are also present in the martian mantle in C1 abundances, as only small fractionation of these elements has been observed in the Earth's mantle and in meteorites. Using element correlations observed in meteorites, the mantle abundances of many siderophile (W, Ni, Co, etc) and moderately volatile and volatile (Na, K, Rb, Cs, F, Br, etc) elements could be calculated (see Table II.2.3/1 and Fig. II.2.3/1).

## II.2.3 The Bulk Chemical Composition of Mars

**Table II.2.3/1. Bulk Composition of Mars Derived from SNC Meteorites.**  
(Dreibus & Wänke, 1987).

Mantle/Crust			
	%		ppm
MgO	30.2	K	305
Al <sub>2</sub> O <sub>3</sub>	3.02	Rb	1.06
SiO <sub>2</sub>	44.4	Cs	0.07
CaO	2.45	F	32
TiO <sub>2</sub>	0.14	Cl	38
FeO	17.9	Co	68
Na <sub>2</sub> O	0.5	Ni	400
P <sub>2</sub> O <sub>5</sub>	0.16	Cu	5.5
Cr <sub>2</sub> O <sub>3</sub>	0.76	Zn	62
MnO	0.46	Ga	6.6
		ppb	
	Br	145	
	I	32	
	Mo	118	
	In	14	
	La	480	
	Tl	3.6	
	W	105	
	Th	56	
	U	16	

Core (21.7% of Mars mass)	
	%
Fe	77.8
Ni	7.6
Co	0.36
S	14.24

H<sub>2</sub>O added during accretion = 3.4%; H<sub>2</sub>O retained in the mantle = 36 ppm, equivalent to a surface layer 130 m deep (100% degassing).

The similar abundances of several geochemically (W to Rb in Fig. II.2.3/2) very different elements in the mantle of Mars is further strong evidence for the formation of the inner planets from two compositionally different components (Ringwood, 1977; 1979; Wänke, 1981; Dreibus & Wänke, 1984). These components are:

*Component A:* Highly reduced and free of all elements with equal or higher volatility than Na, but containing all other elements in C1 abundance ratios. Fe and all siderophile elements are metallic, and even Si might be partly present in metallic form.

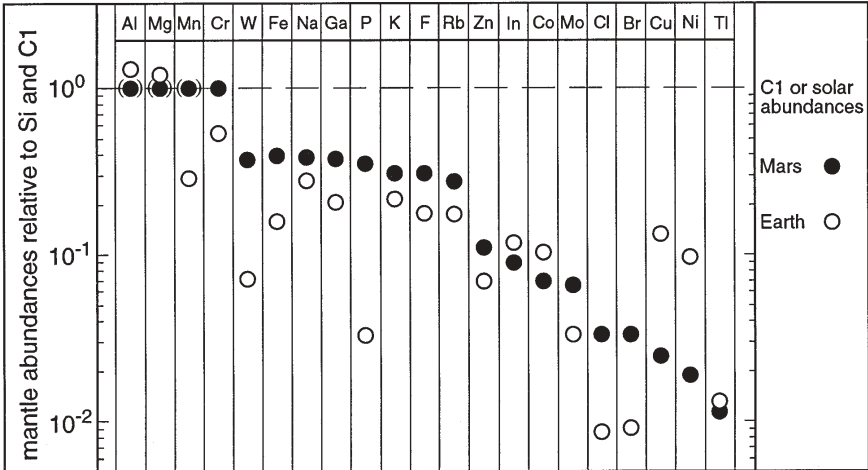
*Component B:* Oxidised and containing all elements (including the volatiles) in C1 abundances. Fe and all siderophile (Co, Ni, Cu, Ga, W, etc) and lithophile elements are present mainly as oxides.

As seen from Table II.2.3/1 and Fig. II.2.3/2, the SNC meteorites indicate abundances of moderately volatile (Na, K, Rb, Zn) and volatile elements (Cl, Br, I) in the martian mantle in excess of those in the terrestrial mantle. A mixing ratio of components A:B of 60:40 is obtained for Mars, compared to 85:15 for Earth. There are, however, a number of elements supposedly derived from component B, which in the Earth's mantle have abundances similar to those of Fe, Na, Ga, K, F and Rb, but in the martian mantle have considerably lower abundances (Co, Ni, Cu, In). These elements all have strong chalcophile character. Their low abundances are taken to indicate a homogeneous accretion of Mars. Hence, contrary to an inhomogeneous accretion of the Earth, as frequently favoured, on Mars the two components had the chance to equilibrate and were probably supplied almost simultaneously to the growing planet. The high abundance of component B, which supplied large amounts of sulphur, was obviously responsible for FeS becoming a major phase and at its segregation extracted all chalcophile elements according to their sulphide-silicate partition coefficients.

The sulphide-silicate equilibrium in the martian mantle indicates its saturation with FeS. The mantle's FeO content is about a factor of 2 higher than that of the terrestrial mantle. As a consequence, the sulphur abundance in the martian mantle is expected to be substantially above the S abundance in the Earth's mantle, because the solubility of FeS in silicates increases with the FeO content. Hence, the observed high concentrations of sulphur in mantle-derived magmas as represented by the shergottites (sulphur content 600-2800 ppm) is not surprising.

The water abundance given in Table II.2.3/1 is derived from element correlations observed in shergottites and cosmochemical constraints. Two quite different approaches yielded an almost identical value. It might, however, be an upper limit only for the water in the martian mantle.

**Fig. II.2.3/2. Estimated elemental abundances in the Martian mantle as derived from SNC meteorites. The higher abundances of moderately volatile (Na, Ga, P, K, F, Rb, Zn) and volatile elements (Cl, Br) compared to those in the terrestrial mantle is obvious. Note the depletion of all chalcophile elements (In, Co, Cu, Ni) in the martian mantle. Note especially the high mantle abundance of P on Mars and its depletion in the Earth's mantle.**



The depletion of chalcophile elements in the martian mantle has been used to infer a homogeneous accretion scenario for Mars. In the same way as discussed above for sulphur, homogeneous accretion had the consequence that water added to the growing planet by component B reacted with the metallic Fe of component A, oxidising it to FeO, while the generated hydrogen escaped. Hence, we should expect a very dry Martian mantle, because water, although added in large quantities to the planet during accretion, was reduced to H<sub>2</sub> except for trace amounts.

Two of the seven known shergottites, Shergotty and Zagami, have compositions very similar to that of the Viking soil (Table II.2.4/1). The other SNC meteorites differ considerably in their chemical compositions.

The large concentrations of sulphur (3.5%) and chlorine (0.8%) in the Viking soil do not seem to be noticeably accompanied by respective cations. The most likely cations for the sulphates, Mg and Ca, have even higher concentrations in Shergotty and Zagami than in the Viking soil. This suggests direct introduction of SO<sub>2</sub> or H<sub>2</sub>S and probably also HCl to the martian regolith via gas-solid reactions.

With respect to the contradictory evidence for a dry martian mantle, supported by the low water content of SNC meteorites but opposed by the martian erosional surface features (which seem to require large amounts of water), the measurements of the oxygen isotopes (Karlsson et al., 1991) and the H/D ratio (Watson et al., 1991) of water extracted from SNC meteorites is of great interest. It was observed that, for all the SNC meteorites analysed and apart from the presence of terrestrial contamination, a large fraction of the water is not derived from the martian mantle, but obviously represents martian surface water: the oxygen isotope composition is up to three times further away from the terrestrial isotope fractionation line than the oxygen in the silicates of SNC meteorites. At the high temperatures of magma generation in the martian mantle, isotopic equilibration between oxygen of the silicates and of water would certainly have been established. Hence, only a fraction of the water found in SNC meteorites can be mantle-derived and the other non-terrestrial part must come from the martian surface. The oxygen isotopes of the surface component might have been created by non-linear isotope fractionation by non-thermal escape of oxygen to space (see Section II.3).

The contradictory evidence of a dry martian mantle (Table II.2.3/1) and the geo-

## II.2.4 The Geochemistry of the Martian Surface Layers

**Fig. II.2.4/1.** A 70 km-wide view of layered deposits in the north polar cap region. Below the ice cap there are successive layers of dust and ice, each about 50 m thick. The layering may be due to period variations in the orbit of Mars causing climatic changes. Erosion of the 500 m-high cliffs, possibly by winds, produced the dark ripple-textured areas at the base.



**Table II.2.4/1.** Comparison of the % Compositions of the Shergotty Meteorite (Dreibus et al., 1982) and Martian Soil (Clark et al., 1976).

	<i>Shergotty</i>	<i>Mars Soil</i>
SiO <sub>2</sub>	51.4	43.0
FeO	19.4	16.2
CaO	10.0	5.8
MgO	9.28	6.0
Al <sub>2</sub> O	37.06	7.2
TiO <sub>2</sub>	0.87	0.6
Na <sub>2</sub> O	1.29	n.d.
P <sub>2</sub> O <sub>5</sub>	0.80	n.d.
S	0.13	3.5
Cl	0.01	<2
H <sub>2</sub> O	<0.02	<1
Sr (ppm)	51	58

n.d. = not determined



**Fig. II.2.5/1.** The Mars Pathfinder landing site, about 300×200 km, outside the mouth of the huge water-cut channel of Ares Vallis (19.5°N/32.8°W). Pathfinder's Sojourner rover analysed rocks and soil washed down from the ancient southern highlands and the basalt plains.



logical evidence of water-erosion surface features can be explained if the surface water is derived from a veneer of water-rich material added late in the planet's accretion stage (Carr & Wänke, 1992). Contrary to the situation on Earth, subduction of crustal material seems not to occur on Mars. Material from a late veneer could therefore have been added to the upper crust only and would remain unrecognised in mantle-derived magmas.

At this point, we can only speculate where all the water that created all of the erosion features now resides. The water in the northern polar cap amounts to only a small fraction of what is required to cause the observed erosion features. A huge fraction was obviously lost to space, as the D/H ratio of the martian atmospheric water (five times greater than the terrestrial ratio) indicates. How much water is still present in the form of ground water or ground ice is unknown and deserves early investigation, especially within the exobiology context.

The high phosphorus concentration in the martian surface rocks (SNC meteorites) and mantle is of special interest. Because of the high mantle abundance of phosphorus, phosphates crystallised as an independent mineral phase and took up many of the geochemically very important trace elements such as the Rare Earth Elements (REE), uranium, thorium, etc. Phosphorus is also an important bioelement. On Earth, phosphate-rich rocks often reflect decomposition of sedimented organic matter.

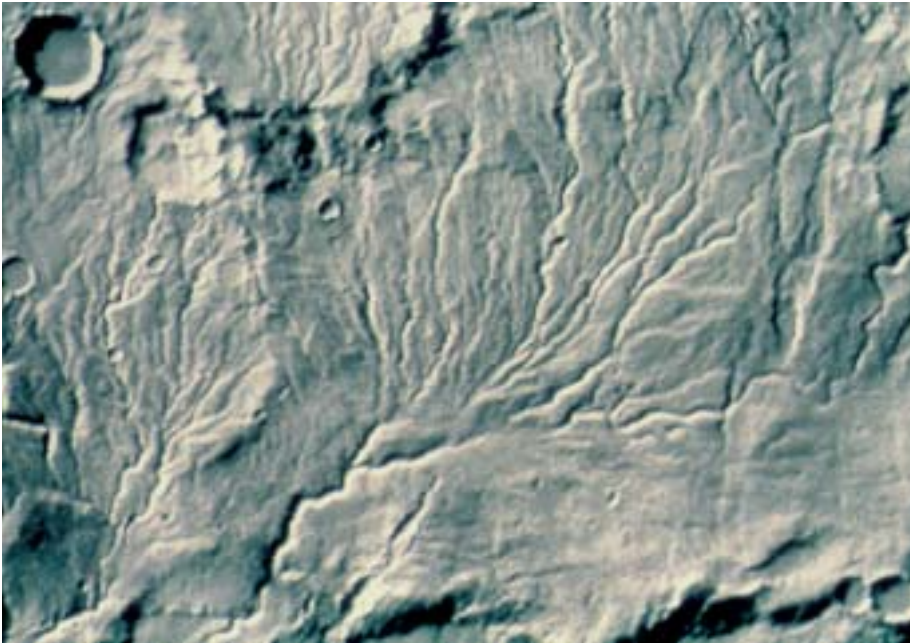
## II.2.5 Water and Ground Ice

The most puzzling features on Mars are those indicating the presence of large quantities of surface water (Carr, 1987; 1996) because the present climate does not allow liquid water on the surface. The present temperatures on Mars range from 150K at the winter poles, where CO<sub>2</sub> freezes out to, a maximum of 290K at summer midday at low latitudes. Under these conditions and with the present mean atmospheric pressure of only 7 mbar, liquid water cannot exist anywhere on the surface.

The most prominent evidence for water erosion are the large dry valleys which are thought to have been formed by large floods. Many of these valleys start from the chaotic terrain in the Margaritifer Sinus region, south of the Chryse basin, and extend northward for hundreds of kilometres. Several converge on the Chryse basin and then continue further north, merging into the low-lying northern plains. The valleys start full-size and rarely have any tributaries.

In other areas, the fluvial features indicate slow erosion (Fig. II.2.5/1). Branching





**Fig. II.2.5/2.** An area about 200 km across of valley networks on Mars. Although they may appear to resemble terrestrial drainage systems, they lack small-scale feeder streams. It is thought that they might have been created by groundwater flow rather than rainwater runoff. Alternatively, because the valley networks are confined to relatively ancient regions, they might indicate that Mars at one time had a warmer and wetter climate. (*NASA/Lunar & Planetary Institute*)

valley networks (Fig. II.2.5/2) are found all over the heavily cratered southern terrain but occasionally also on younger areas. They are similar to terrestrial river valleys as they have tributaries and their widths increase downstream. The networks extend generally only over a few hundred kilometres, hence they are much shorter than terrestrial river systems. Water ice is unstable at the surface at low latitudes but may be present at depths of a few to several hundred metres. The almost global presence of lobate flows around impact craters larger than a few kilometres is taken as strong evidence for the presence of ice at shallow depth at high latitudes and of ice or ground water at greater depth at low latitudes. Under the present surface temperature conditions, water can exist only as fine water ice particles (Fig. II.2.7/1) or frost (Fig. II.2.7/2).

The geochemical evidence suggests that the climate in the early days of Mars, before about 3.5 Gyr ago, was wet and warm, allowing the formation of the observed erosional features. It has been suggested that the temperature has been raised by a strong greenhouse effect owing to a thick CO<sub>2</sub> atmosphere (Moroz & Mukhin, 1978; Pollack et al., 1987). However, the required surface pressure of the order of 5-10 bar would lead to the formation of CO<sub>2</sub> ice clouds at high altitudes. These would reflect sunlight and also limit the amount of CO<sub>2</sub> residing in the atmosphere (Kasting, 1991). Hence, it seems necessary that for the time of a warm and wet climate there were greenhouse gases other than CO<sub>2</sub>. Among others, methane and ammonia have been suggested.

In the light of the high sulphur content of the martian basalts (shergottites), SO<sub>2</sub> or H<sub>2</sub>S are also worth considering as possible additional greenhouse gases (Postawko & Kuhn, 1986). The lifetime of the sulphur compounds in an atmosphere with traces of water vapour is limited to a few months. Although it seems likely that SO<sub>2</sub> or H<sub>2</sub>S dominate the volcanic exhalations, that production rate appears to be too small for any significant greenhouse effect. It has been argued, however, that SO<sub>2</sub> from volcanic exhalation may have been stored in the form of liquid or solid SO<sub>2</sub> tables at shallow depth. A sudden release of the stored SO<sub>2</sub> by impact or volcanic eruption could then bring enough into the atmosphere for a substantial temperature rise that could, supported by feedback effects, last hundreds of years (Wänke & Dreibus, 1994). The large concentration of sulphur in the Viking soil, most likely as sulphates, may be the

## II.2.6 The Climate of Mars

**Fig. II.2.7/1.** Early morning fog in the Noctis Labyrinthis (10°S/95°W). This region, about 300 km wide, lies at the western end of Valles Marineris. This low-lying fog is probably caused by fine water ice particles. (NASA)



signature of such an  $\text{SO}_2$  effect. In this context, it is important to mention that we do not know if the observed erosional features were formed continuously or during individual periods of elevated temperatures separated by millions of years.

It was mentioned above that, at present,  $\text{CO}_2$  freezes out at the winter poles. This translates to very large seasonal variations in  $\text{CO}_2$  pressure of more than 30%. The surface of Mars has an elevation range of more than 30 km, which corresponds to a pressure of 13 mbar in the Hellas basin to 0.2 mbar on top of Olympus Mons.

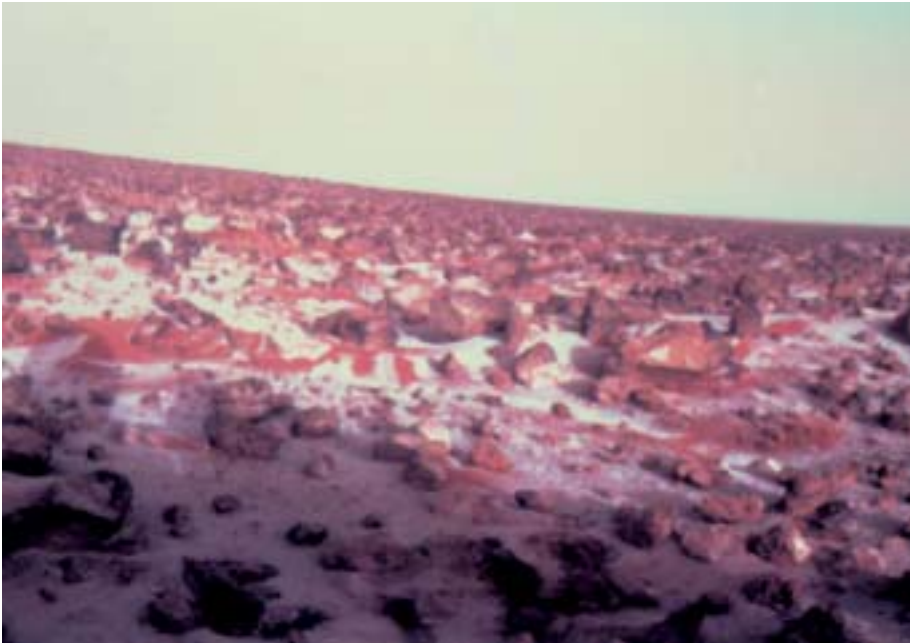
As in the case of water, it is unclear where the  $\text{CO}_2$  from a massive atmosphere went. So far, there has been no clear evidence for large quantities of carbonates in the martian soil. Loss of  $\text{CO}_2$  to space has been observed but it is still uncertain if the rates are high enough to lead to the present situation.

## **II.2.7 NASA-Proposed Sites for Mars Exploration**

The selection of sites for exobiology exploration on Mars is based on two fundamental propositions:

1. early Mars was sufficiently similar to Earth to provide a suitable environment for the inception and development of life;
2. the basic requirements for the existence of martian living systems, or their preservation as fossils, are comparable to those for.

These concepts lead to two domains of investigation: extinct and extant martian life. The other major aspect of the search for life on Mars is the knowledge it can provide on the precursor organic compounds, the traces of which are no longer observed on Earth because of the recycling of ancient rocks by plate tectonics. As no



**Fig. II.2.7/2. Frost deposit at the Viking-2 Lander location (48°N/226°W). This May 1979 image shows a coating of ice a few microns thick. (NASA)**

plate tectonic evidence has been observed on Mars, it is expected that legacies of such compounds can be found in aqueous sedimentary deposits in ancient Martian cratered terrain (NASA SP-530, 1995). Water being the major requirement for life, seeking life equates to searching for traces of water activity (Greeley & Thomas, 1994).

As outlined earlier, the present conditions on Mars do not allow the presence of liquid water on its surface. Today, liquid water may be found only beneath the surface, where there are higher temperatures and pressures. That means at a depth of more than just a few metres and, hence, beyond the access of probes for some time to come. Consequently, we could look today only for environments where ancient life might have been preserved or for natural processes that might have brought extant subsurface life up to more accessible depths.

### **II.2.7.1 Water and a Favourable Environment**

With its current thin atmosphere giving no UV protection and a cold, dry climate, Mars combines the most severe conditions for the inception of life. However, conditions were different in the past, and evidence for water activity and aqueous sedimentary basins have been demonstrated (Cabrol & Grin, 1995; Carr, 1996). The duration of water activity and liquid habitats is also a critical issue (McKay & Davis, 1991). Though the conditions might have been more favourable during the first 15 Gyr of martian history, there is no evidence that large basins such as Elysium were active during the Amazonian era (Scott, 1995; Scott & Chapman, 1991). Even more intriguing is the evidence of the activation of large runoff valleys, and development of smaller-scale lakes in highland craters, such as in the Gusev and Gale craters (Cabrol et al., 1996; Grin & Cabrol, 1997) during the same geological period. Morphologic evidence show these lakes to have existed over long periods and they may have provided oases for life more recently.

Fluvial valley networks and channels in the highlands and on volcano slopes, ancient basins and lakes are, then, the best evidence of a water-rich past. This evidence, coupled with the discovery on Earth of microbiota able to survive in a Mars-analogue environment (Friedman & Ocampo, 1976) and the better understanding of the early evolution of life on Earth, have allowed researchers to identify candidate sites for exobiological exploration on Mars (Greeley & Thomas, 1994). The selection of sites for exobiology is thus based on geologic and geomorphic evidence of paleowaterflows, ponds, ice and hydrothermal activity.

A list of basic exploratory features for exobiology sites has been developed by Farmer & DesMarais:

<b>Deposits</b>	<b>Features</b>
<b>Fluvial</b>	<i>Dendritic drainage networks</i> : simple; complex (higher order) <i>Channel morphology</i> : widening down slope; meandering; flood plains; stream terraces
<b>Lacustrine</b>	<i>Accessibility</i> : layering visible; impact craters <i>Drainage basin fed by</i> : simple channels; complex channels; <i>Shoreline features</i> : lake terraces; deltas
<b>Thermal spring</b>	<i>Accessibility</i> : lava flows; aeolian cover; impact craters <i>Drainage system</i> : simple channels; point source <i>Localised heat source</i> : surface (volcanic centre); subsurface thermokarst
<b>Surface ice</b>	<i>High latitude (&gt;60°)</i> : laminated terrain
<b>Subsurface ice</b>	<i>Mid-latitude (30-60°)</i> : patterned ground; alases; pingos; fluidised crater ejecta.

(After J.D. Farmer & D.J. DesMarais (1994). In *Mars Landing Site Catalogue*, (Eds. R. Greeley & P.E. Thomas), NASA RP-1238, 2nd Edition; with permission of the editors.)

#### II.2.7.2 Exploration for Extinct Life

If Mars and Earth did actually experience broadly comparable conditions during the first 1.5 Gyr of their histories, then it is likely that life appeared during that same period. Evidence of such life on Mars may be preserved in the form of fossils and in various biosignatures.

In the NASA studies for site selection for exopalaeontology, priority is given to landing sites in ancient terrains where hydrological systems involving liquid water appear to have been long-lived and which exhibit a high probability of having surficial aqueous mineral deposits. Preference is given to mineral deposits that might have had a long residence time in the martian crust, i.e. those that are diagenetic stable and resistant to chemical weathering.

On Earth, mineralisation occurred in many Precambrian examples very rapidly, prior to cellular decomposition, and probably when organisms were still viable. The best preservation is observed where organic materials were rapidly perfused with fine-grained silica or phosphate. Evaporites form another group of potential host phases. Whereas on Earth these are readily dissolved in hydrologic cycles, in the very dry martian climate their residence time might be prolonged.

Hydrothermal systems are excellent targets for a search for fossil records of biological activity. Volcanic terrains are abundant on Mars and there is ample evidence for ground water or ground ice. Their combination is the basis of hydrothermal activity, which therefore should also be abundant on Mars. The NASA search for extinct life (fossils) strategy on Mars therefore gives priority to thermal spring sites, which should also provide a good preservation environment for microbial fossils and to stable lacustrine environments and outflow channel deposits. These types of sites give almost a planetwide range for exploration (Greeley & Thomas 1994).

#### II.2.7.3 Exploration for Extant Life

Extant life, if present, probably resides in subsurface habitats where liquid water might be present and, hence, would be most likely of a chemosynthetic form. It seems possible that recent flows of subsurface water have brought such organisms into near-surface environments where they have been cryopreserved in ground ice. It has been suggested that microorganisms may survive in ground ice for millions of years and in evaporite deposits for hundreds of million of years. Nevertheless, the highest priority



**Table II.2.7.4/1. Potential Mars Exobiology Landing Sites.**

	<i>Region (site name)</i>	<i>Location</i>	<i>Priority</i>
Fluvio-lacustrine	Eridania NW	37.0°S / 230.0°W	h
	Parana Valles	22.0°S / 11.0°W	h
	Mare Tyrrhenum	22.8°S / 230.0°W	h
	Terra Tyrrhenum	24.8°S / 285.8°W	h
	Aeolis SE	15.5°S / 184.5°W	h
	Aeolis NE (Gusev)	7.3°S / 305.0°W	h
	Iapygia NW	8.5°S / 159.5°W	h
	Mangala Valles	6.3°S / 149.5°W	h
	Ismenius Laous SW	33.5°N / 342.5°W	h
	Sinus Sabeus NE	8.0°S / 335.0°W	h/m
	Diacria SE	39.5°N / 135.5°W	m
	Iapygia	11.0°S / 279.5°W	m
	Terra Cimmeria	43.2°S / 208.1°W	m
	Candor Mensa	6.05°S / 73.75°W	m
	Eridania SE	57.0°S / 197.0°W	m
	Ares	2.0°N / 16.0°W	m
	Hebes Chasma	1.5°S / 76.5°W	m
	Memnonia NW	14.5°S / 175.0°W	m
	Phaethontis NC	37.5°S / 146.0°W	m
	Oxia Palu NE	21.3°N / 0.8°W	m
Thermal spring	Dao Vallis	33.2°S / 88.4°W	h
	Ares	2.0°N / 16.0°W	m
Ground ice	Ismenius Lacusm SC	44.3°N / 333.0°W	h
	Cassius SE	38.0°N / 256.0°W	m
	Oxia Palus NW	21.1°N / 36.7°W	m

h=high, m=moderate. Adapted, by permission of the editors, from Table 5.4 p16, in *Mars Landing Site Catalogue* (Eds. R. Greeley & P.E. Thomas), NASA RP-1238, 2nd Edition, 1994.

in the search for extant life has the very youngest martian terrains at high latitudes, including the north polar cap, as an objective. However, the recent ice-covered lacustrine activity in the intertropical band of Mars may also offer potential for extant life exploration. Ice-covered lakes, such as those that developed in the Gusev and Gale craters, had their final activity during the Amazonian era but, at least for Gusev, it has been shown that lakes may have occupied the crater episodically since its formation during the Early Noachian period (Cabrol et al., 1996). In Gusev, as in other comparable sites, lifeforms may have had the opportunity to adapt in the ice-covered lakes environment and to survive up to the present, especially if the presence of ice-cored (pingo-like) structures is confirmed by the Mars Global Surveyor mission. Thus, new concepts of drilling and wells may enable us to discover oases of life in the subsurface, even at mid-latitudes.

#### II.2.7.4 NASA Priority Landing Sites for Exobiology

Considering the new developments and results in exobiology and martian geology, NASA has established a list of priority sites for the search of life on Mars. The list is presented in Table II.2.7.4/1 by courtesy of the NASA Ames Research Center (contribution of Dr. N. Cabrol).



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## II.3 The Martian Meteorites

*‘When two great masses come into collision in space it is certain that a large part of each is melted; but it seems also quite certain that in many cases a large quantity of debris must be shot forth in all directions; much of which may have experienced no greater violence than individual pieces of rock experience in a land slip or in blasting by gunpowder.’*

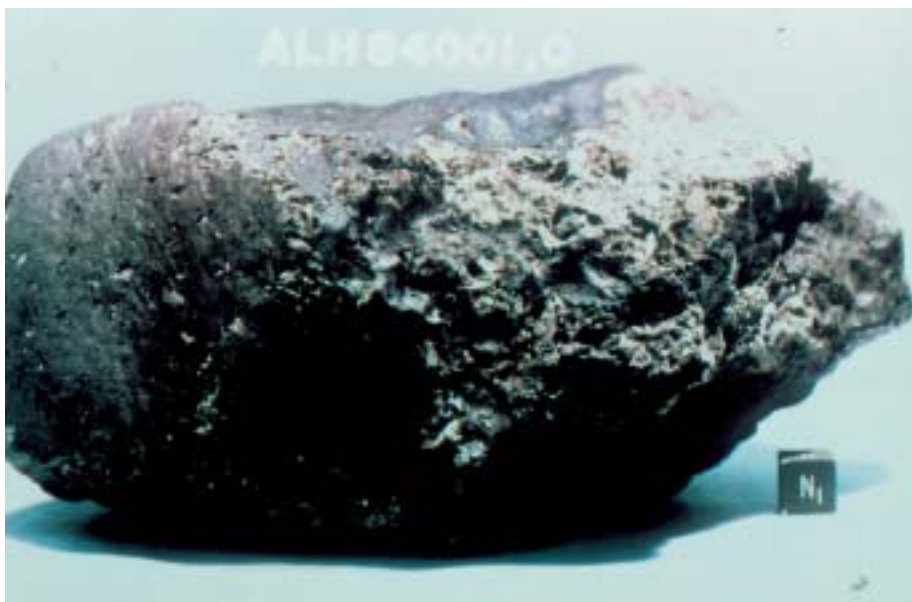
*Lord Kelvin, addressing the British Association in 1871*

Our interest in meteorites here is two-fold. Firstly, as mentioned in Section I.2, they provided the early Earth with substantial amounts of carbon in inorganic and organic forms. Secondly, they can provide information on the constitution and conditions of their original parent bodies. Of especial interest here are those few meteorite samples that appear to have originated from Mars. Indeed, one of the reasons that exobiology is exciting much debate is the announcement concerning the possibility of biological fossils in martian meteorite ALH 84001 (hereafter referred to as A84) (McKay et al., 1996). The obvious implication of this observation, if they are biological fossils, is that there was once life on Mars.

Actually witnessing the fall of a meteorite is a rare event; only 5-8 are reported each year. Other than that, a few meteorites are found simply lying on the ground. This all adds up to a museum-based meteorite collection around the world of about 2500 specimens. In addition, there are the meteorites collected by dedicated expeditions to Antarctica and other deserts, by the US, Japan, Australia and Europe. These may return with 200-1000 fragments per year, but often there may be many separate pieces of a single meteorite. Clearly, therefore, it is difficult to estimate the total number of meteorites retrieved by the collection trips. A best guess of the total number ever collected (i.e. museum-based and by systematic searches) is somewhere in the region of 5000-8000.

Most meteorites originate from bodies in the asteroid belt. Some can even be tracked to a specific asteroid, Vesta. There are a further 18 samples from the Moon. In addition, there are currently 12 others that are lumped together on the basis of a number of common characteristics (some of which make the samples quite clearly distinct from asteroidal debris). Originally, these were called the ‘SNC meteorites’, on account of there being three subgroups that had been recognised historically (the Shergottites, Nakhilites and Chassignites).

### II.3.1 Introduction



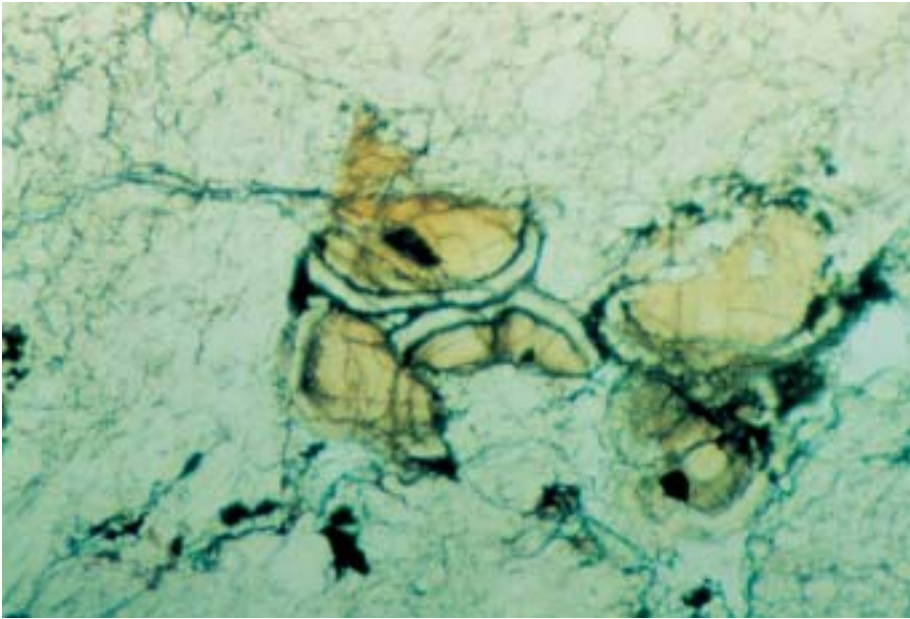
**Fig. II.3.1/1.** Martian meteorite ALH 84001, derived from a lava flow 4.5 Gyr ago. It was ejected from Mars about 16 million years ago and entered the Earth’s atmosphere some 13 000 years ago, landing in Antarctica. (NASA Johnson Space Center)

Clearly, SNC meteorites are numerically rare, so perhaps a better way to assess their relative significance is to compare the total mass of those specimens observed to fall with the total mass of all other meteorite falls. This must be done with falls, because the sample set of those returned by collection trips and found by members of the public suffers horribly from bias. For instance, certain types of samples are more resistant to weathering and so after landing on Earth tend to be more resilient to erosion than others. Thus over about a thousand years, they appear to be more common than they really are. Another biasing effect is that some samples look more like meteorites than others (or perhaps it is more appropriate to say they look less like ordinary terrestrial rocks, enhancing their probability of collection)! Considering only the observed falls, it transpires that SNC meteorites constitute, by mass, some 0.25% of all meteorites. Since the total annual flux of extraterrestrial material to the Earth is estimated to be 40 000 t (much of it in the form of micrometeorites, as will be discussed later), then statistically, some 100 t of SNC meteorites arrive at the Earth each year. So, while SNC meteorites may be relatively rare within the collections, they may be actually quite commonplace.

### II.3.2 The Origin of SNC Meteorites

The fundamental question is, then, where do the SNC meteorites originate? How can we know if they come from Mars? Two things made them stand out to researchers in the 1960s and 1970s. Firstly, they were obviously igneous in origin, being the crystallisation products of volcanic activity. This, in itself, is not unusual. There are other classes of meteorite with this characteristic, representing the end-products of melting events that took place on asteroids early in the history of the Solar System (>4.5 Gyr ago). However, what made SNC meteorites different was that the distribution of the rare-earth elements was more like that of terrestrial basalts than melted meteorites. In other words, they had planetary rather than asteroidal characteristics. The second phenomenon was that their formation ages were substantially younger (i.e. 1.3 Gyr) than the age of the Solar System (e.g. Gale et al., 1975; Jagoutz & Wänke, 1986). The only reasonable way that this could happen was if the samples were formed on a geologically active planetary body, rather than an asteroid. Wasson & Wetherill (1979) are generally acknowledged as first suggesting in print that Mars was the most likely possibility. But there was a problem: at that time no lunar meteorites had been found and the dynamics of transporting samples from Mars (which at its nearest point to Earth is 77 million km distant) are much more difficult than for the Moon (0.4 million km).

This conundrum was resolved early in the 1980s when the first lunar meteorite was identified. (In fact, this was a most uncontroversial sample; all scientists agreed that it had to be from the Moon.) (Lindstrom, 1989). At about the same time, a recently retrieved SNC meteorite, EET A79001 (from Antarctica; hereafter E79), was analysed and found to contain gases that were in most respects identical to those analysed at the martian surface by Viking. In the first instance, it was recognised that the noble gas isotope ratios of gas trapped in what was believed to be shock-produced glass (possibly a product of the impact that ejected E79 from its parent) lay on a mixing line between that of Mars and the Earth (Bogard & Johnson, 1983). It looked very much as though E79 contained martian gases contaminated by species absorbed from the terrestrial atmosphere. This concept was extended and confirmed by a consideration of nitrogen. Nitrogen in the martian atmosphere has a very distinctive isotopic composition, highly enriched in  $^{15}\text{N}$  (McElroy et al., 1976).  $^{15}\text{N}$ -enriched nitrogen was also found in E79 (Becker & Pepin, 1984). But noble gases are trace components, and nitrogen is a minor contributor to the martian atmosphere. If the SNCs came from Mars, then the major species of the martian atmosphere would have to be there in the relevant amounts. The confirming evidence was duly found by Carr et al. (1985) and all the data (Viking and meteorite results) were showed to plot on a trend across more than 10 orders of magnitude. It is now accepted that by studying E79 it is possible to refine the measurements made by Viking and thereby provide new constraints on the martian atmosphere. For example, the  $^{12}\text{C}/^{13}\text{C}$  ratio of the



**Fig. II.3.3/1. Carbonate globules in ALH 84001.** A thin-section view, covering about 0.5 mm. The ‘nanofossil’ structures are found in the rounded brownish and clear globules. The globules are made up of the brownish iron carbonate (siderite) and clear magnesium carbonate (magnesite). The dark rims contain iron oxide and sulphide materials. (*A. Treiman/Lunar & Planetary Institute*)

principal atmospheric component,  $\text{CO}_2$ , now quoted for Mars is, in fact, the meteorite measurement, not from the Viking data.

Once one sample from Mars was identified it was possible to confirm that others in the collection and returned from Antarctica were also martian. Because martian gases are not readily apparent in all of the samples, their presence or absence could not be taken as diagnostic. Indeed, some of the meteorites do not have any residual martian atmosphere. A far more useful and generalised criterion is a sample’s oxygen stable isotopic composition (a measurement requiring only a few milligrammes of sample). Indeed, this is how A84 was confirmed as a martian sample – in most other respects, it is different to the others of the family. Most notably it is extremely old (4.5 Gyr) – as old as the planet. Until recently, old age would almost certainly have consigned it to being of asteroidal origin. Indeed, it is ironic that A84 languished in the Antarctic collection at Houston, unrecognised and believed to be diogenite (Mittlefehldt, 1994).

As already mentioned, oxygen isotope systematics are the vital criterion for recognising meteorite families. Oxygen is 45wt% of any silicate-rich meteorite, so any characteristic discovered among the oxygen isotopes can be considered as diagnostic. Fortunately, oxygen has three isotopes:  $^{16}\text{O}$  (most abundant),  $^{17}\text{O}$  (1/200th of total) and  $^{18}\text{O}$  (1/500th). The exact relative proportions of  $^{17}\text{O}$  and  $^{18}\text{O}$  can be measured very precisely and quoted according to the general formula for  $\delta^{17}\text{O}$  and  $\delta^{18}\text{O}$  (‰, per mil).

As the difference between  $^{16}\text{O}$  and  $^{17}\text{O}$  is 1 mass unit and between  $^{16}\text{O}$  and  $^{18}\text{O}$  is 2 mass units, any samples from a homogenised parent body affected only by geological events must plot on a line of slope 1/2 (actually 0.52). All the rocks on Earth, its water etc define a well-accepted reference line called the terrestrial fractionation line.

Robert Clayton and his colleagues at the University of Chicago (e.g. Clayton & Mayeda, 1983) found that distinct meteorite groups, or groups that were related, had separate lines on an oxygen isotope diagram, because the solar nebula was not adequately mixed before solid silicate bodies separated out. On this basis, Mars might be expected to have a distinguishing line. Indeed it does, although it is predictable that for a terrestrial type-planet, formed in the inner Solar System, the difference between Mars and Earth is small. The important parameter in defining Mars (from martian meteorites) is the difference between the oxygen isotope composition of SNC meteorites and Earth, designated  $\Delta^{17}\text{O}_{\text{Mars}}$ . The  $\Delta^{17}\text{O}_{\text{Mars}}$  has now been measured to

### II.3.3 The Martian Meteorites



very high precision using laser-induced fluorination methods, which can afford repeat measurements on small samples, with decreased contamination and on a rapid duty cycle. Thus  $\Delta^{17}\text{O}_{\text{Mars}}$  has been defined as  $0.32 \pm 0.013$  (Franchi et al., 1997a) from 10 martian samples (including A84) which has a  $\Delta^{17}\text{O}$  of 0.327, thus confirming its membership.

It is now generally accepted that SNC meteorites do come from Mars. Therefore, investigators specialising in low-temperature geochemistry use them for studies characterising martian environmental conditions. (Incidentally, even if ‘martian meteorites’ turn out not to come from Mars, their features are so interesting and relevant for exobiology that it might almost be more exciting!) In the first instance, this means the investigation of martian carbonates, which can be found at levels up to 1wt%. Hydrous minerals have been known about since the late 1970s (Ashworth & Hutchison, 1975), but they are far less abundant and thus more difficult to study.

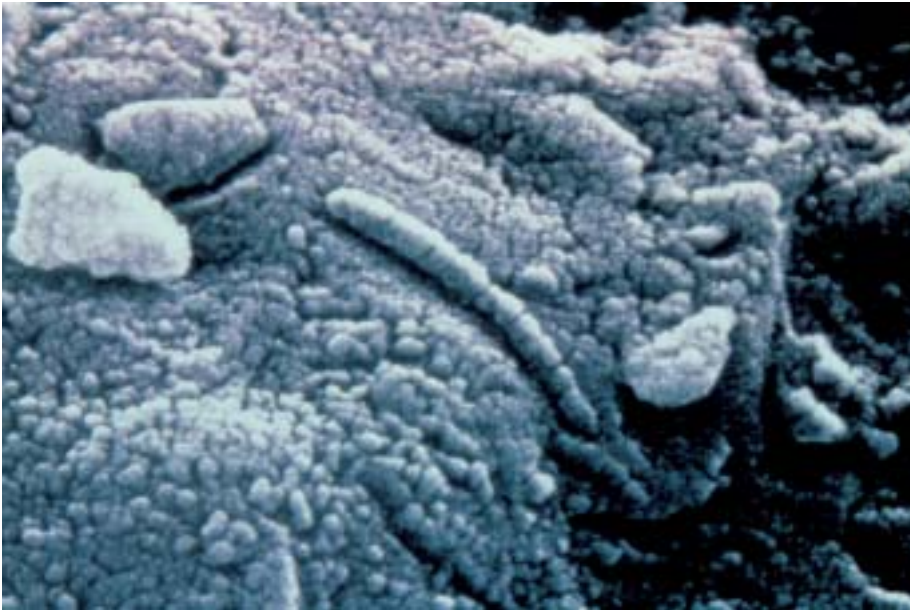
The early measurements of martian meteorites such as Nakhla (Carr et al., 1985), which led to the discovery of carbonates, suggested typical carbonate concentrations of about 0.02wt%. Later, A79 was found to have discrete pockets of the mineral but the veins and cracks in A84 have been found to be rich in carbonates, broken open when the sample is cleaved (Romanek et al., 1994). Mineral chemistry of the A84 carbonates suggested that they formed at high ( $\sim 700^\circ\text{C}$ ) temperature (Harvey & McSween, 1996). However, the oxygen isotopic composition of the carbonates, (a method regularly used on Earth to work out carbonate-formation temperatures), suggested the carbonate veins in A84 formed at low temperatures ( $0\text{--}80^\circ\text{C}$ ) by hydrothermal activity, i.e. a process involving flowing liquid water. Of course, A84 is now under intensive study and the latest results suggest the oxygen isotopic compositions of carbonates are highly variable and possibly therefore of multiple origins. The lower the temperature of formation, the more likely carbonates are a good place to look for evidence of past life on Mars. If the high-temperature philosophy is correct, then the alternative hypothesis for A84 carbonates is that the mineral was generated by impact into  $\text{CO}_2$ -containing permafrosts.

At the same time as studying carbonates in A84, Grady et al. (1994) looked at any other forms of carbon in the sample. There was a very good reason for this. A previous study of E79 uncovered an unexpected phenomenon: the carbonates were accompanied by organic materials. The ratio of carbon in the two forms was about 3 (carbon as carbonates/carbon as organics) (Wright et al., 1989). Clearly, this was a discovery of great significance, but on the evidence available it could not be decided whether the material was biogenic or abiogenic. It is extremely difficult to imagine inorganic ways of making organic deposits together with carbonates on Mars. A84 is a sample containing so much carbonate that the minerals are visible to the naked eye. It also has associated organic material, the relative concentration of organics being even higher than in E79 (carbon as carbonate/carbon as organics  $\sim 1$ ). These results (Grady et al., 1994) along with the isotope data from Romanek et al. (1994) were of milestone importance, and paved the way for the discovery of the putative fossils described by McKay et al. (1996).

### II.3.4 Exobiology and the Martian Meteorites

The images of the purported biological microfossils in A84 (Fig. II.3.4/1; McKay et al., 1996) have been seen worldwide. In fact, the ‘microfossils’ would be more correctly called ‘nanofossils’ on account of their small size. Although similar objects have been observed in terrestrial rocks, there is no evidence as yet that they are owing to living organisms. Their small size, compared to the minimum dimensions for accommodation of the biochemical molecules involved in living organisms, has led many to doubt that living replicating organisms of these dimensions are viable. Nonetheless, the evidence is taken seriously and research aimed at elucidating the nature of the ‘nanobacteria’ of the terrestrial environment is underway. For now, however, it would seem that the A84 images are a matter of faith – you either believe they represent martian biological nanofossils, or they are the result of some other, as yet unknown, process.





**Fig. II.3.4/1.** A possible fossil microorganism observed in the ALH 84001 meteorite. The segmented rod-like object is about 200 nm long. It was found to be in association with martian organic chemicals and is assumed to be of martian origin. Although resembling very small bacteria found on Earth, there is still considerable debate as to the nature and origin of structures of this type in this martian meteorite. (McKay *et al.*, 1996)

Lines of evidence other than images and morphology have been considered by McKay *et al.* (1996). For instance, the polycyclic aromatic hydrocarbons (PAHs) are not the kind of chemical fossils we expect from biology. They are, however, indigenous to the samples. That is, they are more abundant in the interior of carbonate globules than the exterior and give a pattern by laser desorption that is different from contamination. Nevertheless, there could be other explanations for PAHs, and it should be noted that they are ubiquitous, not just on Earth or the Solar System, but also in interstellar space, where few would venture to call them evidence of life.

To assess the likelihood of the long-term existence of life on Mars, before a space mission (which could deliver samples to Earth), martian meteorites of different ages have been studied. As stated above, A84 might be considered to represent an endpoint, having an age almost as old as the planet itself. Nakhilites and Chassigny have intermediate crystallisation ages of around 1.3 Gyr (e.g. Gale *et al.*, 1975). In contrast, the basaltic and lherzolithic shergottites could be as young as 0.18 Gyr (e.g. Jagoutz & Wänke, 1986) but this remains a controversial subject. Regardless, in order to investigate the possibility of recent life on Mars, the evidence found in shergottites has to be considered.

Martian meteorites are hardly ideal samples within which to look for signs of life. They represent relatively fresh samples of igneous rocks, produced by volcanic activity either at high or low levels within the martian crust. Hence from only their primary features, it is apparent that they should not record any evidence of life. In the case of martian rocks, it is the subsequent histories of the samples that are investigated – secondary events such as weathering, hydrothermal activity and atmospheric exposure. In other words, processes that occurred after the rocks crystallised and cooled down. The available martian meteorites have been affected to lesser or greater extents by such secondary alteration. Cracks and veins in A84 record one of the most extensive episodes of secondary processing, and in one model the carbonate minerals have been interpreted as low-temperature hydrothermal products that could preserve evidence of life if it were there in the first place. An alternative scenario suggests they are rapidly deposited high-temperature minerals produced by an impact-driven process within which fossilised life would not be expected to survive. (e.g. Scott *et al.*, 1997)

Another sample with very obvious evidence for secondary activity is E79, which was the first meteorite found to have tangible amounts of carbonate. Moreover, it is associated with amounts of uncharacterised organic matter far in excess of the

potential levels of contamination (Wright et al., 1989). Unfortunately, it is not yet so clear that the carbonate in E79 is martian. E79 appears to contain some modern  $^{14}\text{C}$ , contained in terrestrially produced carbonate. Wherever large deposits of carbonate exist there is a suggestion that  $^{14}\text{C}$  correlates with  $^{13}\text{C}$  abundance and, if so, that a high  $\delta^{13}\text{C}$  identifies the carbonate as indigenous (Wright et al., 1997a). A study of the carbonate-rich samples, including some extracted from completely glass-sealed inclusions, shows they are high in organics. If most of the carbonate is indeed indigenous, then it can be argued that the organic matter came to Earth with the sample. Indeed, the amounts of organic residues encountered are a factor of five greater than for any other martian meteorite, some of which are known to be heavily contaminated. Carbonate-poor fractions of E79 are also among the least contaminated that have been studied. Thus critics of the work on E79 have developed models for selective contamination. For example, flushing the meteorite with large amounts of Antarctic melt water containing low levels of amino acids and PAHs (Becker et al., 1997). There are detailed arguments that can be presented but the most obvious observation against such a mechanism is the fact that the sample does not show any evidence of inundation by the huge quantities of Antarctic melt water required. Wright et al. (1997a) conclude on the contrary that whatever process added the carbonates to E79 also deposited the large quantities of organic compounds measured. Moreover, the event occurred at low temperatures and it involved aqueous fluids.

*If* the organic compounds in E79 are Martian, then the significance of their carbon isotopic composition ( $\delta^{13}\text{C} \sim -25\text{‰}$ ), as compared to a  $^{13}\text{C}$ -enriched atmosphere, estimated from trapped gases and carbonate measurements, cannot be overstated. On Earth, biological activity has been remarkably consistent in terms of its isotopic effects over a period of some 3.5 Gyr. Schidlowski has examined the carbon isotopic composition of more than 1600 samples of fossil kerogenous organic matter of all ages and recorded an average  $\delta^{13}\text{C}$  of  $-25 \pm 7\text{‰}$ , attributing the uniformity to the extensive involvement of the RUBP carboxylase biosynthetic pathway (Schidlowski, 1987). The spread of results is not distinguishable from recent marine sediments even though more modern photosynthetic pathways now contribute to the sedimentary biomass. Over the complete geologic record, marine carbonates vary by no more than  $\pm 2\text{--}3\text{‰}$  around a mean value. Schidlowski has argued successfully (see Section I.3) that a fractionation of  $\sim 20\text{--}35\text{‰}$  in carbon isotopes between dissolved and fixed carbon is therefore a diagnostic signature of life on Earth right back to the earliest Precambrian times. This hypothesis is so well accepted that even when the fractionation is less or greater than the required value, e.g. in Isua metasediments or the Fortescue group, respectively, special pleadings are allowed and the concept remains unviolated.

The possibility of using isotopic fractionation as an indicator of life on Mars has previously been considered (Rothschild & DesMarais, 1989) without reaching a definite conclusion or evaluating the possibility of using data from a martian meteorite. Schidlowski (1992), however, has pointed out that his principle could apply to meteorites from the planet, citing Wright et al. (1989) as a source of data, but with the caveat that various undefined uncertainties mitigate against making a direct linkage to life processes. At the time, this caution was rightly related to the undefined provenance of the carbonate, the organic matter or both. It is now more likely that both are indigenous, in which case it is a reasonable assumption that the carbon isotopic difference of  $\sim 40\text{‰}$  between the components could be indicative of a biologically controlled process. Of course, it is unfortunate that  $\delta^{13}\text{C}$  of the organic matter in E79 is measured at about  $-25\text{‰}$ , in the range of terrestrial contaminants. Even more confidence might have been placed on a potential biological origin if some extreme values had been determined, akin to the values found in some Precambrian samples which have  $\delta^{13}\text{C} < -40\text{‰}$  (Schidlowski, 1987). Low  $\delta^{13}\text{C}$  values have been found on a number of occasions during the stepped combustion of samples of martian meteorites but these are for uncharacterised specimens and impossible to duplicate (Wright et al., 1997b). Unanticipated sources of contamination cannot be ruled out.

If the fractionation between the atmospheric carbon isotopic composition as

indicated by carbonates and the organics in E79 are indicative of life, just as the nanofossils might be for A84, then the conclusion that has to be drawn is of major importance. We do not know the actual age of the carbonate but because it is secondary, younger than the rock of age 180 million years, then one has to accept that life processes are comparatively recent and could be as recent as 0.6 million years, when 79001 was blasted off Mars. It is of great importance to measure emplacement dates for E79 carbonates (and indeed all other secondary features in martian meteorites, especially A84) to restrict the relevant time interval. Until this has been done, it can only be speculated that during the span of the Cenozoic and Mesozoic eras on Earth, a time when dinosaurs lived and died, the first flowering plants evolved, and even perhaps when the first hominids appeared, our sister planet may have had primitive life forms.

It may be that some of the publicity attached to recent findings concerning martian meteorites will be proved to be misplaced. However, using Occam's Razor ('the simplest explanation is the most likely'), the interpretation of the martian meteorite results on the basis that biology was somehow involved may well stand.

Almost all meteorites contain carbon in some form, but it is mostly inorganic. Of special interest for the study of exobiology are the subgroups of the chondrite class, the CI and CM carbonaceous chondrites and the unequilibrated type 3.0 to 3.1 ordinary chondrites. All these groups are 'primitive': they show evidence of the passage or coexistence of water on their parent bodies. Other groups of carbonaceous chondrites might be even more primitive, but they seem to have little extractable organic matter. Their carbon is elemental or inorganic and they are anhydrous. There are a small number of unique or rare samples, many of which have relatively high amounts of carbon, but little or no work has been done to characterise it. CI and CM samples, typified by the Aargill and Murchison meteorites, have been the subject of many studies to characterise their extractable organic compounds: hydrocarbons. Aliphatic, alicyclic, isoprenoid and aromatic structures have all been found. Many other compound classes, normally considered to be related to biological activity when encountered in the terrestrial sedimentary record, have also been recognised. In this category come acids (carboxylic and amino), nitrogen heterocycles, amines, amides, alcohols etc. The early work has been reviewed by Nagy (1975) and more recent work summarised by Cronin et al. (1988). Close to 500 individual compounds have been identified.

The study of extractable organic molecules in samples collected from low-temperature environments and stored in museum collections is fraught with problems related to contamination during the time the samples have been on Earth. This is particularly true for the Aargill meteorite, which fell in 1864. While the Murchison meteorite was recovered in more enlightened times, not all samples have been collected, stored and preserved with equal care. Surprisingly, samples from the somewhat cleaner environment of Antarctica have been less well considered, part of the problem being that a suitable CI meteorite has not yet been located on the ice cap. Indeed, none of the samples from polar locations is available in the relatively large amounts needed for the study of extractable organics. Specimens found in 'sterile' hot deserts tend to be larger but appear to have been leached of their organic content (Ash & Pillinger, 1995).

Early work (e.g. by Nagy et al., 1961) suggesting the presence of biogenic molecules was severely questioned. Since then only very specific problems have been examined in detail. The best-studied case concerns the amino acids. The discovery (Kvenvolden et al., 1970) that these existed as racemic mixtures in the Murchison samples precluded an origin from contamination (all terrestrial biological amino acids are of the L-type). The idea that these amino acids were indigenous to the meteorite was confirmed when several non-protein species (Kvenvolden et al., 1971) were found among the extracts, and recently they have been shown to contain more  $^{13}\text{C}$  and  $^{15}\text{N}$  than their terrestrial counterparts (Engel et al., 1990). The distribution of indi-

### **II.3.5 Carbon Compounds in Other Meteorites (Carbonaceous Chondrites)**

vidual compounds is such that the simplest in terms of structure are most abundant. This implies an abiogenic origin, i.e. built up from single carbon atoms. The same kind of effects are also seen in short-chain aliphatic hydrocarbons, mono and dicarboxylic acids (Yuen & Kvenvolden, 1973) and polyaromatic hydrocarbons. The above interpretation is confirmed by the investigation of the isotopic composition of homologous series (Yuen et al., 1984; Gilmour & Pillinger, 1994), where isotope fractionation effects are in keeping with the greater strength of  $^{12}\text{C}$ - $^{13}\text{C}$  bonds as might be expected if the compound were constructed from simple precursors by a chain-building process. Biosynthesis, while producing isotopic differences between oxidised and reduced carbon, does not seem to show regular fractionation between small and large homologues.

Although the original breakthrough in recognising indigenous compounds in Murchison came from the discovery of racemic mixtures of amino acids, the most recent activities in this area have involved showing that in some instances there are slight excesses (5-10%) of L enantiomers in some compounds (Cronin & Pizzarello, 1996). The great pains taken with extraction procedures suggest that this is a real effect rather than a result of contamination. One proposed explanation of the results is that UV synchrotron circularly polarised light, emitted by neutron stars, might introduce an antiomeric bias. If this explanation turns out to be valid, then while it could account for the origin of optical activity, it possibly calls in to question the whole philosophy of using chirality as a means of detecting exobiology in extraterrestrial environments in the early Solar System.

Although the organic compounds, which are solvent-extractable from carbonaceous chondrites, are treated somewhat warily because of contamination issues, the macromolecular material that comprises the bulk of the carbon is usually considered as indigenous. There is no plausible way that such a large accumulation, up to 5wt%, could have accrued from contamination. In any case, even in the early 19th century, bulk carbon contents were measured soon after the fall of specimens such as the CI chondrite Alais, and high carbon contents were recorded at that time. The carbon isotopic composition of this material is remarkably constant (Smith & Kaplan, 1970; Kerridge, 1985) and relatively high for terrestrial contamination anyway. Nitrogen isotopic composition is well outside of the terrestrial range (Kerridge, 1985). It should be noted that when compared to the carbonates found in the same samples, there is a relatively large fractionation, 50-80‰, in the same sense as used by Schidlowski as an indicator of biogenicity (Schidlowski, 1987). Like kerogen in the Earth's sedimentary rocks, meteorite macromolecules are very intractable, yielding information only about their general structure when broken down by various pyrolytic or oxidative techniques; the building blocks appear to be substantially aromatic. There is a strong likelihood that the material constituting the bulk of the carbon in carbonaceous chondrites and unequilibrated ordinary chondrites originates in dark interstellar clouds. The key results in this respect are the recognition of substantial deuterium enrichments (Kerridge, 1993; McNaughton et al., 1981). Deuterium to hydrogen ratios approaching unity are encountered in small interstellar molecules because of ion-molecule reactions, which encourage H to D exchange (Geiss & Reeves, 1981).

In addition to the organic chemistry discussed above, there have been instances when the existence of microfossils were suggested in carbonaceous chondrites (Nagy et al., 1972), but such reports have been strongly criticised, sometimes justifiably, but perhaps not always. It should not be considered unscientific to indulge in detailed characterisation of the organic matter in carbonaceous chondrites from the point of view of exobiology (Nagy et al., 1961) but as already stated this is almost a taboo area. As can be seen from the amino acid studies above, investigations are almost always motivated by abiogenic interests, with large quantities of information ignored because contamination is perhaps too readily invoked. Methods to recognise contamination should be further investigated, using for example isotopic order, rather than discarding unconsidered large quantities of possibly useful data. Huge amounts of carbonaceous matter from carbonaceous chondrites have been destroyed in the



quest for interstellar grains. Although important in their own right, these studies might be better designed in future to preserve material for other studies. It may be ironic that martian meteorite studies could reawaken an interest in the investigation of the organic material in carbonaceous chondrites. Even more so, if the connection could be made because of the search for martian sediments on Earth.

Although it is highly speculative, a possible association between martian meteorites and type CI chondrites has been suggested (Brandenburg, 1996). Brandenburg's thesis, that Mars is the parent body of the CI chondrites, is based on several lines of thought and both isotopic and geochemical arguments. Top of the list is the similarity in the  $\Delta^{17}\text{O}$  displacements for martian samples and the CI chondrites relative to the terrestrial fractionation line. Other favourable comparisons made by Brandenburg concern the  $\delta\text{D}$ ,  $\delta^{13}\text{C}$ ,  $\delta^{15}\text{N}$  and noble gas isotopic compositions of martian samples, including minerals within this such as carbonates and various equivalent components in carbonaceous chondrites. He also points out that chemical analyses of the Mars regolith by Viking call for an input of CI material and makes the point that Urey (1968) suggested that CI meteorites might have come from an extinct planetary seabed, such as the Moon. Martian meteorites (Burgess et al., 1989) like CIs (Burgess et al., 1991) also contain both sulphide and sulphate minerals. The list of similarities is formidable and the longer it gets one assumes the less chance that serendipity is involved. The Brandenburg idea appeared just before the revelations regarding putative martian fossils (McKay et al., 1996) so the most obvious comparison, with the work of Nagy and co-workers on Orgeuil (Nagy et al., 1972), was not mentioned.

The most crucial test of a relationship between CI chondrites and martian meteorites is the  $\Delta^{17}\text{O}$  value, which Brandenburg suggests as 'similar'. From preliminary reappraisal of only three bulk CI samples, to obtain results of the highest precision, a link looks tenuous: the  $\Delta^{17}\text{O}$  for Argal, Ivuna and Alais has been found to be  $0.64 \pm 0.03$  (Franchi et al., 1997b), i.e. outside the error limits for the martian oxygen line  $0.320 \pm 0.013$  (Franchi et al., 1997a). However, the case will not be fully closed until more definitive measurements with separated and identified fractions have been made.

Recent dust collection, both above the terrestrial atmosphere (Love & Brownlee, 1993) and in the Greenland and Antarctica ice sheet (Hammer & Maurette, 1996), show that the Earth captures interplanetary dust as micrometeorites at a rate of about 50-100 t per day. About 99% of this mass is carried by 'large' micrometeorites in the 50-500 mm size range. This value is about 2000 times higher than the most reliable estimate of the meteorite flux (about 0.03 t per day) recently estimated by Bland et al. (1996). This amazing dominance of micrometeorites already suggests their possible role in delivering complex organics to the early Earth 4.2-3.9 Gyr ago (see Section I.2.1.2.) when the micrometeorite flux was enhanced by a factor of 1000 (Anders, 1989).

The nature of the large micrometeorites, their comparison with meteorites and the basic features of their carbon chemistry have been recently investigated. The tiny micrometeorites collected in the stratosphere, Interplanetary Dust Particles (IDPs), represent less than 1% of the micrometeorites and will not be discussed here.

Mineralogical and chemical studies (Kurat et al., 1994) were first aimed at comparing micrometeorites to meteorites. They afforded two unexpected features:

- micrometeorites are related to the relatively rare group of the most primitive meteorites (carbonaceous chondrites) and predominantly to the CM chondrites, which represent only 2% of the meteorite falls. So the composition of large micrometeorites that constitute the most abundant extraterrestrial matter accreted today by the Earth is under-represented in the most abundant meteorites, the ordinary chondrites and the differentiated meteorites. This reflects major differences in the parent bodies generating the meteorites and the micrometeorites;
- although close to CM chondrites, micrometeorites show marked differences: i)

### II.3.6 Carbon Compounds in Micrometeorites



much higher (about three times) C/O ratios reflecting higher carbon contents; ii) a very strong depletion, by at least a factor of 20, of chondrules that constitute 20% of the mass of CM chondrites; iii) a pyroxene/olivine ratio about 10 times higher. Thus, large micrometeorites seem to represent a population of Solar System objects probably related to comets and not yet represented in the meteorite collections.

Important clues about the carbon chemistry of micrometeorites were recently obtained:

- a detailed analysis of the carbon content of the various groups of micrometeorites (Engrand, 1995) indicates that the flux of total carbon delivered to the whole Earth can be estimated to be 500 t per year. This value is 50 000 times higher than the corresponding value estimated for meteorites;
- Clemett et al. (1998) have used the technique of Microscope Double Laser Mass Spectrometry to study the polycyclic aromatic hydrocarbons (PAHs) in both micrometeorites and major groups of carbonaceous chondrites. The PAH content of micrometeorites is rather similar to that of CM chondrites. This suggests that the amount of PAHs delivered to the Earth by the micrometeorite flux is about 50 000 times higher than the corresponding figure for meteorites. Micrometeorites also show a much larger variety of PAHs, including reactive vinyl-PAHs not yet found in meteorites;
- Brinton et al. (1998) detected amino acids,  $\alpha$ -amino isobutyric acid and isovaline in micrometeorites by high-performance liquid chromatography. In CM chondrites, these two amino acids are found in similar abundances and may have, therefore, been formed via a Strecker-type synthesis. In micrometeorites,  $\alpha$ -amino isobutyric acid is the most abundant amino acid. Its average content is about 10 times higher than in CM chondrites. Thus, the delivery of amino acids to the Earth would be 500 000 times higher than that expected from meteorites. Moreover, the  $\alpha$ -amino isobutyric/isovaline ratio is 20, suggesting that their synthesis is derived from HCN, a compound known to be present in comets.

Micrometeorites therefore appear to be the major source of the extraterrestrial organic molecules delivered to the primitive Earth which may have contributed to the appearance of life. Studies of micrometeorites by analytical electron microscopy bring additional support to this scenario. About 80% of the micrometeorites appear as complex aggregates. For instance, a single 100  $\mu$ m micrometeorite is composed of millions of tiny mineral grains imbedded in the abundant organic compound amounting to 7% of carbon. These grains contain a high proportion of metallic sulphides, oxides and clay minerals belonging to various classes of catalysts. In addition to the carbonaceous matter, the micrometeorites may therefore also have delivered a rich variety of catalysts, having perhaps acquired specific crystallographic properties during their synthesis in the microgravity environment of the early solar nebula. Electron microscope observations also shows that micrometeorites are tightly encapsulated in a thin crust of magnetite, probably gained during atmospheric entry.

In summary, micrometeorites were delivered at an average rate of about 50 000/m<sup>2</sup> per year all over the Earth, between 4.2-3.9 Gyr years ago. They may have functioned as individual tiny chemical reactors when reaching terrestrial liquid water. They probably contained all the ingredients required for the catalyzed processing of their complex carbonaceous matter by liquid water. Their magnetic crusts would have drastically increased their mechanical stability and confined the reactants within the grains. In addition, it probably reduced the access of water molecules inside the grains.

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## II.4 Team I: Exobiology and the Mars Surface Environment

The task of Team I was to define the environment of Mars at the surface and shallow depth, with a view to detecting possible signatures of past life. 'Signature' denotes a large spectrum of evidence from microscopic (bacterial to nanobacterial size) to macroscopic fossils, geochemical indicators of biological activity, and other indicators such as superparamagnetic grains. The evidence of past life might be present at the surface but it will more likely occur below the weathered (oxidised) upper layer. The depth of this upper 'barren' layer can be only guessed and the only way to determine its thickness or to construct a reliable model for it is to dig into the martian surface and collect geochemical and petrophysical data.

The approach adopted by Team I is based on requirements concerning two kinds of evidence of past life: chemical signatures and bacterial fossils. However, a large part of the discussion is also valid for the search of extant life.

In the past few years it has become clear that Mars is an extremely diverse planet in geological terms. The first data from Mars Global Surveyor (MGS) support the existing evidence of an extensive diversification of geological units, features and crustal structures. This geological diversity is of paramount importance in the exobiological exploration of the planet. This new approach is matched by the finding of forms of life in a huge variety of geological settings, environments and ecosystems on Earth. It has been well known for several decades that bacterial forms live in the Earth's crust at depths of several kilometres. Many forms of life live near the hydrothermal vents in the deep oceans and, recently, large living communities have been discovered in deep-sea zones around the bodies of dead whales. It is now clear that life is a strongly colonising factor which, matched with the martian geological diversity, suggests that Mars is actually a place where life was probably present and, just possibly, might still exist in some specialised niche.

The basic requirement for the formation and survival of life is the presence of water and energy (nutrients). This need not be true for an exotic form of life, but in order to establish the environmental constraints for the search for life on Mars it is better to adopt a conservative approach. Therefore, the best candidates are those environments where water has been present for a considerable time. From this point of view, the geological environments should be regarded as any geological setting, regardless of dimension and position, with definite boundary conditions. Thus, geological environments would be obvious features such as lakes and rivers, but we must also include pores, fractures, surface roughness, for example.

### II.4.1.1 Lacustrine Environments

Lakes are ubiquitous on Earth and display a tremendous variety of settings, conditions, shapes, etc. They have also been recognised as having existed on Mars. Martian lakes can be defined as localised areas where water was present as standing bodies. Probably, lakes were scattered on the surface in several geological settings. However, they are easily recognisable when they occur as water-filled impact craters. There are several instances where channels enter large craters and, as frequently, exit on the opposite side. These crater floors probably consist of sediments formed in ponded water. In some cases, there are terraces lining the crater interior walls and these may be interpreted as erosional shorelines. Lacustrine deposits of this kind may be expected to consist of bedded coarse-grained sand, gravel or cobbles near the bottom, grading downstream and upward to more fine-grained material as the flow slowed and particles came out of suspension.

### II.4.1 Environments and Rocks with Exobiology Potential



Fig. II.4.1.1/1. A Gilbert-type delta at the mouth of a short-headed stream. The Gilbert-type delta bodies suggest the presence of standing bodies of water. (Viking image, NASA.)

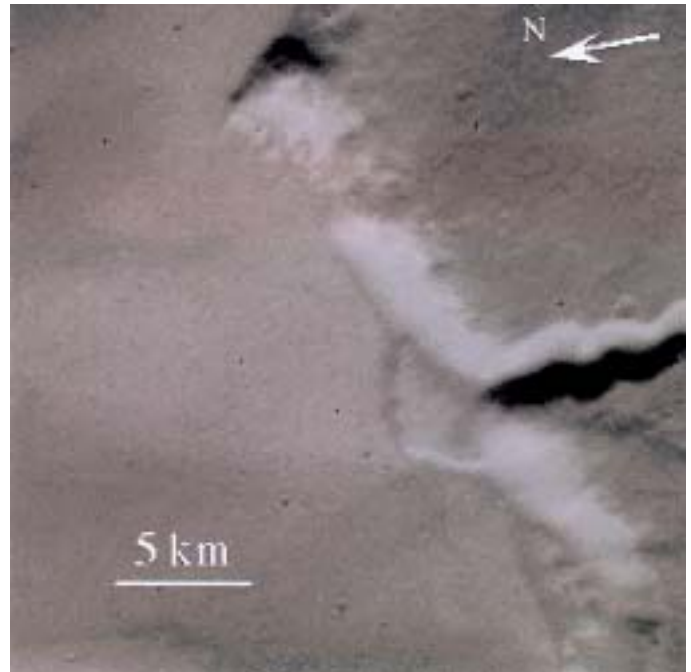
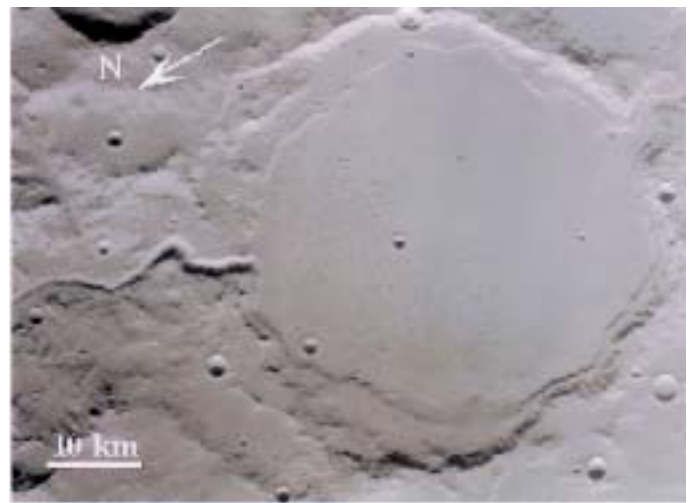


Fig. II.4.1.1/2. Terraces; *Left*: in an embayment of Hydroates Chaos (MGS-MOC image, NASA/MSSS). *Right*: around the rim of an impact crater with an inflow channel (Viking image, NASA.)

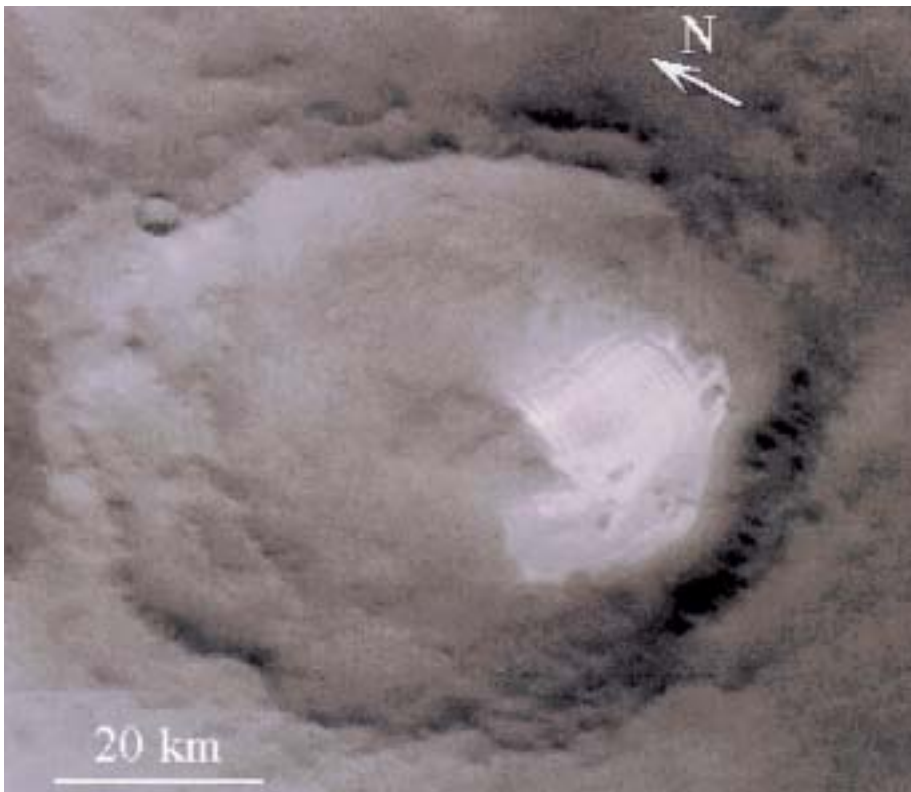


Crater lakes can be isolated, without channels entering the basin. In this case, only the terraces on the craters' inner rims are found. However, most instances show input channels with delta-like structures (Fig. II.4.1.1/1). The morphology of these deltas resembles terrestrial Gilbert-type deltas. These indicate standing bodies of water: when a stream flows into a water-filled basin with steep margins, it forms a delta (Gilbert-type) composed of a flat upper surface (topset) corresponding to the water level and a steep delta front. This type of delta and wave-dominated terraces (Fig. II.4.1.1/2) are the best evidence for bodies of standing water.

The sedimentation pattern could be quite simple, with the coarser deposits near the crater rims and mostly concentrated at the channel mouths (in the deltaic area). The fine sediments (very fine silt or clay minerals) probably remain in the central area. Evaporitic deposits, mainly carbonate, can be present in this zone.

#### II.4.1.2 Sebkha Environments

'Sebkha' is the Arabic word for dry lake. Sebkha on Earth are lakes only occasionally



**Fig. II.4.1.2/1.** The high-albedo area within Wegener crater suggests the presence of evaporitic deposits. The possible sedimentary origin of this area is supported by: i) the presence of ridges at the margin that could be interpreted as shoreline features; ii) the presence of some hills standing far above the high-albedo material. (Viking image, NASA.)



**Fig. II.4.1.2/2.** White rocks: this is one of the most acclaimed putative evaporitic deposits. However, mineralogical data are not available and MGS-MOC images show a rather rough morphology unsuitable for landing. (Viking image, NASA.)

flooded by water and, for most of the time, are dry and exposed to subaerial erosion and weathering. The deposits found in them are evaporitic: mostly calcium carbonate and gypsum or other exotic lithotypes. The sebkha are usually dry and evaporation (with the consequent concentration of evaporitic minerals) occurs within the sediment

Fig. II.4.1.3/1. The source area of Dao Vallis (near Hellas Basin) is one of the most quoted examples of putative hydrothermal vents.



pores, affecting the interstitial water. The evaporation decreases pore pressure which thus draws water from below. This 'evaporitic pumping' can produce massive and thick concentrations of gypsum or limestone, embedding silicoclastic or argilliferous material. Water can be found in shallow ponds even at the surface and direct deposition of salts can occur in these cases.

The sebkha usually exhibit a flat surface with a small supply of wind-blown detritus. They have not been recognised on the martian surface, but some putative deposits have been identified (Figs. II.4.1.2/1 and II.4.1.2/2). Further mineralogical investigation from orbiters needs to be performed in order to establish the presence of sebkha-like environments on Mars.

On Earth, these extreme areas are rich in bacterial life – dispersed in the water or concentrated in microbial mats. The fossilisation potential of these structures, as well as biomolecules, could be quite high if there is not pervasive recrystallisation of the deposits or solubilisation. Microbial fossils would be also preserved by replacement with more stable minerals such as silica.

#### **II.4.1.3 Thermal-Spring Deposits**

On Earth, subaerial thermal spring deposits are localised areas where heated gas-bearing water reaches the surface or the near-surface zone. These areas are strictly linked with volcanic edifices or activity. Several putative thermal springs have been identified on Mars (Fig. II.4.1.3/1), but none has been confirmed by a detailed geomorphologic and geochemical study. Usually, areas near volcanic edifices, having evidence of the generation of channels, are thought to be hydrothermal sites.

These terrestrial environments are rich in microbial material and, if at all present on Mars, could be one of the best targets for exobiological exploration. The large quantity of mineral deposition associated with fast and effective molecule-by-molecule recrystallisation allow the preservation of microbial material. Rocks derived from spring deposits may be rich in fluid inclusions retaining the original liquid and gas as well as microorganism and biomolecules.

#### **II.4.1.4 Duricrusts**

In the presence of surface water, sediments in arid lands tend to form particular soils, generically called duricrusts. These deposits occur when the surface water infiltrates down and brings up soluble compounds such as calcium carbonates, silica, gypsum and sesquioxides (particularly iron). The water can also move upwards in a manner similar to evaporitic pumping of sebkha and, in fact, duricrusts are soil types sometimes associated with sebkha deposits. The evaporation of the interstitial water leads to a large concentration of cement in the form of laterally extensive layers. On Earth, duricrusts are extremely hard and can be several metres thick. Bacterial activity is known to be associated with this type of deposit, which has a strong preservation potential.

On Mars, duricrusts have been not observed directly. However, their presence can be inferred because there is evidence of superficial humidity that can percolate downwards. The extreme dryness and gravity of the planet can enhance evaporation of the interstitial water, with the consequent formation of duricrusts. Iron is largely present at the surface and can assist in duricrust formation.

#### **II.4.1.5 Glacial Deposits**

There is a wide variety of associated landforms on Mars with characteristics resembling glacial features produced by the Pleistocene ice age on Earth. These features are generally restricted to high latitudes, and they form ordered sequences almost identical to those observed for terrestrial glacial landforms. They resemble eskers, moraines, kettles, glacial gouges, kames, drumlins, tunnel channels, outwash plains and glaciolacustrine plains. Of these features, only eskers, moraines, outwash plains and glaciolacustrine plains represent sedimentary deposits. The others are glacial erosion features that occur mostly in glacial deposits. These features will be described in some detail because they are not widely known. Their existence is the subject of debate, mostly because of the important implications for paleoclimatic reconstructions (see below).



Sinuuous ridges resembling eskers are found at various locations on Mars. They are seen best developed on the floor of the Argyre basin, the south polar region and at several locations in the northern plains. Like terrestrial eskers, martian sinuous ridges cross topographic divides, have very low and high tributary junction angles and rectilinear patterns, and show conspicuous longitudinal discontinuities and variations in width and height, producing a segmented or beaded appearance. Also like terrestrial eskers, they sometimes pass longitudinally into, or reside within eroded valleys that are probably glacial tunnel channels. Furthermore, they also show esker-like subhorizontal layering and ridge crests that are sharply crested, rounded, flat-topped or double-crested. Individual ridges range from 10 km to 200 km long, 0.3-3 km wide and 20-160 m high. This is near the upper range for terrestrial eskers, but they could not be seen at the resolution of the Viking images. If the martian sinuous ridges are eskers, they should consist of fine- to course-grained bedded material with occasional boulders.

Smooth plains in the Hellas and Argyre basins might be proglacial lake deposits. In Hellas, they occur at the terminus of the moraines and are associated with fluvial channels and a possible shoreline. There are numerous other areas, including crater floors, that could also be lacustrine deposits. Glacio-lacustrine deposits may have bedded well-sorted fine- to course-grained material, with possible scattered boulders deposited by the glacier near the lake's edge.

In several areas, particularly near the Argyre basin, there are relatively small-scale braided delta-like fluvial erosional and/or depositional complexes. In one instance, a braided delta-like region begins at a breached crater about 50 km in diameter. The crater is the obvious source of the braided complex and may have been the site of a glacier from which meltwater flowed to produce outwash-like plains. Similar braided complexes occur to the west and north. If these features represent fluvial deposits from a melting glacier, then they should consist of fine- to course-grained bedded material.

Glacial deposits are usually associated with large quantities of water, both liquid and solid, and they satisfy the first requisite for the existence of life. If life was present on Mars and glaciation also occurred, it is quite probable that microbial life flourished in glaciated zones (Sharp et al., 1999). The potential for preservation is, however, quite low.

#### **II.4.1.6 Polar Deposits**

There are two types of polar deposits of probable aeolian origin: layered and massive. The layered deposits overlie the more massive deposits and are therefore younger. As these deposits have the lowest impact crater density of any terrain on Mars, they are the youngest deposits on the planet. Both deposits are being eroded by aeolian action. In some cases, older impact craters are being exhumed as the overlying deposits are stripped away. If these deposits are aeolian in origin, they are surely fine-grained, well-sorted sediments. The origin of the layering in the youngest deposits is not well understood, but they could be formed by climate cycles. There is some evidence for angular non-conformities between subjacent bed sets. There is no obvious exobiological potential in these polar layered terrains. However, their high-frequency cyclicity might be a good record of global changes and their study can improve, by analogy with terrestrial global-change studies, our knowledge on the geosphere-atmosphere-biosphere interactions.

#### **II.4.1.7 Ground Ice-Permafrost**

On Earth, permafrost is found widely in the polar areas, with associated extensive bacterial activity. Permafrost is thought to be present extensively in the martian subsurface. If life was present at sometime on the surface, it is quite probable that it is now segregated in the ground ice. The location of the permafrost layer is, however, quite deep and probably out of reach of any direct investigation in the near future. Direct evidence of bacteria in the permafrost could be gathered by the discovery of methane released from the subsurface to the surface. Bacterial activity produces large quantities of methane and this could be released to the surface. It is possible that the



large chaotic areas (that produced the large outflow channels) are created by this type of mechanism.

Owing to the depth of the permafrost, the investigation of this potentially life-bearing environment will probably be postponed until the human exploration of Mars. However, there are areas at high latitudes where the permafrost is thought to exist at very shallow depths. Moreover, steep and high cliffs occur in several areas of Mars, and they might expose a portion of the permafrost.

Current concepts for searching for microbial fossils on Mars are strongly focused on finding the remains of former surface-bound microbial activity in sediments and hot spring environments. While it has become accepted that subsurface life is presently active on Earth to a depth of several km at some places (e.g. Stevens & McKinley, 1995; Stevens 1997), barely any fossil evidence of this has been reported so far, with the notable exception of a paper by Kretzschmar (1982), although there have been scattered reports in the literature since 1782. Recent work has shown that subsurface microbialites similar to those reported by Kretzschmar (1982) are common in terrestrial samples from numerous subsurface environments (Hofmann & Farmer, 1997). This work is in progress and approximately 100 sites have so far been identified. Geological environments containing such suspected fossil remains include

- sites of low-T hydrothermal alteration of volcanic rocks (dominant);
- oxidation of sulphide ore deposits (quite common);
- hydrothermal vein-type ore deposits (rare);
- silica deposits associated with serpentinisation of ultra-basic rocks (rare).

The depth of emplacement of filamentous microstructures of probable microbial origin is unknown in most cases, but reaches 400-800 m in at least three of the better-studied sites. There is a growing body of evidence that subsurface microbial activity commonly results in macroscopic structures similar to surface microbialites (stromatolites). Many of the hitherto recognised possibly microbial structures in such environments are enclosed in microcrystalline silica varieties (chalcedony, agate). The recent identification of agates as products of post-impact hydrous alteration in an impact melt of a Finnish crater (Kinnunen & Lindqvist, 1998) makes it likely that similar silica varieties do exist on Mars, since cooling impact melts interacting with groundwater must have been common. The likelihood of subsurface microbial fossils on Mars is significant and a subsurface biosphere can be expected to be relatively more important on Mars than on Earth because of the inhospitality of the martian surface.

By considering not only the surface-bound but also the subsurface microbial fossils in the process of evaluating landing sites, target selection for sampling, imaging etc enlarges the selection of available possible interesting sites and materials. A fractured or vesicular basalt, for example, would be considered of low interest in existing strategies focused on sediments and thermal spring deposits, but may nevertheless harbour rich subsurface fossil remains.

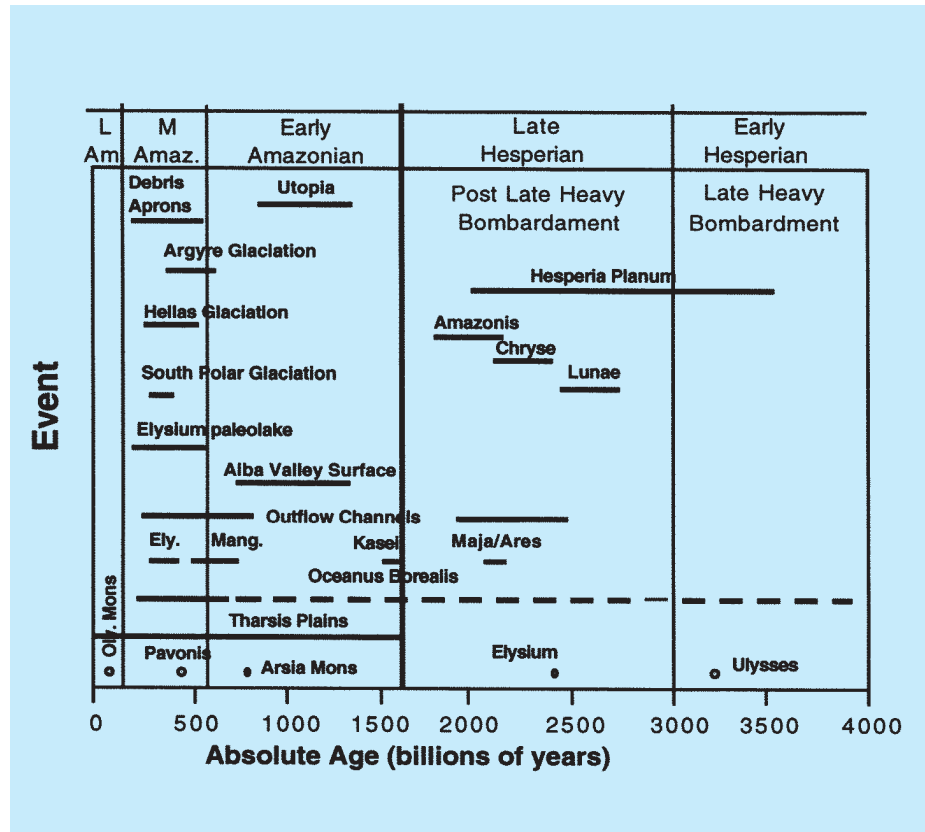
Reconstructing the climatic and environmental changes of Mars should be done through a detailed reconstruction of its geological history. Water is the most important factor among the variables that shaped the planet, and knowledge of the hydrological cycle and long-term variations of the environment are based on geological analysis. Of course, there are serious disagreements on the reconstruction of the geologic history but, basically, two models are proposed.

The first model proposes that the climate before 3.5 Gyr ago was warmer and wetter. A thicker atmosphere was able to sustain a complex climatic and hydrological cycle. In this scenario, rainfall played an important role. Precipitations, in fact, were able to degrade a large part of the geological features (mostly impact craters).

## II.4.2 General Remarks on Subsurface Microbial Fossils

## II.4.3 Climatic and Environmental Models

Fig. II.4.3/1. Ages of several geological units and features. Several events involving large amounts of water are rather young.



Complex fluvial patterns supplied by large-scale underground water circulation, in turn supplied by atmospheric water, formed intricate fluvial systems (channel network). The subsurface circulation of water supplied hydrothermal systems. Water was present later in martian history, but only sporadically and for very short periods. Life, according to this model, developed and was extinguished within the first warmer and wetter epoch.

This warm, wet model at an early epoch is based on two geological observations: 1) the degradation of the craters on the older surfaces ceased abruptly about 3.5 Gyr ago; 2) the valley networks occur on old (older than 3.5 Gyr) surfaces. This model has several shortcomings. Dating the crater degradation is quite complex and somewhat tautological because crater morphologies are being dated by crater counting. The age of the valley network cannot be achieved by defining the age of the unit where the channels occur. The surfaces can be much older, as happens on Earth, where today's rivers cut into even Precambrian geological units.

However, it is quite probable that erosion by flowing water played a major role in the degradation of the Noachian craters and other features (Carr, 1983) and this extensive degradation could be the product of climatic conditions that could sustain a thick and warm atmosphere.

The second model suggests that several episodes of wetter and warmer climate occurred during martian history. Several features indicative of liquid water are scattered over the surface. Outflow channels are a remarkable example, but they can be due to the dramatic and episodic releases of water at the surface and their lives are rather short, although intense. The important question is the fate of the water in the outflow channels. An old hypothesis suggests that the water remains briefly at the surface before sublimating into the atmosphere or percolating below ground. However, there is evidence of extensive coastlines at the border of the northern lowlands where the Valles flow (Parker et al., 1989). This supports the idea of a large ocean in the northern hemisphere (Baker et al., 1991). This northern ocean could have been active

**Table II.4.3/1. Environmental Conditions on Mars during Different Climatic Settings.**

	<i>Icehouse periods</i>	<i>Greenhouse</i>
Duration	long	short
Atmosphere	thin	thick
Water	in subsurface	at the surface
Biota	bacterial activity in ground ice	microbiota and algae at the surface

during several episodes throughout martian history; possibly it was present in epochs as young as the Middle Amazonian. On the other hand, a large number of features suggest the intermittent presence of water (both liquid and solid) on the surface. Outflow channels have been active in Late Hesperian time (Maja and Ares Valles), in Early Amazonian (Kasei and Mangala Valles) and probably in Middle Amazonian (channels of Elysium). Lacustrine deposits show the same age ranges.

It is quite probable that water, released episodically from the subsurface, formed large aqueous environments on the surface. The duration of these events is hard to define. Judging from the extensive feature described above, the surface liquid water remained active for a considerable geological time, allowing the formation of well-defined morphologies. In order to sustain such a complex hydrological system, an atmosphere entirely different from today's must be envisaged.

If life developed on Mars, it is possible it happened before 3.5 Gyr, during the warmer and wetter phase (Carr, 1995). The atmospheric conditions at that time were suitable for liquid water on Mars and life could have developed, as occurred then on Earth (Hansson, 1997). After 3.5 Gyr, the climatic conditions were unsuitable for surface liquid water and consequently for the development and evolution of life. Water has been retained in the subsurface as permafrost. However, during particular events, which may be triggered by an increase of the heat flux in the planet interior, permafrost melted and water was released at the surface. The evidence of water at the surface for remarkable time spans suggests that the climatic conditions changed, allowing the formation of large water bodies. These phases can be described as greenhouse periods with a thick atmosphere maintaining the water liquid. These wet periods contrast with the dry conditions when the atmosphere was thin (icehouse epochs). A sort of cyclicity may be envisaged (Baker et al., 1991), with long-lasting periods of a dry Mars with a thin atmosphere and water in the subsurface, followed by a short-term cycle of wet Mars, with a thick atmosphere and large standing bodies of water. See Table II.4.3/1.

If life was present during the planet's early history (before 3.5 Gyr), the dramatic change in climatic and atmospheric conditions that dried the planet must have affected the life. That probably then followed the fate of the water by entering the subsurface. Therefore, during the icehouse period the biota (if present) were restricted to the water and ice layer underground. On Earth, the permafrost (the subsurface ice) is a place where bacterial activity flourishes, and possibly this could be used as an analogue for the martian permafrost. The release of water at the surface will bring large quantities of bacteria and associated lifeforms back to the surface and, more importantly, it brings large quantities of methane (produced by the bacteria) into the atmosphere.

The energy driving the geophysical, geochemical and, eventually, the biological processes of an inner planet is predominantly of solar origin, except for a minor portion coming from the planet's interior. Depending on its distance from the Sun, its

## II.4.4 The Radiation Environment

orbital characteristics and atmospheric properties, such a planet may have a surface radiation environment substantially different from the Sun's spectral composition and intensity. In addition, every celestial body is embedded in a space radiation environment composed mainly of energetic particles of galactic and solar origin. These particles are modulated by interstellar magnetic fields, the solar magnetic field and the planet's own magnetic field (if the latter exists and is strong enough to deflect charged particles), and interact with the molecules and atoms of the planetary atmosphere. Therefore, the astronomical and physical properties of the celestial bodies that influence their radiation climates will be considered first. Since this study is targeted towards the search for signs of life in the Solar System with particular reference to Mars, this will deal only with the radiation climate of those planets within a 'green belt' – a habitable orbital zone between 0.7 AU and 2.0 AU where, as discussed above, liquid water is likely to have existed at least part of the time during the 4.6 Gyr history of our Solar System. Venus, Earth and Mars are situated in this habitable zone and their radiation climate and its implications for life are discussed below.

On Mars, CO<sub>2</sub> comprises about 95% of the atmosphere and is the major absorber of the short-wavelength UV radiation. Almost no radiation below 200 nm wavelength reaches the surface. The amount of O<sub>3</sub> in the atmosphere varies significantly with season and latitude. Whereas in the equatorial regions no ozone has been measured during any season, in the higher latitudes the O<sub>3</sub> column density increases from summer through autumn to winter, whereafter it decreases through to spring. In high-latitude winter conditions, the maximum observed O<sub>3</sub> amounts to 6 Dobson Units (DU), which is only 2% of the mean terrestrial column thickness. According to the seasonal variation of the O<sub>3</sub> concentration, at high-latitude winter conditions the solar spectrum at the surface of Mars shows a profound minimum in the range of 250 nm (as a consequence of absorption by O<sub>2</sub>), whereas at high-latitude spring conditions nearly all short-wavelength UV radiation above 200 nm penetrates the atmosphere.

Hence, although the solar constant for Mars is only 43% of that for Earth, the fraction of UV radiation reaching the martian surface is very much greater than that reaching the Earth's surface.

#### **II.4.4.1 The Current Atmospheric Radiation Budget**

The effective radiation temperature at the surface of a planet is determined by the solar irradiance impinging on the upper layers of the atmosphere and the albedo of the planet. It increases from Mars (-56°C) through Venus (-44°C) to Earth (-19°C), in all three cases well below the freezing point of water. However, the actual average surface temperature is much higher in the cases of Venus (464°C) and Earth (15°C), and slightly higher for Mars (-53°C). This warming, the so-called greenhouse effect, is based on the fact that optically active gases in the atmosphere allow UV and visible solar radiation to pass through, but absorb long-wave IR emitted by the surface and lower atmosphere. Such greenhouse gases are mainly H<sub>2</sub>O, which is a strong absorber in the 8-12 µm range, and CO<sub>2</sub> with its main absorption band at 15 µm.

On Mars, although the thin atmosphere is rich in CO<sub>2</sub>, its H<sub>2</sub>O content is too low to cause a greenhouse warming. Therefore, nearly all IR radiation, emitted from the surface, is radiated back to space.

Orbital eccentricity and the inclination of the rotation axis to the orbital plane determine the seasonal variations on the surface of a planet. Mars and Earth have similar inclinations to the orbit plane. Therefore, on both planets the seasonal variations of the solar radiation incident on their surfaces follow a similar overall pattern with spring, summer, autumn and winter. However, the martian orbit is more eccentric than Earth's. During southern hemisphere summer, Mars is closer to the Sun than during northern hemisphere summer, so the radiation reaching the surface is about half greater. Comparing the radiation incident at mid-latitudes on both planets, Mars during northern hemisphere summer and winter and southern hemisphere winter receives about 55% as much radiation as Earth during comparable seasons, but up to 84% during southern hemisphere summer.

The rotation rate of a planet obviously determines the length of its days, with Earth and Mars having days of similar lengths: about 24 h. On Mars, the diurnal variation of the surface temperature can be very high, up to 100K: actual ground temperatures at the equator range from 160-180K at night to peak values of about 260-280K at noon.

The surface temperature measured at the landing site of Viking-2 (47.97°N, 225.67°W) averaged over 11 sols agrees quite well with the model calculations. The diurnal temperature profiles measured during the Mars Pathfinder mission showed a good reproducibility from day to day. The temperature is lowest before dawn, then rises rapidly until noon and decreases more slowly during night. In high-latitude winter, the temperature is stable at 148K, the frost point of CO<sub>2</sub>. This low temperature is controlled by the continued condensation of CO<sub>2</sub> during winter at these high latitudes. In spring, CO<sub>2</sub> begins to evaporate. Temperatures rise rapidly after all of the CO<sub>2</sub> has evaporated. At the north pole during summer, a residual water-ice cap remains after the evaporation of the CO<sub>2</sub>. As water sublimates into the atmosphere, the temperature at the water-ice cap is kept close to 200K. Global dust storms are an important factor, reducing the direct transmission of solar radiation by up to 95%. They were recorded twice a year during the Viking mission with roughly a 3-day cycle. There is a rapid rotation of the clouds around the planet, with velocities up to 100 m/s.

#### II.4.4.2 The Current Particle and Radiation Environment

In the vicinity of a planet, the flux of cosmic ray nuclei is modulated by the planet's magnetic field, if it exists. Among the terrestrial planets, the Earth is the only planet with a strong magnetosphere. This causes, on the one hand, deflection of low-energy cosmic ray particles (the cut-off effect), but on the other hand capture of light solar wind particles, forming the radiation belts. Other than the Earth, no other terrestrial planetary body has radiation belts. Recent data from the Mars Global Surveyor mission show a strong remnant magnetisation of parts of the martian crust but no dynamo field. These localised magnetisations are not capable of deflecting charged particles and producing essential radiation belts.

The stochastic solar flares are efficiently absorbed by the atmospheres of Venus and Earth, and even for Mars little reaches the surface. The total annual dose, as the sum of the annual galactic cosmic ray dose and the dose from one large solar flare, has been estimated to be 0.21-0.24 Sv/a. Hence, the surface doses on Mars are about 10-20 times higher than those of the worst case at the surface of the Earth. Below the surface, the martian regolith provides some protection against cosmic rays and solar flares in addition to that already provided by the CO<sub>2</sub> atmosphere.

An additional radiation environment is created by emissions from within the planetary surfaces. On Earth, the primordial radio nuclides are <sup>40</sup>K, <sup>87</sup>Rb, the <sup>233</sup>U series and the <sup>232</sup>Th series. They contribute a total of 2.0 Sv/a to the annual effective dose equivalent from natural sources on Earth. These radioactive elements are enriched in the Earth's crust compared to deeper regions. The abundance of radionuclides on Mars has been assessed from that found in the martian meteorites. Their abundance does not significantly deviate from that of the radio nuclides in the Earth's oceanic crust. It is assumed that the dose equivalent owing to emissions from natural radio nuclides on Mars is less than 1% of that resulting from cosmic rays.

#### II.4.4.3 The Radiation Environment in the Past

From the geological record of the terrestrial planets and observations of young, Sun-like stars, it is inferred that, during the Solar System's 4.6 Gyr history, the radiation climate of Venus, Earth and Mars has undergone dramatic changes. The luminosity of the early Sun during the first 0.1 Gyr is predicted to have been only 0.7 of the current level, and for the following 0.7 Gyr it increased slowly to 0.75.

However, the spectral irradiance at higher energies was much higher for the young Sun. During the first 0.5 Gyr, the soft X-ray flux is suggested to have been 10-100 times than today, and the vacuum UV (30-150 nm) irradiance was 3-20 times.



Several attempts have been made to model the early planetary climates taking into consideration the lower solar luminosity and putative early atmospheric conditions. On Mars, the valley networks and erosion rates provide convincing evidence of liquid water on the early surface. This implies elevated surface temperatures, to allow liquid water to flow across the surface for enough time to cut the valley networks. Most models focus on atmospheres composed mainly of CO<sub>2</sub> and H<sub>2</sub>O, which are efficient greenhouse gases. Other gases, such as NH<sub>3</sub>, CH<sub>4</sub>, and SO<sub>2</sub> are too sensitive to photo-dissociation induced by solar UV radiation to be relevant for greenhouse warming. However, so far, no satisfactory model has been developed to explain the rise of surface temperature to above freezing by greenhouse warming. This is especially complicated by the lower solar constant predicted from stellar models.

## II.4.5 The Rationale for Landing Site Selection

The selection of landing sites is a rather complex procedure and it must take into account several variables. What kind of evidence are we seeking: present life, past life, microbial fossils, geochemical signatures? Where are we looking for evidence of life: at the surface, in the subsurface? Which kinds of instruments are we going to use? At present, we are considering a lander deploying a rover with drilling equipment. Instrumentation includes cameras and geochemical analytical tools to look for chemical indicators of past (present) life.

Two different kinds of landing sites can be envisaged. The first is a particular environment where life could have been present. Several were summarised above. The second is an area where there are a large number of lithotypes representing a random collection of rocks. The first type is based on deterministic thoughts, whereas the second is based on more stochastic considerations. Sites of the first type are required for geochemical investigation and drilling (as well as fossil imaging). The second type is good for a random and extensive search for imaging microbial fossils, but not for chemical investigation of indicators of life. This type of site, similar to the Mars Pathfinder one, is valid for petrological and mineralogical studies.

The first type of landing sites was discussed above, and there is a clear picture of the exobiological potential. Of course, with the current state of our knowledge of the martian environments, the selection of a site of this kind will be based mostly on theoretical work and educated guesses. The *Mars Landing Site Catalogue* (Greeley & Thomas, 1994) lists 150 possible landing sites, only a fraction of which are related to exobiology, however. Landing sites with exobiology interest were selected on the basis of: inferred presence of sediments, often in craters; partial excavation of sediments by younger impacts to allow access to suspected buried sediments; stratified canyon walls (possible sediments); chaotic terrains indicating the presence of water and possibly of hydrothermal activity; high-albedo features in craters (inferred evaporites); ground ice (cratered); polygonal features (sediments/ice?); terracing (water flow). Similar criteria can be applied during the selection of landing sites for ESA landers. However, all of the above criteria are based on the expectation that evidence of former surface-bound life is most likely to be found.

Apart from the possibility of former surface-bound life on Mars, landing site selection should also give consideration to the possibility of fossils of microbes that lived in the subsurface. The geological structures where such fossils might be expected are fundamentally different from those possibly containing surface-bound microbial remains. Such environments have, so far, not been considered in published landing site catalogues, but they should be included in the overall evaluation. The likely presence of a variety of geological materials at many sites makes it possible that both surface and subsurface fossils might occur at a single landing site.

Subsurface environments possibly containing extinct microbial life on Mars are:

- highly porous or diagenetically cemented sedimentary rocks (e.g. conglomerate with low amounts of fine-grained material);
- voids in impact breccias and impact melts;

- voids in volcanic rocks (vesicles);
- fractured rocks of any type.

The chances will be best in rocks that were buried to a considerable depth (several hundred metres or more) and have resurfaced owing to impact ejection, on canyon walls etc. The following landing sites belong to the second type and appear most promising for subsurface fossil microbes:

*Chaotic terrains:* material broken up at a large scale provides insight into the subsurface geology of formerly water-rich environments. Imaging of nearby steep mountain slopes would allow insight into geological context.

*Steep canyon walls:* excellent insight into geological context but virtually inaccessible. Distal debris aprons may be good sites for sampling.

*Channel floors (outflow from chaotic terrains):* possible fragments from microbe-bearing subsurface environments, but with limited geological context.

*Impact crater ejecta:* can provide access to material hidden in the subsurface. Limited geological context.

The selection of the landing site should be the responsibility of a restricted mission team, but the scientific community should be closely involved through meetings and discussions. Of course, the safety of the landing site is of paramount importance. Another task is to understand the types of instruments involved, their operation capabilities and, most important, the scientific background. The mission concept will influence the type of landing sites. The most challenging – and promising – sites are those with environments such as sebkha, lakes and hydrothermal vents capable of sustaining life forms. These kind of sites are identified at present on the basis of their sedimentological and geomorphologic features. Some knowledge about the mineralogical composition of the surface will be extremely useful in refining the interpretation. However, these type of data are lacking and it is difficult to foresee their full availability before 2004, even with the Thermal Emission Spectrometer returning data from the current Mars Global Surveyor mission.

Several basic requirements for a proper landing site can be summarised as:

*Age:* according to conventional wisdom, which sees the Early Noachian as the only geological epoch with climatic conditions suitable for the development of life, the best targets are geological units of that age. This hypothesis is, however, not fully supported by the geological evidence (see above). A large number of lakes, channels and other environments suitable for life are as young as middle Amazonian. Thus, the range of ages of rock units with biological potential actually span a large part of the planet's history;

*Concentration:* it is quite important that the material indicating the past presence of biota, either microbial fossils or biomolecules, is concentrated in appreciable amounts. This concentration occurs in several environments (see above) and depends, in geological terms, on the ability of the lithological substratum to sustain biota;

*Preservation:* the preservation of the evidence of past biota is of paramount importance in the exploration. Microbial material should fossilise rapidly. Environments with crystallisation potential, such as hydrothermal springs or sebkha, are quite efficient. Organic molecules and other chemical evidence may require rapid burial to avoid alteration at the surface;

*Thin aeolian cover:* the Mars Global Surveyor images have confirmed that the martian surface is extensively affected by aeolian deposition. The wind-blown detritus can cover large parts of the planetary surface and the selected landing site should show indications of thin aeolian coverage (that is, high thermal inertia and low albedo);

*Area of the target:* the target for the exploration (a sebkha floor, a hydrothermal spring) must be really extensive in order to be within the landing area uncertainties. Moreover, it should be within the range of the rover capability.

The search for past life can be driven by the experience gathered in terrestrial geological work. This experience suggests that the long-term preservation of biological information on past biota occurs under a fairly narrow range of geological conditions. Detrital systems can preserve fossils or biomolecules by rapid burial, whereas chemical deposits will preserve the evidence of life by coating impermeable material around fossils or recrystallisation. Stable minerals are better, owing to the long residence time – silica and phosphate are particularly suitable. Evaporite materials are rather unstable and only rapid burial allows the preservation of information on biota.

## II.4.6 Rovers and Drilling Operations

The operation of rovers and drilling equipment on the martian surface should take into account the scientific requirements. The environments suitable for exobiology exploration consist of rocks and sediments with characteristics that influence the technical procedures. Our current experience of the surface is biased by the Viking and Pathfinder sites. These are located in plains dominated by former fluvial-related sedimentation and slight aeolian reworking and sedimentation. Moreover, the investigations were limited to exploring just a few centimetres below the surface. It is quite possible that landing sites with exobiology potential will include rather hard lithologies. Duricrusts, limestones and even some sebkha deposits can be considerably hard lithotypes and can provide a technological challenge to the drilling instruments.

Inasmuch as drilling is of paramount importance in the search for life and past life on Mars, a careful analysis of the environmental targets and their geological characteristics should be performed. Duricrusts can be expected in any kind of environments. They can be very hard and could occur a few tens of centimetres below the surface. Even the Viking landing sites could hide hard layers at shallow depths. In fact, even in this environment oxidation processes along with element mobilisation can induce the formation of crust below the surface. Duricrust made up of silica or sesquioxides can be extremely difficult to drill, whereas limestone and gypsum-duricrusts are softer.

Lacustrine deposits can be composed of a number of different lithologies. Limestone beds are as hard as calcium carbonate duricrusts, whereas detrital silicoclastic material is probably very soft. However, clays can resist drilling because of their cohesion capability.

## II.4.7 Landing Sites

Several environments and geological settings with exobiological potential and the previous presence of water have been identified. Climatic models suggest that water was present on Mars throughout its early history, extending the capability for sustaining life up to early (middle) Amazonian.

The analysis of the geological environments on Mars must be pursued using the old Viking data as well as the Mars Global Surveyor images and altimetry data. A detailed analysis of the lithologies where rover and drilling equipment would probably operate effectively is fundamental in order to provide a background to the instruments design.

Four landing sites have been identified as likely to be suitable for exobiological exploration. They are being used as examples to aid the discussion on site selection. In particular, there is no detailed analysis on the safety requirements typical of a lander mission. Mars has plenty of possible landing sites with a large variability in terms of geological setting, exobiology potential, elevation, position, etc. The four represent some of the possible sites and are the compromise among several constraints. They are near the equator and at elevations almost below the datum plane.

### Site 1: Marca Crater and adjacent putative crater lakes (Memnonia) Lacustrine and Deltaic Deposits

*10.4°S/158.2°W*

*Crater diameter: 84 km*

*Elevation of the substratum: 2 km*

*Elevation of the crater floor: about 1 km or less*

These craters are located in the northern area of Memnonia in a Noachian geological unit. It is quite probable that Marca Crater formed during the Late Heavy Bombardment. There is evidence that the crater has been filled with water in more recent times. The floor shows a crater population largely below 1 km in diameter, and the possible age for the floor unit lies in the Amazonian. The main evidence for the presence of water is the Gilbert-type delta at the rim. This evidence, coupled with the flat young floor and the remains of the eroded and flooded central peak, suggests that the crater acted as a lake in one or more episodes.

The crater floor probably contains ridges of young wind-blown sand. However, this loose detritus is probably in patches, as suggested by the variation in albedo that strongly supports the possibility of the lacustrine beds being exposed.

The nature of the lacustrine deposits cannot be identified; there is probably clastic detritus (sand and pebbles) from the erosion of the crater rims matched by an internal drainage pattern. There could also be limestone and clay. The site would allow old Noachian rocks and younger lacustrine deposits to be investigated.

In the area there are several craters that have been lacustrine basins during the Late Hesperian and Amazonian eras (Farmer & Landheim, 1994), including the Gusev Crater (Grin & Cabrol, 1997). All of them are alternative landing sites because the settings are basically similar to Marca Crater. Gusev Crater is actually the only crater of this type that received a large amount of sediment by a large-scale fluvial channel.



**Fig. II.4.7/1. Site 1: Marca Crater is one of several impact structures in Memnonia showing evidence of standing bodies of water. (Viking image, NASA.)**



## **Site 2: Apolinaris Patera**

### **Volcano and Volcanogenic Units**

*8.6°S/187.5°W*  
*Elevation: 0-1 km*

Apolinaris Patera is a volcanic complex in the equatorial area of Mars at a relatively low elevation. The age is probably Hesperian (Scott et al., 1993). The patera itself is not of great exobiology interest, but there is a lot of evidence for the presence of water (Gulick, 1998): chaotic zones, lobate ejecta and channels. Owing to the volcanic activity, coupled with the presence of water, the most probable processes are hydrothermal. These hydrothermal systems probably existed well into the Amazonian era (Gulick, 1998), witnessed by the pristine crater associated with the volcanic edifice. Fluids were likely ejected from the volcano on to the surrounding plains, forming a rather large hydrothermal site. Evidence of (hydrothermal) sapping occur in the southern area.

Among the several possible landing areas, the southern portion of the patera probably has the greatest potential for exobiology. There is a large fan-shaped channel complex in this area. Erosion of the volcano's flanks has probably exhumed large quantities of old volcanic units, and hydrothermal springs are rather probable. A landing site at around 12°S/185.5°W (Gulick, 1998) would be suitable for exobiological exploration.

**Fig. II.4.7/2. Site 2: Apolinaris Patera is a volcano with extensive features suggesting the presence of water and, consequently, hydrothermal activity. (Viking image, NASA.)**





### **Site 3: SE Elysium Basin** **Channel and Lacustrine Deposits**

*3°N/185°W*

*Elevation: -1 km to -2 km*

The south-east portion of the Elysium basin is a flat area of the northern lowlands near the planetary dichotomy (Dohm et al., 1998). The basin was a catchment area for a large part of the Amazonian area, with a number of outflow channels into the basin. It is quite probable that volcanic and sedimentary rocks coexist, forming a complex basin with a wide variety of geological units.

The presence of channels is firmly recognised, whereas the evidence for lakes is scarcer. However, it is quite probable that the area contained bodies of water intermittently in epochs as young as Amazonian (Scott et al., 1995).

The coexisting lacustrine deposit, outflow channels and volcanic (also pyroclastic) deposits make this landing site quite attractive. The possibility of large lava flow covers cannot, however, be ruled out entirely.

**Fig. II.4.7/3. Site 3: the flat morphology of this part of the Elysium Basin has been suggested to be the result of a standing body of water. (Viking image, NASA.)**



**Site 4: Chasma area, east of Valles Marineris**  
**Large Range of Sedimentary Environments**

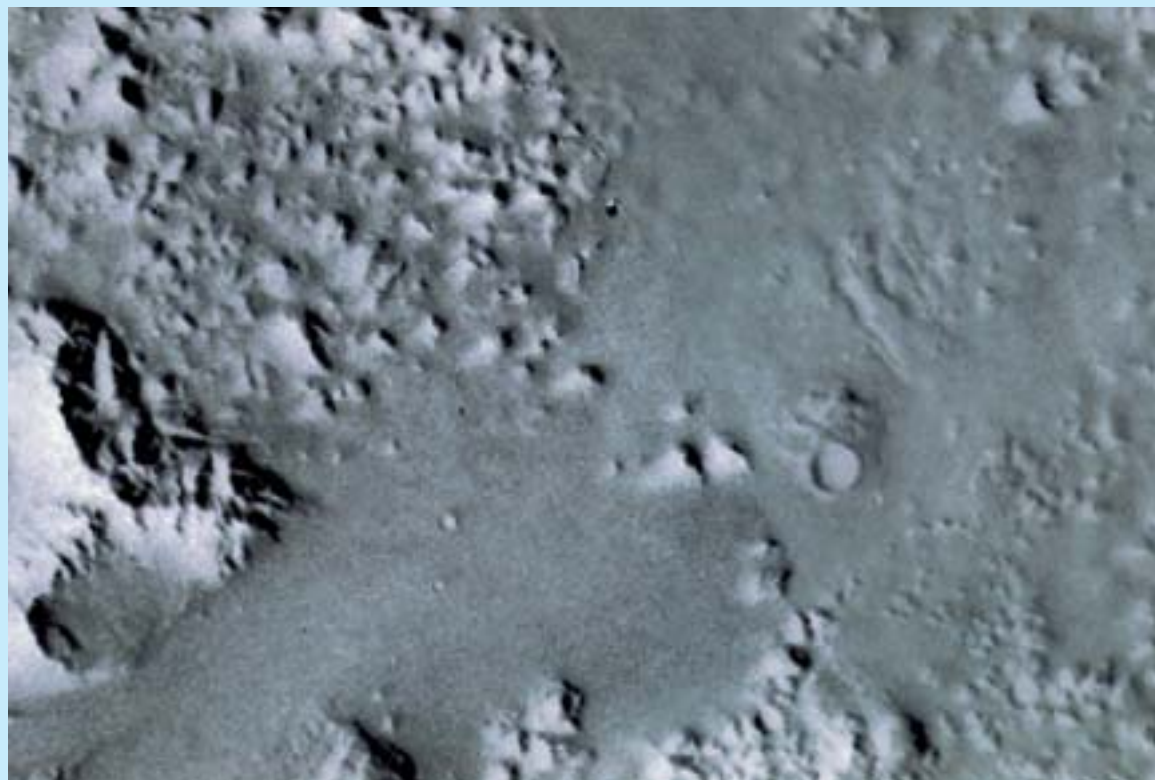
*8°S/35°W*

*Elevation: below -3 km*

This area is one of the deepest chaotic zones in the equatorial belt of Mars. It is quite complex and includes some attractive zones such as Capri Chasma and Gangis Chasma. Moreover, it is dominated by the channel Chaos Complex that connects Valles Marineris to Hydroates Chaos. The area consists of typical chaotic features suggesting the presence of mass-wasting material that is supposed to represent rocks from a large time span judging from the elevation of the surrounding cliffs. The outflow channel would provide debris from remote areas as far as Valles Marineris. From this point of view, the landing site is remarkable for the possibility for analysing rock samples from different geological units. Moreover, the basin's depth and the link with the possible lacustrine deposits of the Hydroates Chaos (Mosangini & Ori, 1996) suggest the possibility that the area has been affected by standing bodies of water.

The exobiology potential is not as high as the other landing sites but the possibility of collecting a variety of lithologies, plus the possible presence of lacustrine deposits, make this an interesting site.

**Fig. II.4.7/4. Site 4: the floor of the chasma area east of Valles Maineris is rich in sedimentary features ranging from putative lacustrine deposits to aeolian sand sheets. Fluvial activity has left a large number of structures. (Viking image, NASA.)**



Some do not offer tremendous exobiology potential, but for other reasons could be suitable for a landing.

One of the most promising environment types has been not included in the selected sites: the sebkha dry lake. These lakes probably existed on Mars but the evidence is still poor – some white patches are observed at visible wavelengths (Figs. II.4.1.2). Examples of these features are relatively common (Section II.4.1.2; O'Connor & Geissler, 1996), but without direct mineralogical (and/or elemental) analysis the interpretation of these deposits is still dubious.

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## II.5 Team II: The Search for Chemical Indicators of Life

### SCIENTIFIC OBJECTIVES

The main scientific objectives of Team II's task is to define a full and detailed, but realistic, instrumentation package to search for chemical indicators of past and/or present life. This includes a sampling subsystem to drill and sample the subsurface martian soil. It should also include several dedicated subsystems to perform:

- elemental analyses;
- mineralogical determination;
- isotopic and molecular analyses of inorganics and organics in connection with structures and life processes.

It is essential to have access not only to surface but also to subsurface samples, to a depth where :

- the possible effect of UV radiation on the chemical indicators of life is negligible;
- the concentration of oxidising agents such as  $\text{H}_2\text{O}_2$ , a potential source of degradation of organics, including bio-organics, is negligible.

So far, information about the UV radiation climate on the surface of Mars has been obtained only from radiative transfer models. Owing to the absorption of shorter wavelengths by the  $\text{CO}_2$  atmosphere, almost no radiation below 200 nm reaches the surface. The amount of UVC ( $190 \text{ nm} < \lambda < 280 \text{ nm}$ ) reaching the surface depends on the atmospheric concentration of  $\text{O}_3$ , which varies significantly with season and latitude. Whereas in the equatorial regions no ozone has been measured during any season, the  $\text{O}_3$  column density in the higher latitudes increases from summer through autumn to winter, whereafter it decreases again through spring. However, the maximum  $\text{O}_3$  amounts to 6 DU, which is only 2% of the mean terrestrial  $\text{O}_3$  column thickness. According to the seasonal variation in the  $\text{O}_3$  concentration, at high-latitude winter conditions the solar spectrum at the surface of Mars shows a profound minimum at around 250 nm (as a consequence of absorption by  $\text{O}_3$ ), whereas at high-latitude spring conditions and in equatorial regions almost all short-wavelength UV radiation above 200 nm penetrates the atmosphere. Hence, although the solar constant for Mars is only 43% of that for Earth, the fraction of UV radiation reaching the surface of Mars is very much greater. UV radiation is easily absorbed by martian dust and soil, so it will not penetrate to depths greater than a few mm, provided there are no cracks in the upper layers. The case is different for  $\text{CO}_2$  and water ice, where the spectral UV attenuation strongly depends on the degree of pollution in the ice. More insight into the UV penetration will be obtained from models being developed and from laboratory measurements under simulated martian conditions.

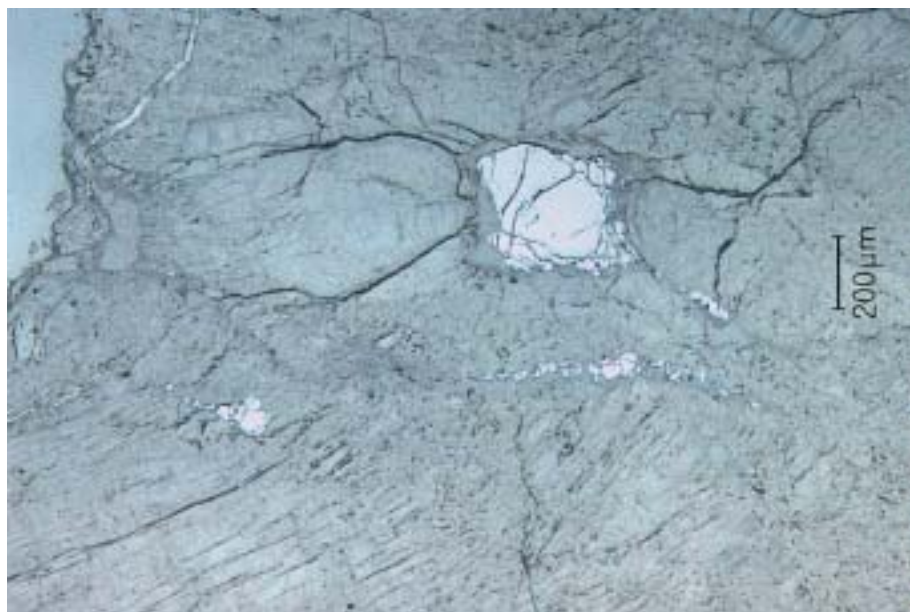
A model of the  $\text{H}_2\text{O}_2$  concentration as a function of depth in the near-surface martian soil is an absolute prerequisite for estimating the minimum depth to which such conditions are likely to be present.

To be able to correlate such a model with the observations, it is also essential that the sample acquisition subsystem should be able to provide a stratigraphic concentration measurement of minerals and organics, especially of the biologically significant compounds.

### II.5.1 Sample Acquisition and Distribution Subsystem



Fig. II.5.1/1. The polished surface of ALH 84001 at low magnification. Easily visible are a large chromite grain (light grey) surrounded by feldspar-like matter (dark grey) in a rock consisting of orthopyroxene (main mass grey). Such a surface offers the possibility of *in situ* analysis of the different minerals, making mineral mappings and analysing the fillings in the cracks, which could carry life or life remnants and mineral precipitates of an organic origin. Elemental and isotopic analyses of the minerals and life remnants allows the rock's formation conditions and its history to be estimated. (Courtesy Gero Kurat)



The sample acquisition subsystem should also be able to penetrate the surfaces of the rocks. Because the rock-atmosphere interface is a prime reaction zone, its chemical and mineralogical composition and physical state are likely to be very different from those of the shielded rock underneath. Samples for the various analyses outlined below will have to be taken from both of these sites. Furthermore, the sampling process has to take into account the need for flat surfaces for certain techniques to be applied, i.e. rocks or chips thereof will have to be ground flat (for APX, microscopy, Raman spectroscopy, IR spectroscopy). Loose, fine-grained matter will have to be placed on to flat sample supports before analysis by the above methods can be performed properly.

## II.5.2 Mineralogy, Petrology and Geochemistry

### II.5.2.1 Mineralogy and Petrology

#### II.5.2.1.1 Extinct Life

Surface and subsurface mineralogical, petrological and geochemical analysis provides indispensable basic information on the general planetological setting of a site, the local environment and traces of biological activity. A mineralogical-petrological analysis can help to evaluate a site for its potential to host life or life remains.

Life can leave its imprints on rock surfaces as etch pits, reaction product deposits and organic matter deposits (bio-crusts), and it can leave them below the surface. Any place, from fractions of a millimetre up to many metres below the surface, is possible for biological activity and thus for biodeposits. Any space from micropores to macro-cracks can be used by living organisms.

In loose sediments, macrobiomarkers could be present in intergranular space at a depth where living organisms and their remains are protected from degradation. Such markers could comprise fossilised hard parts, such as skeletons, shells and spines, or organic matter (kerogen-like material).

A search for such biomarkers has to be accompanied by a proper mineralogical and petrological characterisation of the environment. Principally, the following situations and derivatives can be envisaged:

- the primary hard rock (basalt, andesite, etc) environment;
- the sedimentary (sand, mud, chalk, conglomerate, etc) environment and the large free space (large vugs, caves, fissures, etc) in both rock type environments.



**Fig. II.5.2/1.** Sediments are formed via different processes. Volcanic tuff at Monte Leste, Sal Island, Cabo Verde. Tuff has been cemented and hardened at different degrees depending on original mineralogy and porosity of tuff layers. White crust: late formation of calcium sulphate. (Courtesy G. Kurat)



**Fig. II.5.2/2.** Sediments governed by fluid circulation. Tuff, Monte Grande, Sal Island, Cabo Verde. Hardening and cementation of tuff below the surface (here opened in a pit) is governed by fluid circulations (down and up). It will not be detectable at the surface but will influence drilling. (Courtesy G. Kurat)

The different types, naturally, require different analytical approaches. All environments need, however, to be characterised in detail in order to provide a proper basis for estimating the local history of biological activity. Such characterisation has to comprise:

- mineralogy, texture and bulk chemistry of primary rocks;
- mineralogy and sedimentology of the soil-, wind- and water-deposited sediments, regolith, etc (grain sizes, grain shapes, secondary minerals such as clays, carbonates, zeolites, hydrates, chlorites);
- mineralogy of mobile phases and hard ground cements: halogenides, sulphates, nitrates, silica, carbonates, Fe oxyhydrites, etc;
- search for biomarkers: framboidal sulphides or oxides, biophosphates, oxalates, silica, biogenic magnetite, barite, fossils.

#### ***II.5.2.1.2 Extant Life***

In a search for extant life beyond the Earth, microorganisms are the most likely candidates for a biota in an extraterrestrial habitat. Microbial communities leave discrete vestiges of their existence in the surrounding inorganic habitat. Biomineralisation and biodegradation of minerals are examples of life's interaction with the lithosphere. Therefore, a search for biogenic minerals would provide valuable information about ongoing or past activities of life. The biogenic nature is imprinted in the distinctive crystallographies, morphologies and/or isotopic ratios that make these minerals distinguishable from their abiotically-produced counterparts of the same chemical composition. Those minerals resulting from genetically-controlled mineralisation processes and formed within a preformed organic framework would have non-interchangeable characteristics, such as the orientation of the crystallographic axes and the microarchitecture. Terrestrial examples are the strontium sulphate skeletons of the unicellular marine Acantharia, the amorphous silicate shells of diatoms and the biogenic magnetite formed by bacteria. It is important to note that minerals produced under biologically-controlled processes are not necessarily in equilibrium with the extracellular environment. By contrast, those minerals formed extracellularly by biologically-induced mineralisation processes can be less easily distinguished from their abiotically-formed counterparts. They are formed in an open environment and are in equilibrium with this environment.

#### **II.5.2.2 Geochemistry (Elemental Composition Analysis)**

The quantitative analysis of as many chemical elements as possible will provide essential information on the elemental bulk composition of the rocks and soil. Major, minor and trace element abundances will allow depiction of the location's geological history and the analysis of the oxidation state of certain elements (e.g. Fe, Mn, S, N) will provide information such as the ratio FeIII/FeII on the redox conditions and their historical development. Knowledge of the relative abundances of the biologically significant elements C, H, N, O, S and P, and their distribution between organic and inorganic matter is particularly important.

Also of great interest is the determination of:

- the abundance of carbon in relation to carbonates (the importance of which is drastically increased by the problem of the carbonates in the SNC meteorites);
- the abundance of nitrogen and its oxidation state in the martian soil, to understand what happened to the initial atmospheric nitrogen.

In all cases, determining the variation in abundance with depth is crucial for extrapolating the data and estimating the abundances in the soil's deeper layers.

### **II.5.3 Isotopic Analysis**

Isotopic ratios are a very valuable set of chemical biomarkers (Schidlowski et al. 1983; Watanabe et al., 1997).

#### **II.5.3.1 Carbon and Hydrogen**

The  $^{13}\text{C}$  depletion produced by the Calvin cycle of photosynthesis is a clear signature of biological activity. Other carbon-fixing pathways involving photosynthesis, such as the C4 (Hatch Slack) pathway, also fractionate in favour of  $^{12}\text{C}$ , but to a lesser extent. The  $\delta^{13}\text{C}$  values of average biomass are about 20-30‰ more negative than those of inorganic carbon. As discussed in Part I, such negative values have been maintained over the whole history of life on Earth.

Methanogenic microorganisms generate substantial fractionations that prefer hydrogen to deuterium by sometimes as much as 30% relative to the aqueous substrate. Carbon is isotopically lighter than that fixed by photosynthesis in such systems. Methylotrophic organisms thus produce matter that is isotopically light in both carbon and hydrogen; kerogens of this kind have been encountered in Precambrian formations.



Similarly, the determination of  $^{15}\text{N}/^{14}\text{N}$  can provide very important information on possible biological activities in the analysed samples. The same is true for the  $^{34}\text{S}/^{32}\text{S}$  isotopic composition change between sulphides and sulphates.

### II.5.3.2 Sulphur

The element sulphur represents an important constituent of the terrestrial biosphere. Its presence and even more so the characteristic isotope signatures of sulphur in various oxidation states give testimony to biologically-controlled processes, mostly in low-temperature sedimentary environments.

Sedimentary pyrites often display negative to strongly negative  $\delta^{34}\text{S}$  values (e.g. Chambers, 1982; Habicht & Canfield, 1997; Kaplan & Rittenberg, 1964; Strauss, 1997), and these are correctly interpreted as resulting from bacterial sulphate reduction. However, the geological record provides sufficient evidence for sedimentary (biogenic) pyrites with positive or even strongly positive  $\delta^{34}\text{S}$  data that are equally a result of bacterial sulphate reduction (e.g. Lambert & Donnelly, 1990; Ohmoto, 1992; Ohmoto et al., 1990, 1993; Schidlowski et al., 1983; Strauss, 1993). In addition, other biologically-controlled processes within the sulphur cycle (e.g. sulphide oxidation) are associated with only minor displacements in the isotopic composition.

Consequently, a full understanding of the martian sulphur cycle and, thus, possibly evidence for biological participation, can be obtained only through a thorough study of abundances and isotopic compositions of both oxidised and reduced sulphur species.

### II.5.4.1 Inorganics

The quantitative analysis of water in the soil will allow derivation of a water concentration profile and could give access by extrapolation to the water concentration in the deeper layers.

This is a key point for studying the possibility of extant life in the present martian subsurface, water being one of the essential ingredients for life. The analysis of water is also important for the estimation of the mineralogical composition (hydrate content). Similarly, it is important to measure the martian soil abundances of  $\text{CO}_2$  (carbonate content),  $\text{NO}_2/\text{NO}_3/\text{N}_x\text{O}_y$  (nitrate content),  $\text{SO}_2/\text{SO}_3$  (sulphates) and phosphates.

It is essential to measure the abundances of oxidants in the soil, and determine their concentration gradients with depth (Fig. II.5.4/1). If our models of the evolution of organics on the surface and in the near-subsurface are correct, the oxidant concentration should be anti-correlated with the concentration of organics in the soil. These oxidants are mainly:  $\text{O}_2$ ,  $\text{O}_3$  and principally  $\text{H}_2\text{O}_2$  (with eventually organic peroxides).  $\text{Fe}^{3+}$ ,  $\text{Mn}^{x+}$ , carbonates, nitrates and sulphates should also be mentioned in this context.

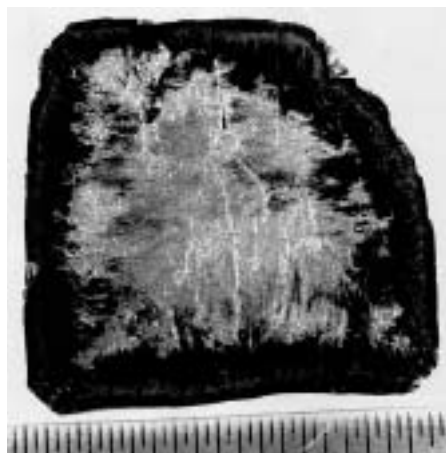
### II.5.4.2 Organics

The discovery of organics in the martian soil would be of prime importance for exobiology. In the very oxidised martian environment, the abiotic formation of organics through atmospheric chemical processes is unlikely. The only likely major abiotic source of organics on the surface and in the near-surface is extra-martian importation, from the impact of carbonaceous meteorites. However, by differentiating a meteoritic abiotic origin from a bio-origin, the detection of organic carbon in the martian soil could provide the evidence of former life processes.

After the death of organisms on Earth, their primary biopolymers (such as proteins and polysaccharides) undergo a complex process of degradation and condensation (humification) to give complex and chemically stable macromolecular materials (kerogens). In addition, stable lipid-rich biopolymers survive this process to contribute directly to kerogens, which are amorphous condensed aggregates made up mainly of aliphatic and aromatic moieties (de Leeuw & Largeau, 1993). Kerogens typically comprise by far the majority (>90%) of sedimentary organic matter and their

## II.5.4 Molecular Analysis

**Fig. II.5.4/1.** A pyrite crystal that oxidised inwards from the surface. The surface consists of iron oxides/hydroxides – very different from the inside. It demonstrates the necessity of sampling the interiors of minerals and rocks. (Courtesy G. Kurat)



chemical composition depends, *inter alia*, on the thermal history they have experienced. Naturally-occurring thermal breakdown gives rise to lower molecular weight components (gas and oil), and this process can be mimicked using pyrolysis techniques.

Sediments also contain stable organic compounds, especially lipids and metallo-porphyrin pigments which survive in whole or in part the processes of alteration, or which are produced by kerogen thermal breakdown. Many of these components can be classified as biomarkers on the basis of their chemical structures and/or carbon isotopic compositions. Hydrocarbon biomarkers and kerogens of recognisable biological origin have been found in sediments as far back as Precambrian in age (>0.5 Gyr; Imbus & McKirdy, 1993).

Based on terrestrial experience of organics of microbial origin, realistic criteria for assigning a biological origin to organic matter are the presence of:

- compounds occurring as such or derived from pyrolysis, with structures characteristic of an origin in a biosynthetic pathway (e.g. acyclic isoprenoids, terpenoids, steroids);
- a  $^{13}\text{C}$  depletion in the isotopic composition of bulk organic matter, fractions therefrom or compounds present as such or released by pyrolysis (see Section 5.3);
- chiral components with a biological configuration, present as such or released by pyrolysis (e.g. steroids and terpenoids);
- homologous series of components, present as such or released by pyrolysis, with a non-random carbon number distribution (e.g. straight-chain hydrocarbons or alkanic acids with a pronounced carbon number maximum, restricted carbon number range, even/odd or odd/even carbon number predominance, resulting from acetate C2 units in the acylpolymalonate biosynthetic pathway).

The search for volatile low molecular weight organics, such as alkanes (particularly  $\text{CH}_4$ ) is also of importance. The discovery of simple organics such as  $\text{CH}_4$  in near-surface samples, despite the oxidising environment of the surface, could be a sign of current biological activity. The same could be true for  $\text{H}_2\text{S}$ .

The selection of target organics related to biological structures or/and activity has also to take into account the relative chemical stability of the compounds in a highly oxidising environment. In particular, among the building blocks of biomacromolecules, amino acids are relatively stable, nucleotides and their sugars (ribose or deoxyribose) are not, and purines are more stable than pyrimidines.

Taking these criteria into consideration, a priority order of organics to be sought is:

- i. Volatile low molecular weight compounds:
  - hydrocarbons, especially  $\text{CH}_4$ ;
  - alkanic acids ( $\text{RCO}_2\text{H}$ );
  - eventually peroxy acids ( $\text{RCO}_3\text{H}$ ) which could be present in an oxidising environment.
- ii. Medium molecular weight compounds:
  - hydrocarbons (e.g. straight- and branched-chain, isoprenoids, terpenoids, steroids, aromatics);
  - alkanic acids and alcohols;
  - amino acids;
  - purines.
- iii. Macromolecular components:
  - kerogen-like material;
  - oligo and polypeptides.



Homochirality can also be a crucial signature of life, extant or extinct. Biological systems use only one mirror image form (R or S enantiomer, also named in some cases D or L) of a chiral molecule (e.g. proteins are made of only L amino acids, not D), in contrast to non-living systems which are normally racemic (contain equal numbers of L and D molecules). The enemy of finding homochirality as a signature of past life is the process of racemisation, i.e. D/L interconversion. Although the racemisation half-life of amino acids is typically  $10^6$  years in the wet conditions prevalent on Earth, this rises to  $10^{13}$  years or even more in the dry, frozen conditions of Mars – so homochirality may be preserved even if the extinction occurred 3-4 Gyr ago.

The existence of an enantiomeric excess could be established simply by detecting optical activity in bulk martian soil, but a separation technique such as chromatography would be necessary to determine the actual D/L ratio for individual organics. Finding optical rotation and life-like isotope ratios *together* would be a particularly powerful indicator of life that would be difficult to mimic abiotically.

The discovery of pure enantiomeric forms in the martian soil would be a clue for the presence of very recently active living systems. The discovery of an important enantiomeric excess would be a clue to the presence of an extinct life.

A direct measurement of optical activity of organic matter in the martian soil is less likely to provide very useful information. To obtain chirality information, measurement of the R/S ratio in enantiomers or diastereoisomers of certain of the organics listed in II.5.4.2, which are able to exist as such, would be required.

## II.5.5 The Search for Homochirality

### INSTRUMENTATION TO SATISFY THE SCIENTIFIC OBJECTIVES

The technology exists in principle, but does not fulfil all of the scientific requirements: depth – sample acquisition – sample preparation – micro analysis – transfer. The crust must also be removed from rocks and the subcrustal surface inspected. The requirements are:

- a sample acquisition able to provide mineral and organic stratigraphy;
- a drilling system able to reach down to 1.5 m (depth to be confirmed);
- a sample and *in situ* rock-preparation system able to expose rock interiors to provide a surface suitable for optical investigation and micro-analysis.

The MOLE concept seems to be able to drill down to 1.5 m, but not through rock, and it must be investigated if drilling can be performed vertically. Incorporating a drilling system on a martian rover will be demanding, in terms of mass, dimensions and power requirements.

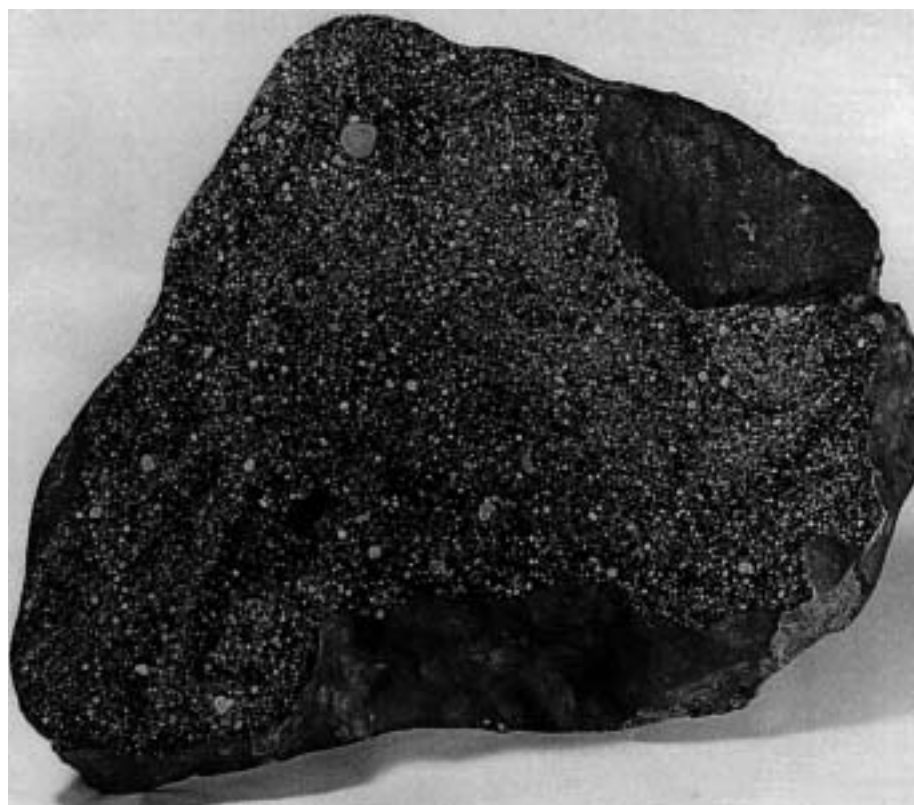
## II.5.6 Sample Acquisition and Distribution Subsystem

### II.5.7.1 General Considerations

The constraints on mineralogical studies (and the search for fossils) require a good sample documentation and preparation (smoothing or polishing) for optical microscopy examination and selection of grains for microchemical analysis. In principle, two fundamentally different samples will be encountered: hard rocks (primary and lithified secondary) and soil (unconsolidated regolith and sediment). The nearby geological environment will be represented by hard rock boulders, whereas the soil will tend to represent almost planet-wide coverage. Naturally, both samples are of great interest and a thorough study of a planetary surface from samples of one landing site has to be based on a detailed knowledge of both sample types. Although the unconsolidated soil carries information on close and very distant locations this is also true for the consolidated sediments that are possibly widespread at the surface of Mars, as documented by Mars Pathfinder. However, investigations of hard rocks or

## II.5.7 Mineralogy, Petrology and Geochemistry

Fig. II.5.7.1/1. The Mező-Madaras meteorite, about 30 cm across. The visible surface does not allow the rock to be identified, but the chipped surface at lower right provides some insight. However, only cutting and (semi) polishing allows the full beauty to be seen. This provides full information on the rock texture and a wide range of analyses can be made on carefully pre-selected spots. (Courtesy G. Kurat)



unconsolidated soil need different sample preparations. These are briefly outlined below.

- The surface of hard rocks is likely to be covered by dust and a weathering crust (desert varnish and/or radiation-damaged rind and/or hydrated/carbonated crust and/or biogenic encrustation, etc). For a proper mineralogical-petrological investigation of such rocks, the dust and the crust must be removed (Fig. II.5.7.1/1). This could be achieved by either grinding or core drilling. The first method could produce ground samples of the crust and of successively deeper rock layers for analysis. The second could provide cross-section samples perpendicular to the surface, allowing a detailed analysis of the rock, its constituents and the changes in properties with depth. This would be the ideal sample for the *in situ* analysis of hard rocks.
- Soil samples need particle size-sorting before processing (combined with grain size analysis). The fine-grained part (fine sand 60-200  $\mu\text{m}$ , silt 2-60  $\mu\text{m}$  and clay <2  $\mu\text{m}$  particle diameter) will have to be processed as such and preparations for analysis could be made on sticky or very soft metal tapes. The coarse-grained part (coarse sand 600-2000  $\mu\text{m}$ , gravel 2-60 mm) will have to be crushed to expose the interior and will have to be processed in a similar way to the fine-grained particles (the usual terrestrial preparation by embedding and cutting/grinding does not seem to be feasible). The ideal sample preparation equipment therefore should include:
  - a grinding device to take off the crust of rock boulders and smooth the surface of boulders and drilled cores;
  - a core drilling device to probe the interior of hard rocks and regolith;
  - a fine grinding/polishing device to smooth the ground surface for optical investigation and micro-analysis;
  - a magnetic device to separate minerals/grains with a variety of magnetic susceptibilities (ferromagnetic, paramagnetic, diamagnetic);

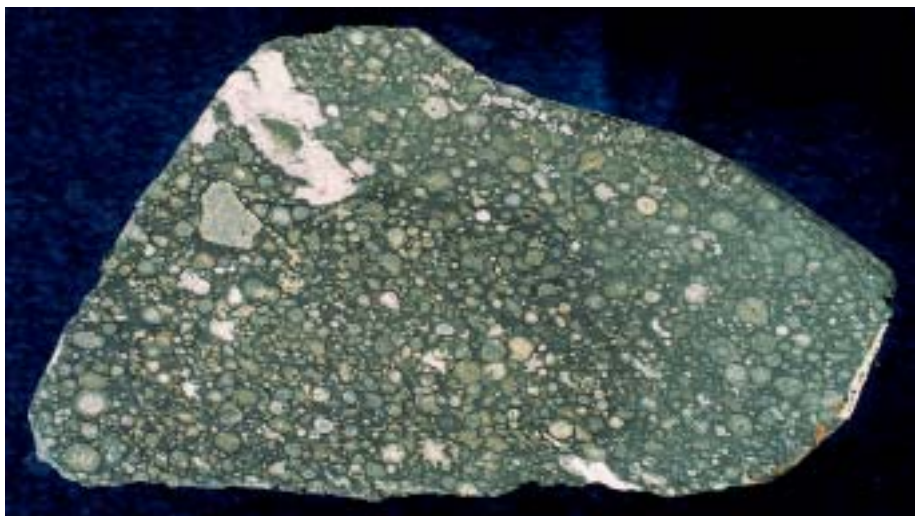


Fig. II.5.7.1/2. The cut and polished surface of a piece of the Allende chondrite, demonstrating the richness of objects and the choice of spots to be analysed. About 9 cm long. (Courtesy G. Kurat)

- a sampling device to allow samples to be taken from flat ground or polished surfaces (small core drill, chipping, etc.);
- a sample transfer system (manipulator) to transfer prepared samples and selected grains to the chosen analytical instrument.

As for investigating the sample, a combination of the following methods would be ideally suited:

*Optical microscopy:* with several magnifications to see the morphology of the samples, select them and look at details.

*Electron scanning microscopy:* surfaces, shapes, etchings, coatings, textures, microfossils, mineral abundances (average Z analysis – BSE), micro analysis of major and minor minerals, cathodoluminescence, etc. The method is applicable to small single-grain mounts on metal and for polished surfaces (for imaging only if not coated with conductive light element, e.g. Be or C). If equipped with a Si (Li) detector or equivalent CCD major element analysis of grains mounted on metal foil could be performed.

*Ion probe analysis:* surface analysis (coating, hydrogenated or carbonated surfaces or rock-forming minerals), major and trace elements of rock-forming minerals, secondary minerals, hard and soft biogenic matter, isotope analysis of minerals and organic matter (H/D, Li, B, C, N, O, S, etc.), impact glasses, ice?, salts and cements, major and trace elements, isotopes, elemental imaging, isotopic imaging. If laser ablation and inductive coupled plasma (ICP) techniques are used for ion production, the method can be applied to single-grain mounts and polished surfaces and will give good and quick results for major, minor and trace element abundances. However, isotope analysis will be difficult with the same instrument.

*X-ray analysis:* identification of minerals by X-ray diffraction. Ground-up mineral grains or mixtures will be needed or the method will be applied only to the fine-grained part of the soil.

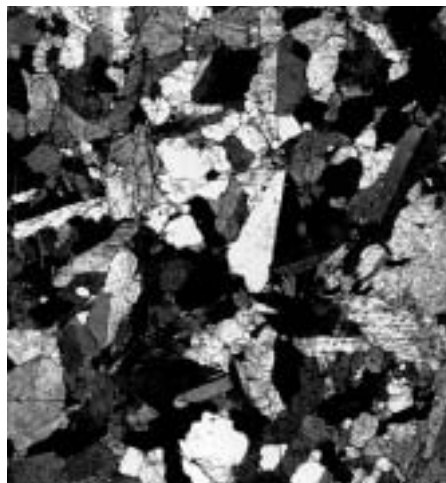
*Differential Thermal Analysis (DTA):* identification and analysis of H<sub>2</sub>O-, OH-, CO<sub>2</sub>- etc bearing minerals (volatiles released could be analysed by a mass spectrometer).

*APX spectroscopy:* bulk major and minor element (above He) contents of rock and soil samples. A reasonably flat surface is required.

*Raman spectroscopy:* mineral identification on selected grains from loose or solid samples, surface coatings, cements, etc. This method is applicable to single-grain mounts and polished surfaces.

*IR spectroscopy:* reflected or emitted IR: identification of pyroxenes, olivine, amphibole, carbonates, hydrous minerals, etc. The spectrometer could be attached to the

Fig. II.5.7.1/3. Ideally, rocks should be investigated in thin sections and transmitted light, making the texture clearly visible. In this photograph, crossed polarisers allow individual crystals to be seen. However, views like this are not essential, because texture and mineralogy can also be depicted on polished surfaces, although in a more cumbersome way. This is a polished thin section of an amphibole-orthopyroxene rock from Zabargad Island in the Red Sea; grain size is about 0.5 mm. (Courtesy G. Kurat)



optical microscope. Both single grains and grains in a polished section could be analysed.

*UV spectroscopy*: reflection spectroscopy, fluorescence of single grains and grains in polished mounts.

*Mössbauer spectroscopy*: determination of the  $\text{Fe}^{2+}/\text{Fe}^{3+}$  ratio in Fe-bearing minerals and mineral type.

*Atomic Force Microscope (AFM)*: high-magnification surface imaging of selected grains, identification of condensates, investigation of radiation damage, etc.

### II.5.7.2 Optical Microscopy

*Low magnification (20-80×)*: bulk documentation, colour analysis, texture, fossils, grain size analysis, grain shape analysis, selection of samples and/or sites for X-ray and microchemical analysis. A smooth surface on hard rocks is necessary.

*High magnification (100-500×)*: details of major minerals, minor phases, accessories, surface of grains, twinning, mineral intergrowths, exsolution and micro reaction textures, microfossils. Ideally, hard rocks should have a polished surface.

At least two, preferably more, magnifications should be available on a well-centred revolver or sledge, or on two or more in-line microscopes. They could be equipped with colour diodes, a laser (Raman), IR source and UV light source.

Space instrumentation for optical microscopy already exists. In particular, the Close-Up Imager from DLR in Berlin (Dr. Michaelis, Prof. Neukum), which is part of the Instrument Deployment Device (IDD) (MPI Mainz, Dr. Rieder, Prof. Wänke) that was planned for ESA's InterMarsNet mission, will have a spatial sampling of about 50  $\mu\text{m}/\text{pixel}$  with 3-colour images. The mass, including all electronics, will be of the order of 100 g, and the power consumption about 1 W.

### II.5.7.3 Alpha Proton X-ray Spectrometer (APXS)

The APXS can determine all elements except H and He with good accuracy. Hence, in the case of an ill-defined geometry, relative elemental abundances can easily be converted to absolute abundances. In particular, it seems to be able to analyse carbon down to about 0.1wt%. It can thus provide an important input on the puzzling question: where did the atmospheric  $\text{CO}_2$  go to?

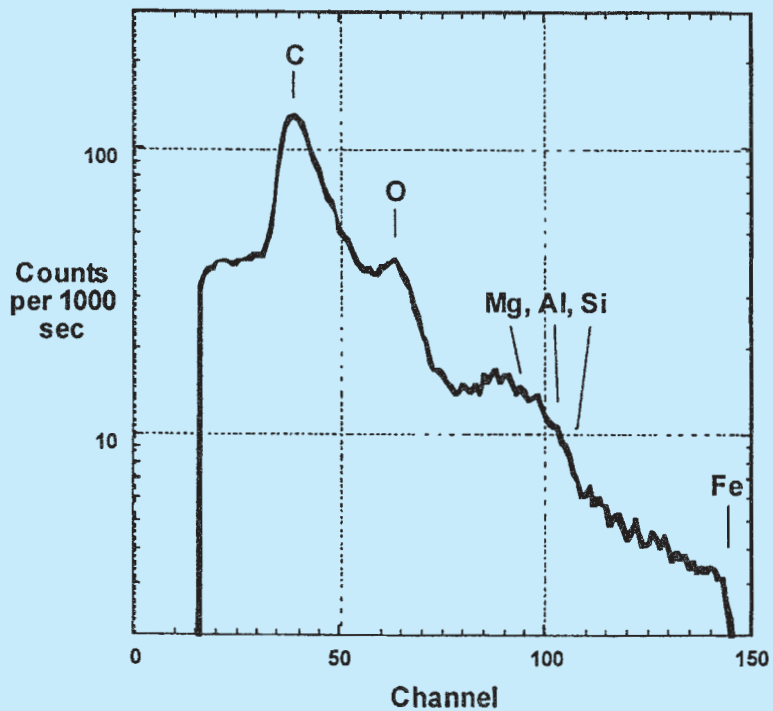
Inclusion of such an instrument within the proposed package would allow these measurements to be extended to cover the subsurface samples. In fact, since the APXS can determine the absolute concentration of oxygen, it will also be able to shed light on the absolute oxidation state of the subsurface material as a function of depth. Apart from these specific tasks, the general versatility of the APXS makes it a natural candidate for inclusion in this package.

A particular practical advantage of the APXS is that it does not require important sample surface preparation, other than the need to ensure that the sample surface region is not contaminated (although it must be cleaned of dust). Hence, it should be readily accommodated above one of the sample presentation positions of the sample carousel. The APXS in backscattering mode can determine all elements except H and He with good accuracy. The alpha mode is most sensitive to the light elements, such as C, O and N, whereas the X-ray mode is more sensitive to the higher z-value elements.

*The alpha mode*. Elastic collisions between the alpha particles and nuclei of the target sample result in Rutherford scattering. For a scattering angle close to  $180^\circ$  (backscattering), the maximum energy of the backscattered alpha particles is characteristic for the scattering element. The Alpha Mode is best suited for the light elements C, N and O, for which the maximum energies of the backscattered alpha particles differ most. For elements with higher masses, only groups of elements can be determined.

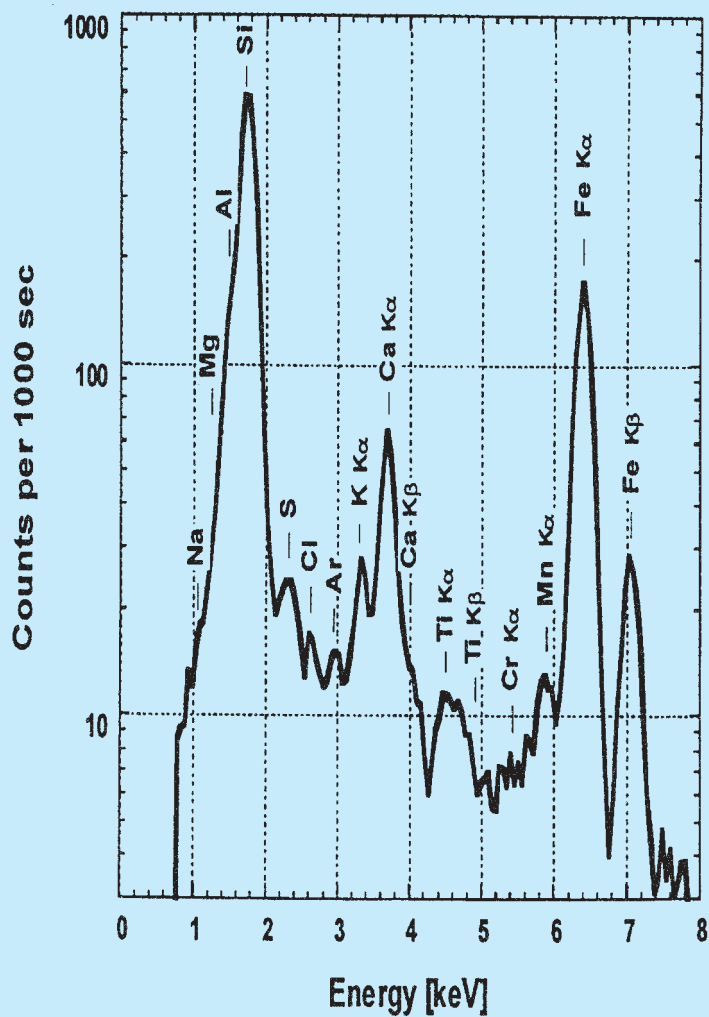
*The proton mode*. After elastic collisions, the alpha particles give rise to nuclear reactions of which the (a, p) reactions are of analytical importance as they yield





APXS Team

Fig. II.5.7.3/1. Alpha backscattering spectrum of soil sample A-2, during the Mars Pathfinder mission. The contribution of atmospheric carbon and oxygen are not yet corrected, so most of the carbon comes from atmospheric carbon. (Courtesy G. Klingelhöfer)



APXS Team

Fig. II.5.7.3/2. X-ray spectrum of the 'Barnacle Bill' rock obtained during the Mars Pathfinder mission, showing the elemental composition of the rock's near-surface material. (Courtesy G. Klingelhöfer)



protons of discrete energies. The proton spectra can be measured independently of the alpha spectra because of the different ranges of alphas and protons. The proton mode is of interest for the determination of Na, Mg, Al, Si and S.

*The X-ray mode.* The alpha particles from the radiation sources are also used as a very efficient excitation source for the production of characteristic X-rays. Charged particle excitations are, in fact, highly superior to any other kind of X-ray excitation as they give by far the best signal-to-noise ratio.

The combination of all three modes allows determination of all elements heavier than helium present in concentration levels greater than 1%. In order to achieve the highest accuracy and sensitivity, a measuring time per sample of up to 10 h is required. That will tend to define the rate of sample presentation from the drilling systems. While the alpha mode has a good resolution for the light elements C, N and O, the resolution decreases for heavier elements. On the other hand, the resolution of the X-ray mode increases for heavier elements.

The instrument is well developed, has flown on several missions and is accepted for future Mars missions. There was an APXS onboard Viking, the Mars Pathfinder rover Sojourner (Rieder et al., 1997) and on the Russian Mars-96 mission. Sojourner's version contained nine  $^{224}\text{Cm}$  sources with a total activity of about 50 mCi ( $1.85 \times 10^9$  decays/s). There will be a similar instrument on NASA's Mars Surveyor 2001 mission as part of the ATHENA payload, and another APXS on ESA's Rosetta comet lander for launch in 2003. This demonstrates the good heritage of the instrument.

The total APXS mass, covering the sensor head and electronics box, is 570 g. Power consumption is 340 mW and data output is 16 Kbytes per sample analysis.

However, in the case of Mars there is a potential problem concerning the quantitative analysis of C and N. The atmospheric  $\text{CO}_2$  and  $\text{N}_2$  signals can interfere with those from the soil and rocks. To minimise the atmospheric signals, the original Pathfinder instrument design has been modified using additional collimators for the alpha particles. Finally, the instrument needs to be calibrated under real environmental conditions to account for the remaining atmospheric carbon and nitrogen contribution. Such a modified APXS, which will be part of the Mars Surveyor 2001 mission, is able to analyse carbon down to the 0.1wt% level. The sensitivity for nitrogen is less because of the proximity of the nitrogen and oxygen signals (similar mass numbers).

#### II.5.7.4 Mössbauer Spectrometer

Mössbauer spectroscopy (Klingelhöfer et al., 1996) is a powerful tool for analysing Fe-bearing minerals and iron compounds. In particular, the oxidation state of the iron and the  $\text{Fe}^{2+}/\text{Fe}^{3+}$  ratio in the soil and rocks will be determined directly. With Mössbauer spectroscopy, the  $\text{Fe}^{2+}/\text{Fe}^{3+}$  ratio can be determined for samples taken at different depths in the soil, which provides complementary information with respect to the  $\text{H}_2\text{O}_2$  oxidant depth profile determination.

Biogenic magnetite very often (in most cases) shows up in Mössbauer spectra as a superparamagnetic component having strongly temperature-dependent Mössbauer parameters. Finding such signatures in the Mössbauer spectra will be an indication of previous biological activity. However, there are inorganic processes that also produce such nanophase magnetic particles. So, we must be careful in the interpretation of the data.

The general scientific objectives of *in situ* Mössbauer spectroscopy are:

- determination of the oxidation state of iron-bearing minerals;
- identification of Fe carbonates, sulphates, nitrates, etc that would provide information on early martian environmental conditions;
- identification of iron-bearing phases with low detection limits;
- determination of the iron oxides and the magnetic phase in the martian soil;
- detection of nanophase and amorphous hydrothermal Fe minerals that could preserve biological materials.

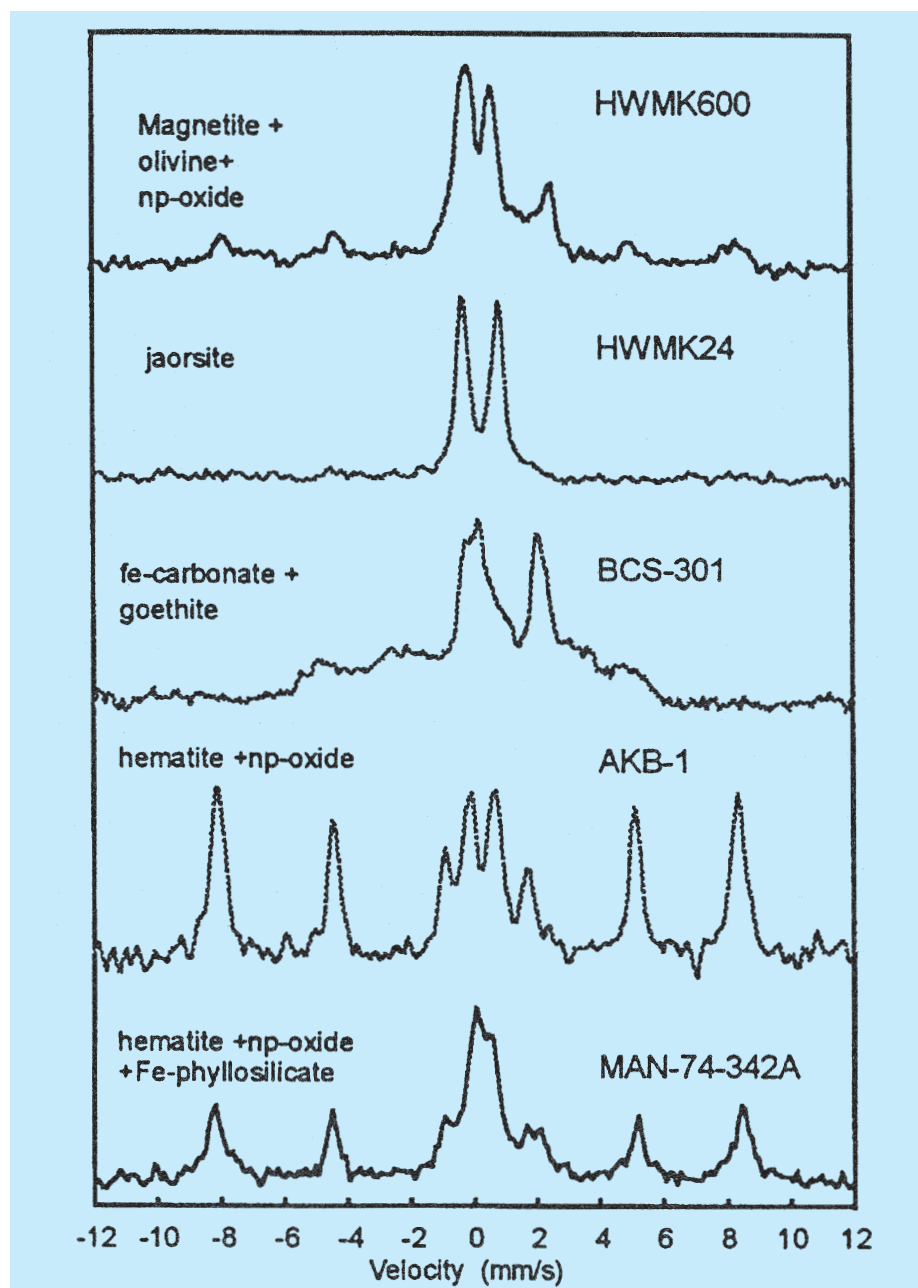


Fig. II.5.7.4/1. Backscatter Mössbauer spectra of martian analogues obtained with MIMOS II. From top to bottom: HWMK600, palagonitic tephra sample from Mauna Kea; HWMK24, jarositic tephra sample from Mauna Kea; BCS-301, chemical standard derived from an iron ore deposit; AKB-1, amygdaloidal basalt from the Keweenaw peninsula; MAN-74-342A, impact melt rock from the Manicouagan crater in Canada. (Morris et al., 1998) (Courtesy G. Klingelhöfer)

Measurements with the Mössbauer instrument will be done by placing it against the sample. Physical contact is required in order to minimise possible microphonic noise on the velocity-modulated energy of the emitted gamma-rays. The field of view is circular (diameter about 1.5-2 cm). The average information depth for Mössbauer data is of the order of 100-200  $\mu\text{m}$ , assuming basaltic rock composition. The thickness of dust layers as found on rocks during the Viking and Pathfinder missions may exceed this. To get at least some information from a possible weathering rind and the host rock, the dust needs to be removed by appropriate devices. Measurements before and after dust removal should be performed.

The Mössbauer parameters are temperature-dependent. Especially for small particles exhibiting superparamagnetic behaviour (e.g. nanophase Fe-oxides, which may be of biogenic origin), the Mössbauer spectrum may change drastically with temperature. The observation of such changes will help in determining the nature of the iron-bearing phases. Therefore, Mössbauer measurements at different temper-

atures should be performed: at least one during the lowest temperature period (night) and one during the highest temperature period (day).

To acquire one spectrum will take approximately 8-12 h depending on the phases and the total iron content. The temperature variation for one spectrum should be not larger than about 10°C. In case of larger variations, spectra for different temperature ranges will be stored separately, resulting in an increased total measuring time (depending on the number of temperature intervals required).

The MIMOS II instrument developed at the Technische Universität Darmstadt, Germany, was scheduled to fly on Russia's Mars-98 rover mission. This instrument has been developed and a prototype built and partially qualified for the Russian-German Nanokhod instrument deployment device (IDD; Max-Planck Institute for Cosmochemistry, Mainz, Germany), which was scheduled to fly on Europe's Inter-MarsNet mission. A MIMOS II prototype was successfully tested on the Rocky-7 prototype rover during the May 1997 field tests in the US Mojave Desert, taking data under semi-real conditions.

The Mössbauer spectrometer is a simple instrument. The main parts are the radiation source, the velocity transducer (drive), the Silicon PIN (positive-intrinsic-negative) radiation detectors and their pre- and main-amplifiers, and the instrument control and data acquisition system.

The standard  $^{57}\text{Co}$  radiation source is used, embedded in a solid rhodium matrix, which is mounted in a titanium holder. This design is intrinsically simple, rugged and is made very safe through the shielding design. The drive is a unique miniature double-voice coil electromechanical drive, developed at TU Darmstadt. The drive system has already undergone environmental testing at +20°C to -80°C, as well as vibration and shock tests in the frequency range specified for the Russian Mars-98 lander mission. The Si-PIN-diodes together with the pre- and main-amplifiers and the bias supply have been tested in the same way (shock, frequency, temperature). Prototype versions operated successfully at even unfavourably high temperatures during the Rocky-7 field tests. The instrument control unit and data acquisition system, including the software, successfully operated in a number of laboratory measurements as well as the Rocky-7 field tests in May 1997. For the Mars Surveyor 2001 Athena mission, the MIMOS II instrument will be split into two parts: a detector head mounted on the arm, and the instrument control and data acquisition unit located in the Rover's warm electronics box.

The total weight is about 500 g, with the sensor head itself about 400 g. The power consumption is of the order of 1.5 W. The radioactive Mössbauer source will have an intensity of about 300 mCi at launch.

#### **II.5.7.5 Ion/Electron Probes, X-ray Spectroscopy**

For chemical composition, the ion probe is heavy and an alternative could be electron probe analysis. Information on crystal structure could be provided by X-ray spectroscopy, if miniaturised for application to planetary exploration missions. There are two US teams from the NASA Ames Research Center and Los Alamos National Laboratory working on the miniaturisation of X-ray diffraction instruments (XRDs). There are no prototypes available yet. The development of flight instruments will require at least 2 years, if successful at all.

#### **II.5.7.6 IR Spectroscopy**

##### ***II.5.7.6.1 Molecular Spectroscopy***

IR spectroscopy at 0.8-4.0  $\mu\text{m}$  can determine the abundances of many types of minerals, including clays, hydrates, and carbonates. Carbonate materials such as magnesite, calcite and siderite can easily be distinguished from one another. All have near-IR features in the 1.7-2.5  $\mu\text{m}$  range attributable to the  $\text{CO}_3^{2-}$  anion. Sulphates such as gypsum and anhydrite have absorptions in the 1.0-1.5  $\mu\text{m}$  and 4.0-4.7  $\mu\text{m}$  ranges. Ferrous absorptions of pyroxenes are also evident in the 1.0-2.0  $\mu\text{m}$  region. The ratio of the 1  $\mu\text{m}$  to 2  $\mu\text{m}$  absorptions is diagnostic of the composition. Although olivine has been found in only one martian meteorite, the structure of the broad absorption

feature is also a diagnostic of composition. The absorptions of C-H and C≡N bearing organics can be found throughout the 1.7-4.0  $\mu\text{m}$  range. A spectral resolution of  $>100 (\lambda/\Delta\lambda)$  would be sufficient.

Good spatial resolution is needed to investigate variations within a sample and we estimate that a resolution of 200  $\mu\text{m}$  should be attainable.

A secondary objective for IR spectroscopy is investigation of the atmosphere.  $\text{CO}_2$ ,  $\text{H}_2\text{O}$  and CO all have characteristic absorption bands in the IR. The variations of these gases and the dust cycle are strongly coupled in the martian atmosphere and hence could provide significant additional information on the time variability of the atmosphere. Local sources of these gases may be present, indicating hydrothermal activity.

It should be noted that the highest spectral resolution near-IR spectroscopic measurements of Mars have come from the Hubble Space Telescope, with a spatial resolution of around 200 km per pixel. These data show variations in ferric alteration minerals and primary ferrous silicates but are limited by their coarse spatial resolution. It is not clear whether we will see low contrast in high spatial resolution data (as was seen in the optical with Mars Pathfinder's IMP imager) or whether IR spectroscopy at the 200  $\mu\text{m}$  level will be extremely revealing.

#### **II.5.7.6.2 Instrument Requirements**

Low-mass IR spectrometer systems have been proposed for several missions, including the RoLand lander on the Rosetta comet mission and some systems are now commercially available. The system may require illumination devices such as light-emitting diodes in order to compensate for absorption lines in the solar continuum at the surface caused by dust in the atmosphere. The system should, however, be comparable in mass and power consumption to the microscope.

Because transmission IR spectroscopy may not be applicable (requiring the preparation of thin sections), the less diagnostic reflection mode may have to be selected. Even in this mode, however, identification of mineral and organic compounds and detection of ( $\text{H}_2\text{O}$ ) and (OH) in silicates should be relatively easy. On the other hand, a Raman microspectrometer would probably provide most of that information in a more unambiguous way.

#### **II.5.7.6.3 Thermal IR Spectroscopy**

Thermal IR spectroscopy in the 6-9.5  $\mu\text{m}$  region can be used to investigate specific absorptions/emissions of carbon-bearing molecules. Strong features are evident at 6.18  $\mu\text{m}$  from the C=C and C=O bonds, 6.86  $\mu\text{m}$  ( $-\text{CH}_2-$ ,  $-\text{CH}_2-$  scissor), 7.26  $\mu\text{m}$  (C-H bend) and 7.90  $\mu\text{m}$  (O-H or C-H bend). This wavelength range could therefore provide a direct test for the presence of organics.

Thermal emission spectroscopy can also determine whether geothermal or hydrothermal activity is occurring. Finding warm surface regions would be extremely important in the search for extant or extinct martian biota (Walter & DesMarais, 1993). Is Mars geologically dead or is there sufficient heat produced to drive a subsurface hydrothermal circulation system? If this circulation is on a small scale, thermal emission spectroscopy may be a requirement in order to look in the optimum place for life.

#### **II.5.7.6.4 Instrument Requirements**

We are at present unaware of any proposal for an instrument designed to exploit this possibility at high resolution on a rover. Some additional investigations need to be made. The potential difficulties, however, are substantial. For example, the detector and instrument may have to be cooled. The sample itself will be in the transition region between absorption and emission within this wavelength range and hence detailed investigations need to optimise the wavelength region to be investigated and to control the temperature of the sample accordingly. It does not appear to be a realistic option at present.

Temperature anomalies might be investigated using IR spectroscopy but this would require extension of the device out to a wavelength of 5  $\mu\text{m}$  with reasonably high signal to noise.

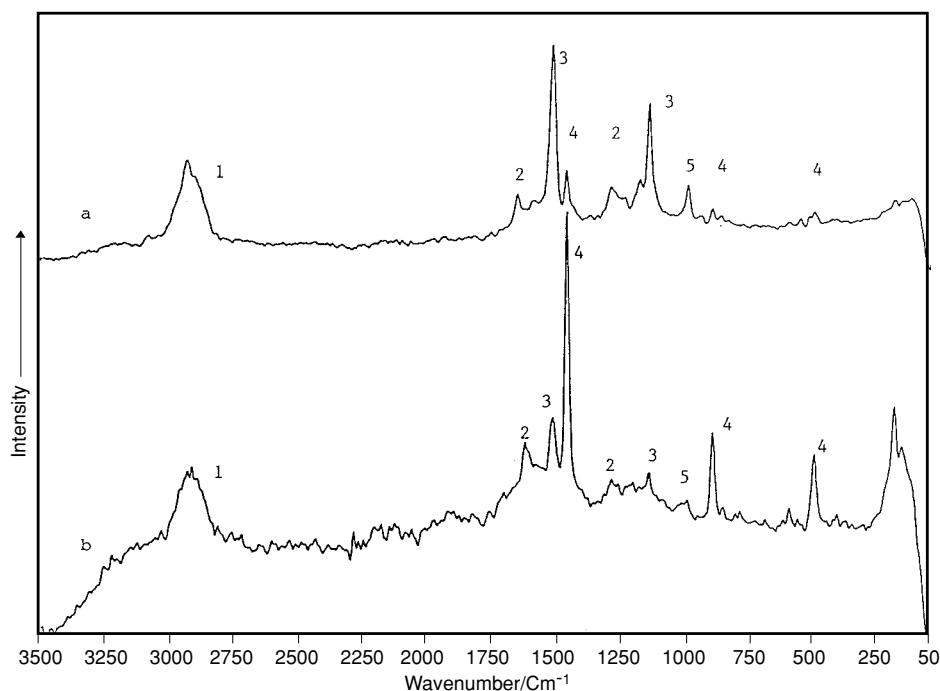
Fig. II.5.7.7/1. Example of Fourier Transform-Raman spectra of *Dirinia massiliensis* forma *sorediata* encrustations from regions of (a) cortex, (b) medulla/oxalate interface.

FT-Raman spectra were obtained using Nd<sup>3+</sup>/YAG laser excitation at 1.06  $\mu\text{m}$  with a Raman microscope attachment offering a spatial resolution of about 10  $\mu\text{m}$ .

Key to spectroscopic bands:

1. n(CH) of lichen celluloses and organic metabolic by-products. 2. metabolic by-products of chemical biodeterioration. 3. lichen pigmentation (carotenoid). 4. calcium oxalate. 5. substratal incorporation (calcite: 1086  $\text{cm}^{-1}$ , gypsum: 1007  $\text{cm}^{-1}$ ). An interesting spectroscopic difference between the cortex and medulla sampling regions (a) and (b) is the different ratio of lichen carotenoid pigment (3) to oxalate (4). The latter region contains more calcium oxalate than the former for similar amounts of organic lichen material (1).

(Courtesy H. Edwards)



The technology is already under development. The CIVA-M instrument on the Rosetta comet lander combines an ultra-compact and miniaturised visible microscope and coupled IR spectrometer. The spectrometer operates in the range 1-4  $\mu\text{m}$  at a resolution of 50 nm, using a rotating grating. Its mass is 650 g, size 155×75×65 mm, average power of 3.4 W.

#### II.5.7.7 Raman Spectroscopy

IR and Raman spectroscopic techniques are complementary in their specificity of molecular information provision. The origins of the two techniques are very different: molecular transitions occur in the IR and give rise to a spectrum through direct absorption of electromagnetic radiation in the submicron region of the IR, whereas Raman spectra arise from molecular scattering processes from high-intensity laser radiation. IR spectra arise from dipole moment changes in the molecular vibrations, whereas Raman spectra arise from polarisability changes in the molecular vibrations.

The result is that IR spectroscopy is an excellent technique for the detection of polar molecular groupings such as -O-H and -C=O, whereas Raman spectroscopy is better for nonpolar groupings such as -C-C- and -C=C-. This also means that IR spectra are strongly dependent on the presence of water, which can swamp weaker absorption signals, whereas Raman spectra are insensitive to water.

The major difference in the operational instrumentation required for these two techniques is that IR spectroscopy is confined to the IR region, whereas Raman spectroscopy can be accomplished using UV, visible or near-IR excitation sources. Since Raman scattering intensity (sensitivity) is dependent on  $n^4$ , where  $n$  is the frequency of the exciting radiation, this means that for the same laser power there is a 160-fold increase expected for molecular scattering in the UV at 300 nm compared with the near-IR at 1064 nm. However, there must always be a balance between the excitation wavelength chosen and the ability of the specimen to withstand the radiation energy falling on it. In the case of some sensitive organic materials, a dose of UV-radiation at 300 nm is sufficient to break the chemical bonds and 'burn up' the sample. Also, fluorescence processes are several orders of magnitude stronger than Raman scattering. These are much more prevalent the nearer one approaches the blue, green ends of the visible spectrum and are common in the UV. Fluorescence exci-



tation at shorter wavelengths can swamp the weaker Raman scattering from organic molecules.

In the Raman work carried out at the University of Bradford, (Edwards et al., 1999), blue, green and red excitation have been used and, most recently, 1064 nm excitation in the near-IR from a Nd<sup>3+</sup>/YAG laser has been used in conjunction with Fourier Transform techniques for data processing and accumulation.

Sampling for Raman spectroscopy is much easier than for IR absorption and this has enabled its rapid application to geological problems. Raman spectroscopy is essentially a surface technique and sample spots from 100 mm diameter down to less than 1 mm are now being achieved using microscope systems and remote sensing probes with optical fibres of 1-50 m length.

A big advantage of Raman over IR spectroscopy for geological studies is that the whole spectrum from about 100-4000 cm<sup>-1</sup> can be obtained simultaneously using either FT-techniques or CCD detectors. The vibrations and characteristic signatures of minerals, crystals and inorganic species are generally within the region 100-1200 cm<sup>-1</sup>, a region which is not fully covered by non-specialist IR instruments.

A vital factor in the assessment of the capability of Raman spectroscopy to provide information about extraterrestrial molecules of life is engineering restrictions on instrument size and weight. This is already being addressed in other quarters and there are claims of ultra-small, micro-Raman spectrometers that could be employed in a Lander payload. However, a critical test must be the use of these systems on terrestrial problems in order to evaluate their potential against their bigger, heavier research laboratory-grade instruments.

Miniaturised Raman spectrometers are under development in the US, and for the Mars Surveyor 2001 rover a Raman spectrometer is expected to be included in the Athena payload. This instrument is currently under development at JPL and Washington University, St. Louis (Wang et al., 1997). It will operate at a wavelength of about 685 nm, where there is high Raman scattering effect, excitation of relatively little fluorescence and high CDD detection efficiency.

It is an essential part of the molecular structural analysis that the development of a miniaturised Raman spectrometer is undertaken for both mineralogical and biological organic materials *in situ* analysis. This will be a novel piece of instrumentation for a European lander programme – it is not being envisaged fully elsewhere – in that the examination of material in the martian subsurface is perceived as an integral part of the molecular structural investigation.

The major problem concerning isotopic analysis in space is that of isobaric interferences if the mass-resolution of the instrument is not high enough. In the simplest case, this is the effect of protons attached to light isotopes being confused with heavy ones. For example, <sup>12</sup>CH has the same integer mass as <sup>13</sup>C. Thus, unless the two species can be distinguished, an incorrect assessment is made. In practice, on Earth, the separation of <sup>12</sup>CH and <sup>13</sup>C can be achieved by a high-resolution mass spectrometer. However, higher resolution almost always means a heavier instrument package. Efforts are underway to improve resolution of the folded time-of-flight mass spectrometer, under development for the COSAC instrument on the Rosetta lander.

The alternative approach is to process chemically species of interest down to molecules that do not give isobaric problems with a low-resolution mass spectrometer. On Earth, for example, carbon in a complex organic macromolecule such as kerogen has to be combusted. Combustion reactions are feasible under a chemical pre-treatment scheme called MODULUS (Methods Of Determining and Understanding Light elements from Unequivocal Stable isotope measurements). The principle also applies to other processing activities needed for species related to organic analysis, e.g. the determination of nitrogen in organics or oxygen and hydrogen composition of water.

By using the stepped combustion method, information is yielded on the nature of the carbon-bearing samples, and detection is possible of organic matter in rocks and

## II.5.8 Isotopic Analysis

soils. In this approach, the sample is exposed in an oven to aliquots of oxygen as the oven temperature is increased from ambient. All known forms of carbon are combustible, organic matter being the most easily changed to carbon dioxide. Aliphatic-rich material burns at the lowest temperatures and highly crosslinked (aromatic) kerogens at something approaching 600°C. The combustion temperature level is a continuum right up to where organic matter becomes amorphous carbon (poorly crystalline graphite), so it is possible to recognise natural kerogens from their stability with oxygen. Although organic matter overlaps with the decomposition of carbonates, the latter are easily recognised because they are still degradable in the absence of oxygen. Stepped combustion is thus a universal method for detecting organic remains even when they have begun to degrade through geological processing. It provides valuable information on the nature and origin of carbon compounds. This technique is also included on the MODULUS experiment.

Mass spectrometers can operate in a variety of ways to measure isotopes. All require the elements under study to be presented in a gaseous form. The procedure generating the greatest accuracy is the 'dual-inlet dynamic' mode, where a sample and standard are repetitively compared by introduction from separate inlet systems. The highest sensitivity method is the 'static' mode, where a standard is inserted into a mass spectrometer closed off from its pumping system after a sample analysis has been completed and any residual specimen pumped away. A compromise situation is afforded by GC-IR-MS (gas chromatography-isotope ratio-mass spectrometry). All the specimens are burned in an interface reactor as they are eluted from a gas chromatography column (see below) on the fly. Standards are introduced as spikes in appropriate regions of the gas chromatogram where no component is present. The disadvantage of GC-IR-MS is that it can be applied only for carbon species that are sufficiently volatile to be chromatographed. It is, however, compatible with pyrolysis-gas chromatography-mass spectrometry (PYR-GC-MS; see below).

Methods of measuring isotopes using lasers tunable to the absorption frequency of specific carbon bonds, e.g.  $^{12}\text{C-O}$  or  $^{13}\text{C-O}$ , are under consideration but their application even in terrestrial laboratory situations is still limited by the accuracy of measurement. Although all kinds of mass spectrometers are able to detect and measure isotopes, the accuracy will always be greatest with magnetic-sector instruments because only this format is capable of simultaneous detection to eliminate fractionations during ion source fluctuations. New developments of miniaturised magnetic mass spectrometers, based on Mattauch-Herzog geometry double-focusing, is under study. A breadboard instrument, including ion source, electric sensors and magnetic sector with an array detector is already available in Europe, with a total mass of only 1 kg. Its application to an exobiological package for the martian environment still requires the development of a powerful pumping device.

Laser ablation (LA) inductively coupled plasma (ICP) mass spectrometry (MS) could be a very attractive technique. It needs only minimum sample preparation and could be done directly from the microscope. If the mass spectrometer could be furnished with an LA-ICP-MS ion source, it could be utilised for isotopic analyses on inorganic and organic matter such as:

- the major rock-forming minerals;
- secondary minerals replacing primary minerals and forming crack fillings, etc;
- organic fillings of cracks, etc.

Such *in situ* isotopic analysis of H, C, N and O will reveal conditions of formation of primary and secondary phases as well as organic matter.

*Action needed:* a study of the feasibility of a low-mass high-performance pump, such as a miniaturised turbo molecular pump, for space applications, and the possible use of LA-ICP-MS.

Previous investigations by the Viking missions provided no evidence for any reduced species, in principle because of the strongly oxidising surface conditions on Mars. The investigation of subsurface material represents an important advantage,

provided that a depth below the strong oxidising conditions can be reached. This approach offers the possibility of determining a depth gradient of redox-sensitive geochemical parameters. This is particularly true for sulphur. In addition, the presence of sulphur phases in different oxidation states and their subsequent isotopic analysis will yield information on the mode of redox reactions, specifically a possible participation of biologically-controlled processes. The analytical approach could include previously applied techniques such as stepped combustion and isotopic analyses of resulting SO<sub>2</sub> fractions and sulphur isotope measurements using an ion microprobe. The full analytical spectrum, however, is still possible only in Earth-based laboratories, requiring a sample-return mission. Spacecraft analytical facilities might, however, obtain sufficient data through combustion followed by isotope ratio mass spectrometry of sulphur-bearing compounds (i.e. mineral phases). It should be possible to perform this type of analysis on surface and subsurface material. Sample material would have to be crushed (better: pulverised) before delivering it to the furnace. Since the sample material will likely contain other oxidisable components, a chromatographic separation of the gas mixture (such as in elemental analysers with a conflow-setup) is a prerequisite in order to obtain reasonably pure SO<sub>2</sub> for mass spectrometric analyses. A temperature-based separation, analogous to the stepped combustion approach, might be used to study different mineral phases of different sulphur oxidation states.

In conclusion, a MODULUS-like instrumentation, based on chemical transformations, including stepped combustion and pyrolysis, coupled to gas chromatography-mass spectrometry techniques can fulfil most of the scientific objectives described in II.5.3.

The analysis of inorganic and organic compounds can be efficiently carried out by gas chromatography coupled to pyrolysis and mass spectrometry (PYR-GC-MS).

## II.5.9 Molecular Analysis

### II.5.9.1 Gas Chromatography (GC)

Gas chromatography is a powerful technique for analysing complex mixtures of gases or volatilisable constituents, and it will be a crucial component of the proposed exobiology instrumentation package. Schematically, a gas chromatograph includes: (i) a carrier gas reservoir and pressure controller; (ii) an injection subsystem, where a sample of the mixture to be analysed is introduced and carried by the carrier gas into (iii) one or several (in series or in parallel) GC columns – the heart of the GC system, where the separation occurs – and (iv) a detector, connected at the exit of the column, able to indicate systematically elution of compounds (other than the carrier gas) from the column(s).

The carrier gas is usually He, N<sub>2</sub>, Ar or H<sub>2</sub>. It flows continuously through the column, carrying the different constituents at different rates, depending on their chemical structures and molecular weights. The column can be a tube of a few metres length and one to a few mm internal diameter, packed with an absorbent (GSC: Gas Solid Chromatography) or with an absorbent impregnated with a stationary phase, a liquid of very low vapour pressure (GLC: Gas Liquid Chromatography). It can also be a longer (10-100 m) and open tube (wall-coated open tubular), with smaller internal diameter (0.5-0.1 mm), coated with a film of a solid absorbent (GSC) or of liquid phase (GLC). The chemical nature of the absorbent or the liquid phase is directly related to the chemical nature of the compounds to be analysed. The best universal detector is probably a mass spectrometer, able to provide the mass signature of each eluted compound and hence allowing a secure identification of each GC peak. The GC-MS coupling requires that most of the carrier gas be removed from the sample before it enters the mass spectrometer.

In the case where multi-GC columns are used in parallel, specific GC detectors must be used (in addition to MS coupling, as planned on Rosetta's COSAC instrument). Nano-thermal conductivity detectors (nano-TCDs) are commercially available in Europe, but they need to be space qualified. Detectors able to

discriminate chiral compounds have recently been described (Bodenhoefer et al., 1997) (see Section II.5.10, below). They also need to be studied.

#### II.5.9.2 Mass Spectroscopy (MS)

A variety of mass spectrometers (magnetic, quadrupole, time-of-flight, ion trap) are able to differentiate and analyse the resulting ions, depending on their mass-to-charge ratio. The ion trap spectrometer is best suited for use in conjunction with a gas chromatograph, because its optimum pressure conditions are much higher (about  $10^{-3}$  mbar) than those required with the other MS types (typically  $10^{-6}$  mbar or lower). Other mass spectrometers could also be used if a powerful pumping device is available (see below).

#### II.5.9.3 Pyrolysis (PYR)

Pyrolysis is a powerful technique for the analysis of non-volatile compounds of low thermal stability, in particular organic compounds of high molecular weight, such as macromolecules or oligomers, when the thermal pulse fractures the bonds between separate entities to produce recognisable sub-units (Irwin, 1982; Meuzelaar et al., 1982). There are many pyrolysis variants, most of which readily lend themselves to space applications. The simplest for space application is furnace pyrolysis, using a small oven.

Combustion can also convert non-volatile material into gaseous species. This method, when used in stepped fashion, can distinguish between different forms, e.g. organics vs carbonate, sulphides vs sulphates. Because it is quantitative, it gives absolute abundances of elements of biological significance (e.g. C, H, N, S) and allows bulk isotopic compositions to be established.

It should be pointed out that the use of pyrolysis techniques with small ovens could also allow differential thermal analysis measurements to be carried out. This is a powerful tool for determining the nature of the source of volatiles (such as  $\text{H}_2\text{O}$ ,  $\text{SO}_2$ ,  $\text{CO}_2$ ), which are released during stepped heating of the samples, and thus to infer, in particular, the molecular structure of inorganic compounds.

#### II.5.9.4 Available PYR-GC-MS Techniques

Chemical sensors based on GC and MS instrumentation have already been used in atmospheric probes and surface landers for analysing extraterrestrial environments, including Venus and Mars surface materials. Until recently, they involved only chromatographic packed columns with mainly magnetic, then quadrupole, MS.

The Titan atmosphere Huygens Probe of the Cassini/Huygens mission launched on 15 October 1997 to reach the Saturnian system in 2004 includes a GC-MS instrument (Niemann et al., 1997) coupled to an aerosol collector and pyrolyser (ACP) (Israel et al., 1997). It will determine the nature and abundance of the organic and inorganic compounds present in Titan's atmospheric gas phase and aerosols. This will be the second GC-MS instrument, and PYR-GC-MS instrument, ever flown in a planetary mission (after Viking). The GC subsystem uses three GC columns in parallel: two wall-coated open tubular (WCOT) columns and one micro-packed capillary column based on up-to-date chromatographic technology. One WCOT capillary is 10 m-long (MXT type, Restek) of very narrow bore (0.15 mm), with special stainless steel tubing (Silcosteel) (Afflalaye et al., 1996). It allows separation of  $\text{C}_3$  and higher hydrocarbons and nitriles. The other (14 m  $\times$  0.18 mm internal diameter) uses an adsorbent material newly developed for capillary GC applications: glassy carbon. It allows the quantitative analysis of  $\text{C}_1$ - $\text{C}_3$  hydrocarbons. The third column (2 m  $\times$  0.75 mm i.d), packed with Carboxen, allows the separation of permanent gases, in particular CO and  $\text{N}_2$  (Afflalaye et al., 1997). The MS subsystem is a quadrupole mass spectrometer with five ion sources: three are dedicated to each of the GC columns, one allows direct MS analysis of the atmosphere, and the fifth is dedicated to direct MS analysis of ACP samples. The ACP instrument includes a collector, which will collect Titan's atmospheric aerosols on a filter, an oven where the filter is introduced after the collecting phase for heating the sample at different temperatures, and a

**Table II.5.9.4/1. New PYR-GC-MS Developments for Space Applications.**

<i>Experiment</i>	<i>MS type</i>	<i>Mass</i>	<i>Power Requirement</i>
MODULUS	Ion Trap	3.5 kg	
COSAC	Time-of-Flight	4.5 kg	8-16 W

carrier gas to transfer the resulting gases to the GC-MS for analysis. Such a system has sensitivity and resolution capabilities of one to several orders of magnitude higher than Viking's PYR-GC-MS, with less mass and volume.

New instrumentation involving PYR-GC-MS techniques and using the heritage of Huygens is under development for space application, in particular for *in situ* analysis of cometary nuclei.

The COSAC and MODULUS instruments, the two GC-based experiments on the Rosetta lander, use pyrolysis oven GC techniques with several columns and a coupling to mass spectrometry. COSAC uses a new concept of time-of-flight MS, based on a specific geometry of the MS. MODULUS uses a ion-trap MS; mass is 10-20 g (excluding power supplies).

Pyrolysis ovens with loading mechanisms will be available for the COSAC and MODULUS experiments. Some ovens will have the facility for optically viewing the sample before the heating process starts. Other types, which have exciting potential for studies at predetermined temperatures, without using circuitry for control, are hot wire and curie point pyrolysis units. Here, the properties of the material, rather than feedback loops, decide the temperatures to which the sample is heated. Although not yet tested in space, such methods should be easily adaptable.

PYR-GC-MS instrumentation on the exobiology package will be able to analyse:

- inorganics: HO, CO<sub>2</sub>, NO<sub>2</sub>, N<sub>x</sub>O<sub>y</sub>, SO<sub>2</sub>, SO<sub>3</sub>, H<sub>2</sub>S, ...
  - volatiles adsorbed in the soil and released by light heating
  - or chemically bound in minerals and released by pyrolysis.

Only the analysis of H<sub>2</sub>O<sub>2</sub> may be a problem and specific analytical techniques have to be found for this compound.

- organics:
  - low molecular weight organics (RH, RCO<sub>2</sub>H, RCO<sub>3</sub>H, ...) can be analysed by GC-MS and MS
  - higher molecular weight organics:
    - hydrocarbons, including PAHs can be analysed by GC-MS and MS
    - amino-acids and purines by PYR-GC-MS/PYR-MS
  - macromolecular compounds:
    - kerogens and oligopeptides can be analysed by PYR-GC-MS and PYR-MS.

An instrument based on the COSAC and MODULUS concepts seems a good solution:

- small furnace pyrolysis;
- multi-GC columns system sets of 2-4 columns in parallel;
- ion-trap MS.

By extrapolation from the Rosetta instrumentation, the expected mass is 4 kg.

#### **II.5.9.5 Laser Ablation-Inductive Coupled Plasma-MS (LA-ICP-MS)**

Major and trace element determinations of organic and inorganic phases could be



determined in individual phases *in situ* by adding an LA-ICP ion source to the MS. This method is very fast and, if a separate MS can be dedicated to that task, it will give by far the most data for analysing the phase equilibria and formation conditions of:

- primary phase assemblages;
- secondary phases;
- atmosphere-related phases (coatings, efflorescences, etc);
- organic matter.

This instrument should also be capable of performing isotopic abundance measurements and thus could be very important in the search for life or life remnants.

#### II.5.9.6 Other Techniques

Other techniques can be envisaged for analysing compounds of very low volatility or non-volatile compounds (inorganics, PAHs, amino acids, purines, macromolecular organics). They include derivatisation system-GC-MS, High-Performance Liquid Chromatography (HPLC) and Supercritical Fluid Chromatography (SFC).

Derivatisation processes are often used in the laboratory to analyse non-volatile organics by GC or GC-MS. This is the case with amino acids. A two-step chemical derivatisation (esterification of the acidic group followed by trifluoroacetylation of the amino group) transforms the non-volatile amino acids to volatile N-TFA esters, which can be quantitatively analysed by GC or GC-MS. However, this has never been used in space and an automatic derivatisation system compatible with space constraints has yet to be developed.

HPLC is also a powerful technique for analysing complex mixtures of high molecular weight, low-volatility compounds. It uses liquid solvent (instead of the GC's carrier gas), a requirement that might be very difficult to qualify for space activity. However, if developed for space application, it would be an instrument of tremendous importance for exobiological studies. Thus, there is a clear need for research and development of space HPLC and HPLC-MS systems.

A related method to HPLC is SFC. In this case, a very unreactive gas is compressed to a temperature at which it becomes supercritical. Under such circumstances, some species such as CO<sub>2</sub>, can become an extremely powerful solvent, with properties that can be varied extensively by the addition of small amounts of modifying agents. Compounds in solution in supercritical fluids can be separated chromatographically. An interesting possibility for Mars might be utilising the atmosphere itself as a supercritical reagent for carrying out organic analysis. Landing spacecraft would then not have to transport hazardous consumables for chromatographic separations, only the hardware and an appropriate pump. SFC has also not been used in space, but it might be somewhat easier to develop than HPLC.

European laboratories are studying the feasibility of these different concepts.

#### II.5.9.7 The Analysis of H<sub>2</sub>O<sub>2</sub>

The quantitative analysis of H<sub>2</sub>O<sub>2</sub> will be difficult by GC, MS or GC-MS techniques and, owing to its scientific importance, it will require a specific instrumentation.

Hydrogen peroxide is usually analysed in environmental sciences by complex techniques based on chemiluminescence or UV fluorescence. These techniques involve, after air sampling, chemical derivatisation, using liquid transfers, chemical reactors, injection valves and enzyme storage. Furthermore, they require very accurate calibration. Thus, although their sensitivity is very high (a few ppt – parts per trillion, 10<sup>12</sup>!), they do not seem adequate for space application.

Another type of instrumentation can also be used to analyse H<sub>2</sub>O<sub>2</sub> quantitatively, although it is less sensitive (parts per million range). It involves electrochemical measurements with:

- i. rotating electrode (amperometric titration),
- ii. specific H<sub>2</sub>O<sub>2</sub> electrode (oxidation or reduction on a platinum disc electrode),

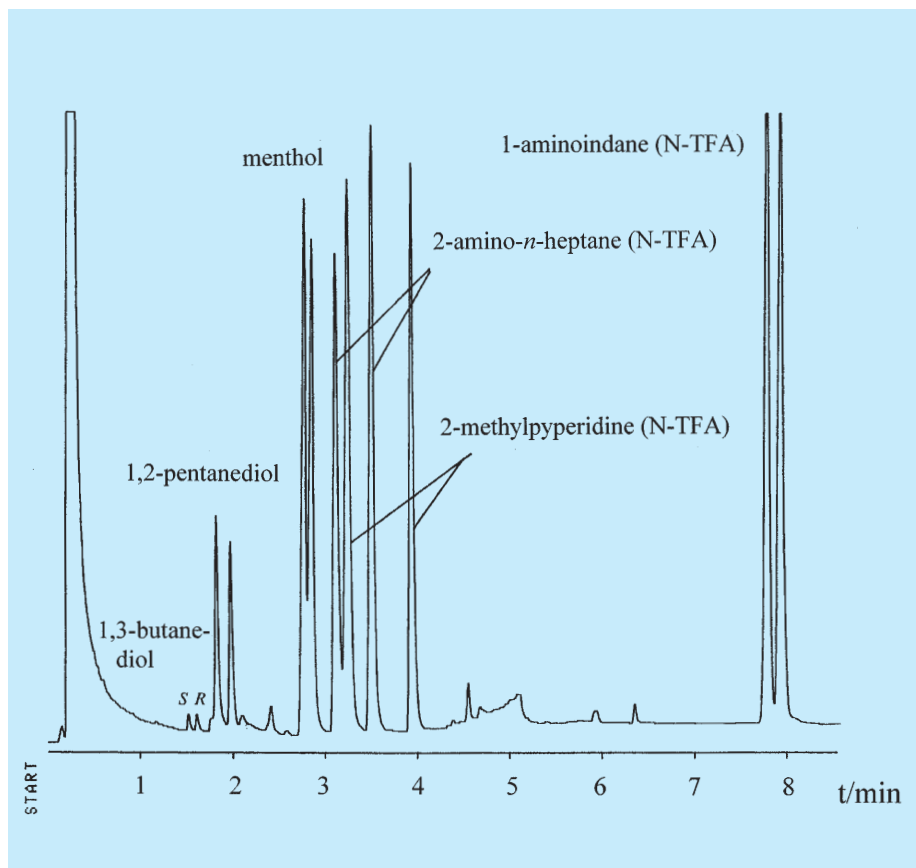
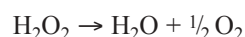


Fig. II.5.10.1/1. Overall view chromatogram for some diols, one alcohol and three derivatised amines by the use of a G-TA Cyclodextrin phase, 10 m × 0.25 mm internal diameter, (T = 3 min at 85°C, 15°C/min to 115°C, 5 min at 115°C, p = 60 kPa). (Courtesy W. Thiemann and U. Meierhenrich)

iii. specific oxygen electrode ( $O_2$  produced by  $H_2O_2$  decomposition).

This last method uses the enzymatic reduction of  $H_2O_2$  with catalase:



followed by the quantitative measurement of the produced oxygen by an  $O_2$  electrode (diffusion of  $O_2$  through the Teflon membrane of the electrode and its reduction on the platinum electrode).

This last technique can easily be miniaturised (microcathode), has a high selectivity to  $H_2O_2$  (enzyme) and then to  $O_2$  (selective permeability of a Teflon membrane). Although its space qualification is probably not easy (liquid handling, temperature dependence, need for internal calibration with standard solutions, cathode passivation, enzyme utilisation), it seems to be a possible way for space application and should be explored.

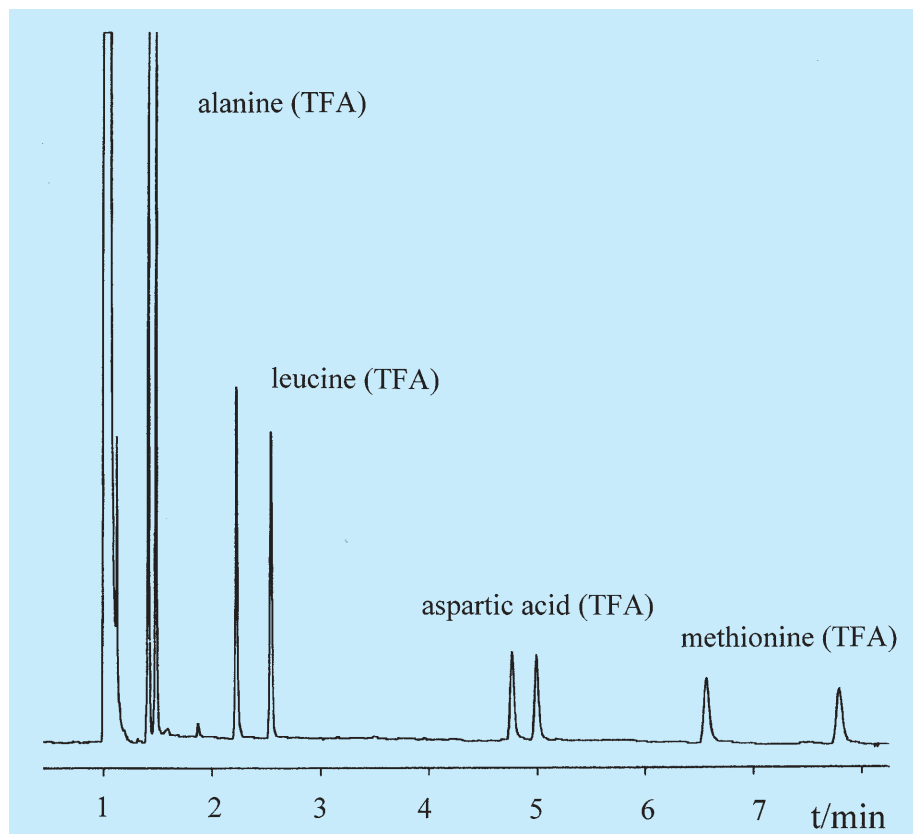
New technological developments are under study at NASA's Ames Research Center, based on specific detectors, to analyse different kind of oxidants, including  $H_2O_2$ . It is recommended that ESA should make sure the technology is available from European sources.

### II.5.10.1 Bulk Chirality Measurements

A measurement of bulk optical rotation could be provided by the 'SETH Cigar', a miniaturised space polarimeter (MacDermott et al., 1996). Moving parts are avoided by replacing the normal rotating analysing polarisers by multiple fixed polarisers at different angles, used with a diode array detector. Measurements at several different wavelengths, using three or four different diode sources, could provide clues to the

## II.5.10 Chirality Measurements

**Fig. II.5.10.2/2.** Enantiomers of trifluoroacetylated amino acids (alanine, leucine, aspartic acid and methionine) are baseline separated by the use of a Chirasil-L-Val phase, 25 m  $\times$  0.25 mm internal diameter, Chrompack. (Courtesy W. Thiemann and U. Meierhenrich)



identity of the soil constituent responsible for the optical activity. Complexing agents could be used in the solution to enhance the optical rotation of important target molecules such as amino acids.

### II.5.10.2 Enantiomeric Separations

Enantiomers can be separated using chiral GC columns, such as the COSAC GC-MS system, which will use pairs of columns: one has a homo chiral coating, while the other has a racemic coating of the same substance. If two peaks are obtained through the homo chiral column but only one through the racemic column, they are very likely enantiomers, and the D/L ratio can be obtained from the peak areas. However, different columns are required to separate different enantiomers, and amino acids require derivatisation, which is difficult in space.

A GC system cannot be directly coupled to a polarimetric detector because GC samples are too small. But the SETH Cigar polarimeter could be used separately to complement the chiral GC (e.g. to detect amino acids). However, HPLC is ideal with polarimetric detection: the output from chiral HPLC columns could go directly into a SETH Cigar-type polarimeter to give a more powerful system, able to separate a wider range of enantiomeric compounds, including amino acids, without prior derivatisation. Development of space HPLC instrumentation should become possible in the coming years with increasing miniaturisation and improved handling of liquids.

However, such a development is still very tentative. On the contrary, the use of GC in space has been fully demonstrated. GC (chiral columns) coupled to optical rotation measurements or/and MS should provide the separation of enantiomers, their quantitative analysis and the determination of the R/S isomers ratios.

One or several chiral column(s) in parallel on a PYR-GC-MS system can provide such a measurement. However, this technique (except if a chemical derivatisation system is used) is not compatible with the analysis of amino acid enantiomers. It should be noted, however, that diastereoisomeric separation can be carried out on an

## RECOMMENDED PAYLOAD AND TECHNOLOGY RESEARCH PROGRAMME

Taking into account the mass, power and volume restrictions, the following instrumentation appears to be essential for reaching the exobiological objectives of the package as described earlier in this document.

### FIRST PRIORITY

*Microscope:* for general examination of the samples

- resolution: 3  $\mu\text{m}$
- mass: 250 g (100 g, with the close-up imager but with 50  $\mu\text{m}$  resolution)
- heritage: Beagle-2

*Raman spectroscopy:* molecular analysis of minerals and organics (with IR)

- must include near IR excitation for biological applications, as well as geochemical
- spectral range: 200-3500  $\text{cm}^{-1}$
- resolution: 8  $\text{cm}^{-1}$
- projected mass: 1 kg
- heritage: Mars Surveyor 2001

*APX Spectrometer:* elemental analysis, detection limit a few 0.1%

- mass: 570 g
- power: 340 mW
- data output: 16 Kbytes per sample analysis
- heritage: Mars Pathfinder, Mars Surveyor 2001, Rosetta

*Mössbauer Spectrometer:* quantitative analysis of Fe

- mass: 500 g
- power: 1.5 W
- radioactive source of about 300 mCi
- heritage: Mars Surveyor 2001 Athena mission (MIMOS II instrument)

*PYR-GC-MS system:* isotopic, elemental, organic and inorganic molecular composition, and chirality measurement

- PYR: includes several ovens (for pyrolysis, combustion and chemical transformation of the samples)
- GC: a minimum of four columns, in parallel, likely to be capillary open columns for separation, respectively of:
  - permanent gases and very low molecular weight organics
  - volatile higher molecular weight organics of small polarity
  - volatile higher molecular weight organics of high polarity
  - column for enantiomer separation (chiral or, if derivatisation with chiral reactant, non-chiral column)
- heritage: COSAC
- additional detectors (nano-TCDs, chiral detectors) for each GC column
- MS: ion trap (heritage: MODULUS) or magnetic (Beagle-2) or Quadrupole (heritage: CHARGE). In all cases, a pumping device (such as a miniaturised turbo molecular pump) will have to be developed.
- total mass: 5.5 kg
- power: 10-20 W (to be confirmed)

*H<sub>2</sub>O<sub>2</sub> and other oxidant-dedicated sensors (still to be fully studied and developed):*

- expected mass: 100 g (to be confirmed)

### SECOND PRIORITY

*Infrared spectroscopy:* molecular analysis of minerals and organics (with Raman)

- wavelength range: 0.8-10  $\mu\text{m}$
- spectral resolution:  $>100 (\lambda/\Delta\lambda)$
- spatial resolution: 200  $\mu\text{m}$
- expected mass:  $<1$  kg
- expected power: 3-4 W
- heritage: CIVA-M (Rosetta lander), ISIS-M (Champion-Deep Space 4)

*Technology research programme items:*

- turbo molecular pump
- LA-ICP ion source
- H<sub>2</sub>O<sub>2</sub> specific sensor
- GC detectors (nano-Thermal Conductivity Detectors, chiral)
- grinding/sample preparation system

achiral column (i.e. for compounds with more than one chiral centre).

Let us also mention the recent development of piezoelectric and optical gas sensors that 'are capable of recognising different enantiomers and of qualitatively monitoring the enantiomeric composition of aminoacid derivatives and lactates in the gas phase' (Bodenhoefer et al., 1997). Such a technique could be applied to a GC-MS system including a solid- or gas-state derivatisation process for analysing amino acids after derivatisation into volatile compounds.

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## II.6 Team III: The Inspection of Subsurface Aliquots and Surface Rocks

The basic objectives of this study were to define methods and instrumentation:

- to search for evidence of extinct microbial life at all scales down to 0.01  $\mu\text{m}$ ;
- to document the mineralogy, petrography, secondary soil components and mobile phases, their compositional changes and their changes in abundances with depth;
- to determine the dust particle characteristics and any potential danger to human life.

### SCIENTIFIC JUSTIFICATION

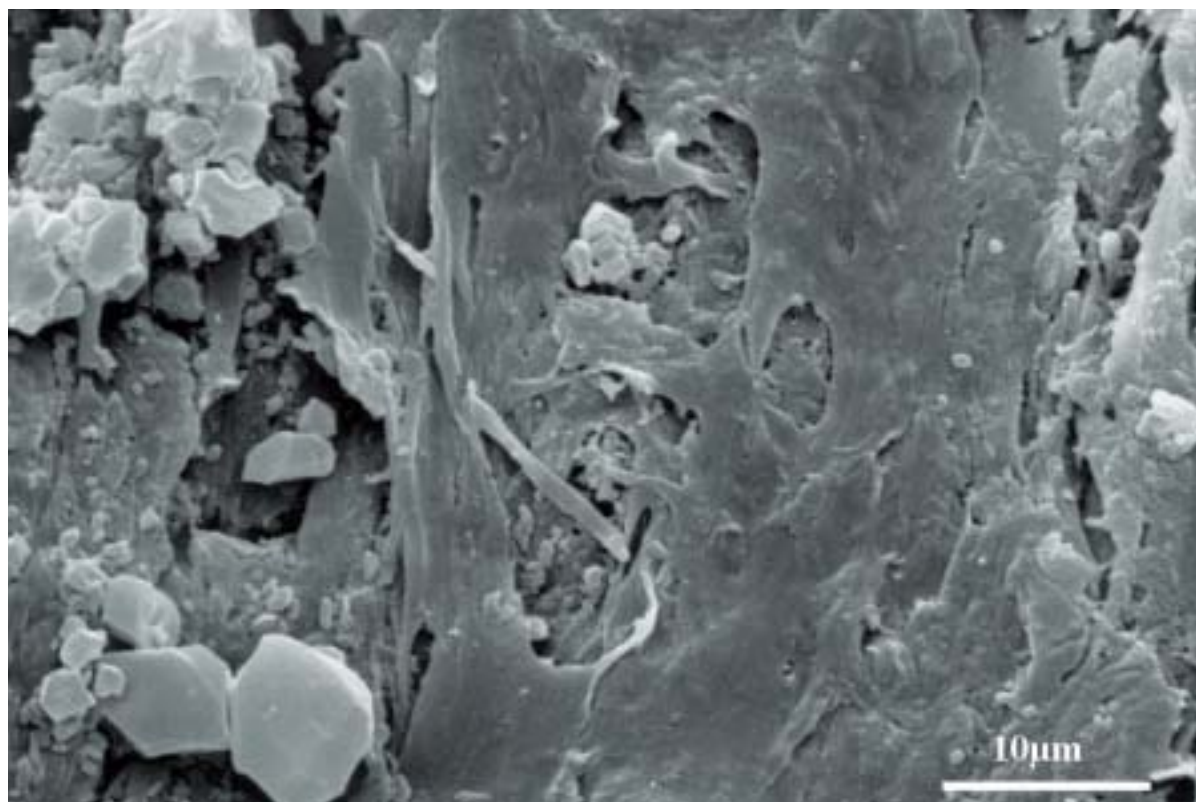
One of the major drawbacks of the analytical data from the Mars Pathfinder mission was the uncertainty concerning the extent to which the Alpha-Proton-X-ray Spectrometer (APXS) was affected by a possible silicate-bearing weathering rind on the surface of sampled rocks. There is now no evidence that the APXS ever sampled unweathered material. The surfaces of many rocks facing to the north-east appeared less red in the initial panorama. However, it has been recently shown that the remarkable brightness of the sky produced by airborne dust affects the illumination of the surface in such a way that rocks appear redder when in shadow or oblique sunlight. The initial interpretation of the less red faces of rocks as dust-scoured (and hence unweathered) surfaces must therefore be questioned and cannot be justified without a more sophisticated treatment of the illumination. Future missions must resolve this problem by making a concerted effort to remove any rind and/or dust coverage in order to sample unweathered rocky material. Argument about contamination of sampled materials will always occur unless the sampling device provides a method of exposing unaltered, unweathered material. Once the unweathered surface has been exposed, the full capabilities of the remote sensing and sampling investigations can be used on it. Given that drills, saws and polishers can be implemented within a relatively small mass budget, their incorporation into the mission is mandatory.

The subsurface environment on Mars may contain records of extinct life as well as possible extant life in the form of endolithic microorganisms. Given the environmental development of Mars, it is unlikely that forms of life much higher than algae, fungi, mosses or lichen ever evolved in the relatively short time span of continuous, life-supporting conditions that existed. Although conditions conducive to life probably occurred (and may still occur) intermittently, as discussed in Section II.4, they were probably not of long enough duration to permit further evolution. Rather, they may have permitted simple reproduction or overturning of the extant populations, wherever they survived.

It should be noted that there is a common misconception that if all the organics have been oxidised or eliminated by UV radiation it will not be possible to substantiate the presence of erstwhile cells if there are no more organics. This is not the case, because most prokaryotes are preserved by mineral replacement, with the subsequent degradation of the organic matter. Experiments have demonstrated that the morphology of prokaryotes may be faithfully recorded by the nucleation of minerals onto the surfaces of cells, as well as within them (Westall et al., 1995). Moreover, not only are the organisms preserved but also the exocellular organic substances they

### II.6.1 Investigation of Unweathered Rocky Material

### II.6.2 Imaging of Fossilised Material in Sediments



**Fig. II.6.2/1. Biofilm seen using a scanning microscope.**

produce (Westall et al., 1995). Most microorganisms, such as bacteria, live attached to substrates and build up layers of slime containing cells, authigenically precipitated minerals (e.g. carbonates, pyrite) and trapped particles.

These layers are known as biofilms (Fig. II.6.2/1) and they may often be preserved even when the microorganisms that produced them are not preserved (Gerdes & Krumbein, 1987). Stromatolites are famous examples of such microbially-mediated, laminar build-ups and examples are given in Part I. Thus, the presence of macroscopically biolaminated deposits is a valid indicator for the presence of life, even though the microorganisms may not be visible (either because they are below the limit of visibility of the method employed or because they were not fossilised).

Apart from the experimental simulations of microbial fossilisation, there are numerous examples of mineralogically replaced fossils in the rock record: the oldest coming from 3.3-3.5 Gyr sediments from the Early Archean of South Africa and Australia (Westall, 1999). These early terrestrial fossils are, however, very small (typically 1  $\mu\text{m}$  but sometimes up to 4  $\mu\text{m}$ ) and are close to the limit of optical resolution. Other examples of 1-2  $\mu\text{m}$ -sized fossil bacteria are found in siderite (Wuttke, 1983), phosphate (Liebig et al., 1996), calcite (Barbieri et al., in press) and silica (Monty et al., 1991).

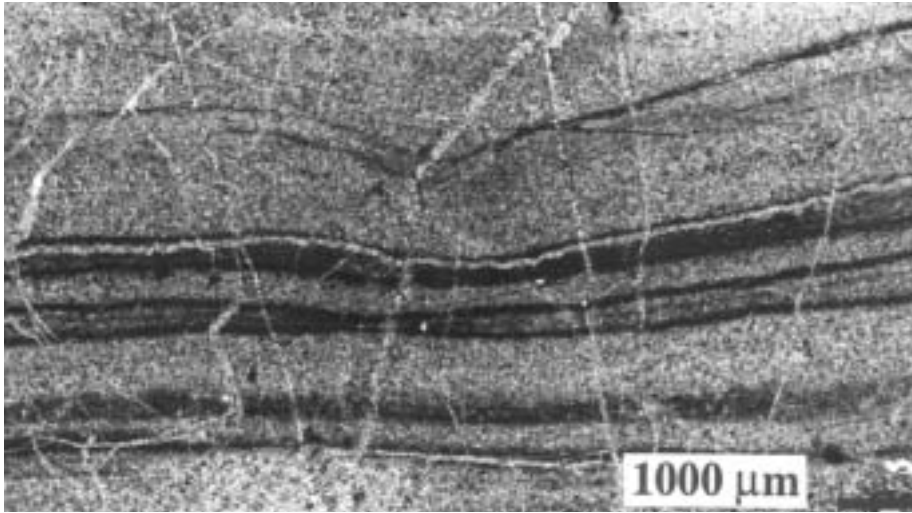
### II.6.2.1 Macroscopic Scale

On a macroscopic scale, two biogenically produced features can be observed:

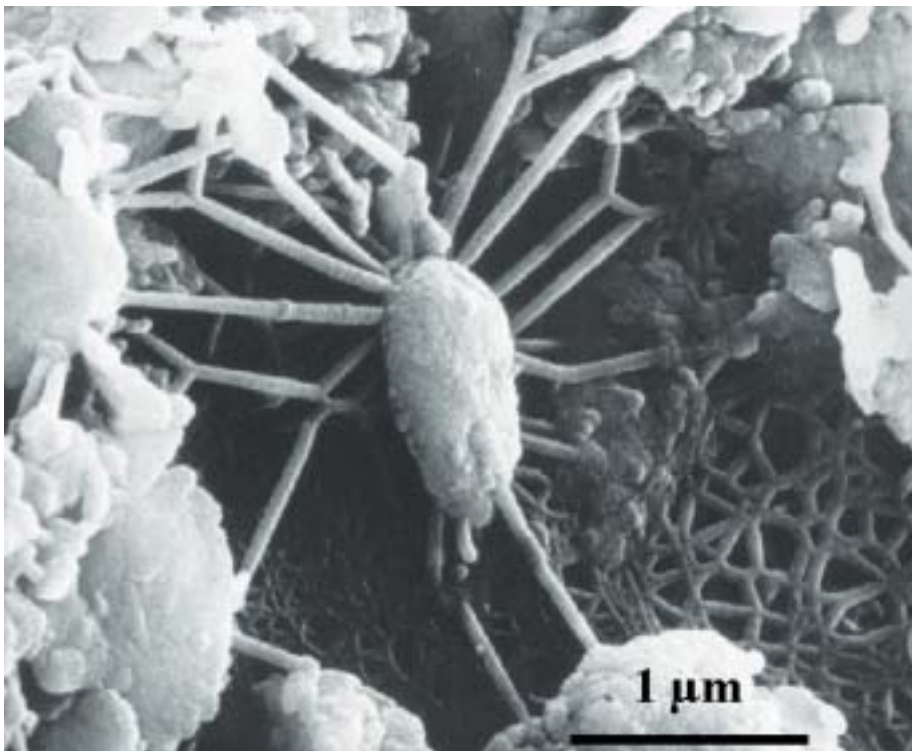
- biolaminae (biofilm layers) of the order of a few hundreds of microns to about 1 mm in thickness but extending for tens of centimetres or more;
- fossils >1 mm in width and tens of millimetres in length (cyanobacteria, algae, mosses, lichen etc).

#### *Biolaminae*

- large biolaminated structures, such as stromatolitic mounds of the order of > 0.5 m



**Fig. II.6.2.1/1. Thin section of biolaminated structures.**



**Fig. II.6.2.2/1. A modern bacterium attached to its biofilm substrate and surrounding sediment particles by filaments.**

in width and height will be visible at a distance of 3-5 m with a resolution of 10 cm per pixel;

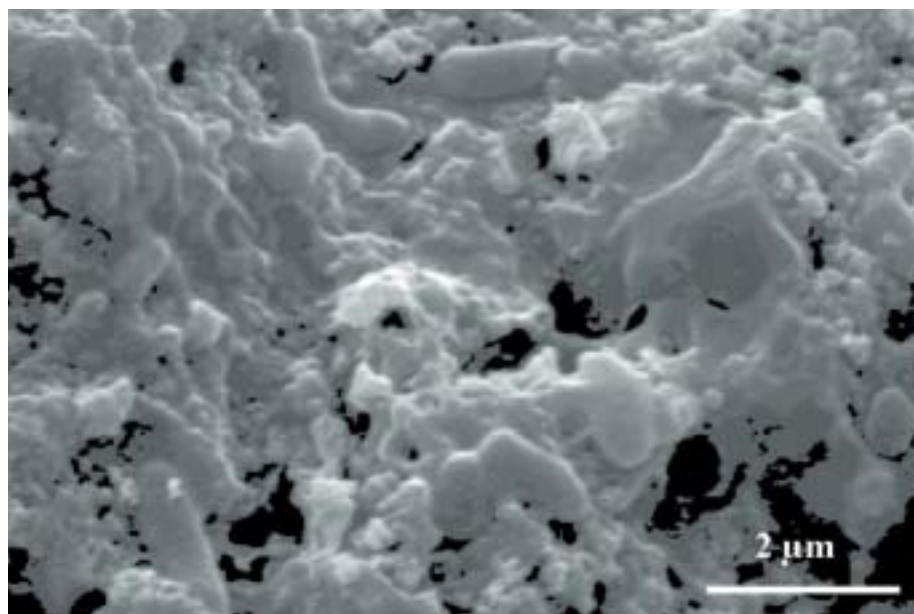
- smaller biolaminated structures with packets of 2-3 mm-thick, fine layering will be visible at 50 cm with a resolution of 1 mm per pixel (Fig. II.6.2.1/1).

#### *Fossils*

- smaller filamentous organisms can be macroscopically visible even though their width may be <1 mm if their lengths are at least 10 cm. These organisms usually have a sinuous structure and they occur in associations or colonies, thus facilitating their observation. Encrusted filamentous organisms about 0.5-1 mm or more thick, such as cyanobacteria and algae, will be visible at a distance of 50 cm with a resolution of 100-250 mm per pixel;
- other types of primitive organisms such as mosses/lichen will be visible at a distance of 2-3 m with a resolution of 1 mm per pixel.



Fig. II.6.2.2/2. Fossil rod-shaped bacteria (sausage-shaped structures) embedded in grainy biofilm. From the Hooggenoeg Formation, Onverwacht Group, South Africa (age 3445-3472 Myr).



#### II.6.2.2 Microscopic Scale

On a microscopic scale, biogenic structures that can be seen include:

- undulating biofilm laminae of the order of 100 μm;
- microfossils with a maximum dimension <5 μm.

##### *Biofilm*

The undulating structure of biofilm will be brought out by a magnification of 10-20 times and a resolution of at least 25 μm per pixel (Fig. II.6.2.2/1).

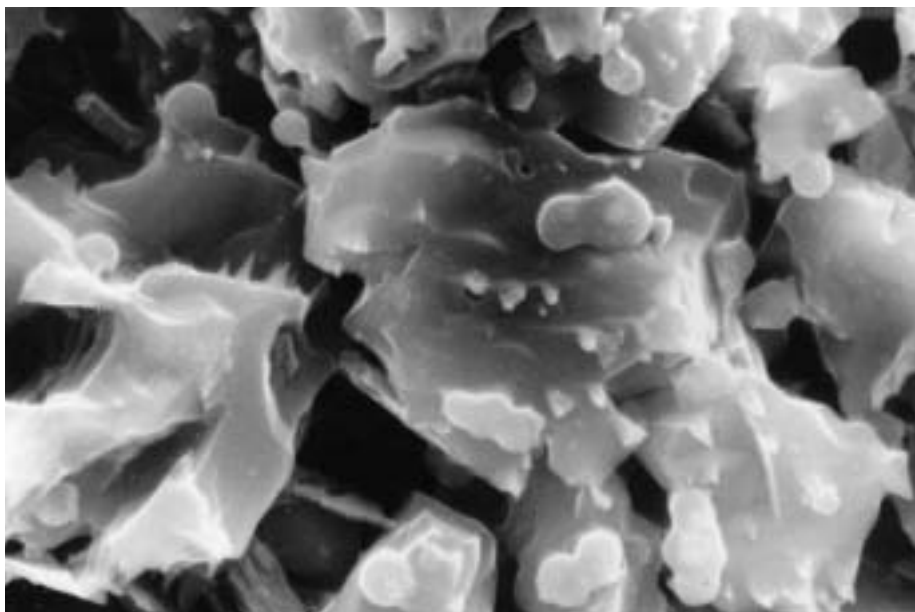
##### *Microfossils*

- microfossils 1-5 mm: these include filamentous cyanobacteria and algae. Structures at the larger end of this size range can be examined on a one-to-one basis or with magnifications up to 5-10 times for 1 mm structures, needing resolutions of 1 mm per pixel for 5 mm structures to 250 μm per pixel for 1 mm structures;
- microfossils 0.1-1 mm: microfossils in this range include filamentous cyanobacteria, algae and fungal hyphae. These need to be magnified 10-20 times at a resolution of a minimum of 25 μm per pixel;
- microfossils 50-100 μm: including cyanobacteria, fungal hyphae and algae. These need to be imaged at 50-100 times with at least 25 μm pixel resolution, or 50 times with 10 μm pixels;
- microfossils <50 μm: cyanobacteria, fungal hyphae and smaller, non-cyanobacteria. Such structures need imaging with magnifications of at least 50-100 times and high resolutions of 1-5 μm per pixel.

Most prokaryotes are preserved by mineral replacement and it does not matter whether there are still organics. It is still possible to see fossil unicells even if they no longer contain organics. An example of this type of process has been found in fossils from 3.3-3.5 Gyr-old sediments from the Early Archean of South Africa (Westall, 1998) (Fig. II.6.2.2/2). Experiments to simulate the fossilisation of prokaryotes have also been performed which show that they are mostly mineral-replaced and still very identifiable in terms of typical prokaryotic features (such as size, shape, association, cellular division).

These very old terrestrial fossils are, however, very small (typically 1 μm) and close to the optical resolution limit, although an example 4 μm long has been found in the Warrawoona sediments of Australia of similar age.





**Fig. II.6.2.2/3.** Fossil coccoid bacteria 1  $\mu\text{m}$  in diameter showing cellular division. These structures have a size typical for terrestrial bacteria. High magnifications are needed to image them ( $>100$  times with resolutions of at least 0.25  $\mu\text{m}$  per pixel).

Searching for signs of extant or extinct life includes the evaluation of direct evidence in the form of preserved fossils, either mineralised or as organic remains, as well as a more indirect approach utilising geochemical and/or isotopic proxies for past biologically-driven processes and their metabolic products, as discussed in Section II.5. As such, the presence of organic compounds and/or certain chemical elements that represent important building blocks of life (i.e. C, H, N, S, P), their specific isotopic compositions and their presence as mineral matter of presumed biological origin might provide evidence for (or against) the presence or past presence of life.

In addition to the exclusive search for evidence of life, a thorough characterisation of the present and past environmental conditions should be a prerequisite. In comparison to terrestrial settings, a wide variability in habitats do show flourishing life. However, certain physical and chemical parameters provide boundary conditions that limit its survival.

Of particular interest are minerals that presumably formed through biological processes, i.e. biominerals. Pyrite ( $\text{FeS}_2$ ) is a ubiquitous mineral in terrestrial sediments and frequently of biogenic origin. A key process in its formation is the bacterial reduction of sulphate to  $\text{H}_2\text{S}$  and subsequent reaction with iron minerals, to form iron sulphide. The formation of sedimentary pyrite, specifically of biological origin, and the relationship between pyrite morphology, origin and depositional environment, has been studied for many decades (e.g. Berner, 1970; Goldhaber & Kaplan, 1974; Berner, 1984; Morse et al., 1987; Schoonen & Barnes, 1991; Sawlowicz, 1993; Wilkin et al., 1996; Wilkin & Barnes, 1997). Furthermore, pyrite has been considered as a potential origin of life (e.g. Wächtershäuser, 1988; Russel et al., 1989; 1990).

Of all the possible biogenic morphologies, framboidal pyrite consisting of minute pyritic grains resembling raspberries (Rust, 1935) has received most attention. Generally, the sizes of these framboids range from 5  $\mu\text{m}$  to 20  $\mu\text{m}$ , but framboids as large as 250  $\mu\text{m}$  have occasionally been observed. Alternatively, framboids as small as 1  $\mu\text{m}$  were also seen. Agglomeration of individual framboids might result in the so called polyframboids with sizes ranging from 35  $\mu\text{m}$  to 900  $\mu\text{m}$  (average 50–200  $\mu\text{m}$ ). Thus, a continuous spectrum of sizes, microframboids–framboids–polyframboids, exists (e.g. Sawlowicz, 1993). In terrestrial settings, framboidal pyrite is most common in recent and ancient sedimentary environments, but has also been reported from magmatic rocks or hydrothermal occurrences (e.g. Sassano & Schrijver, 1989). The framboidal texture is usually associated with pyrite, but has also

## II.6.3 Investigation of Biominerals

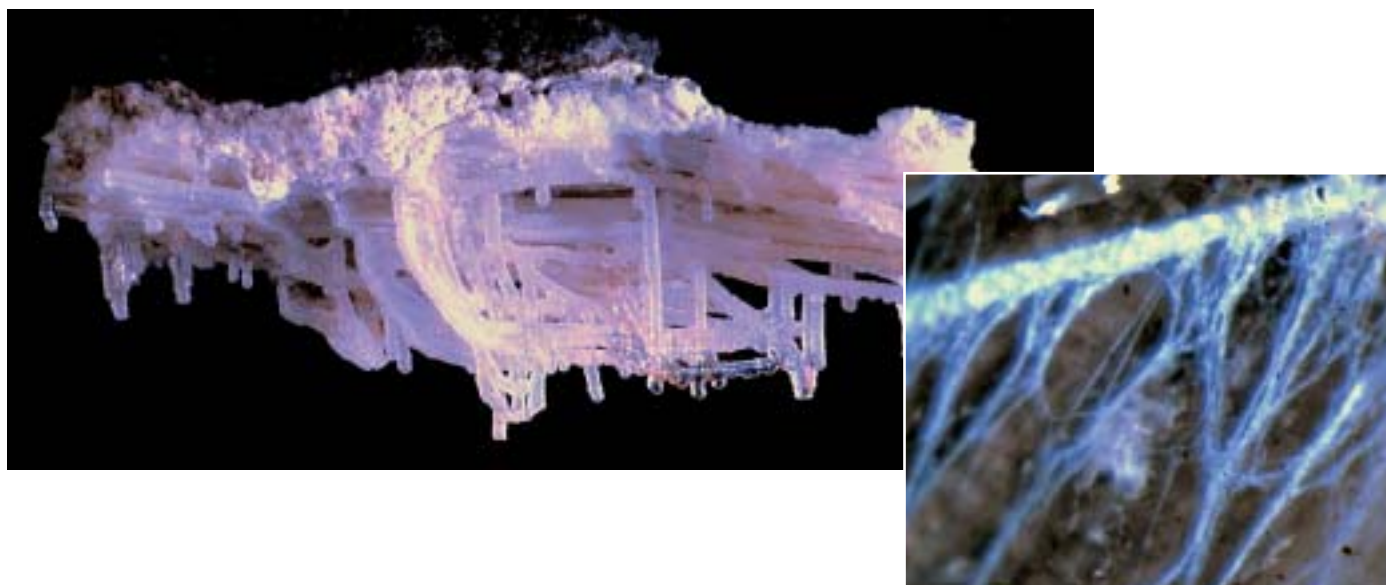
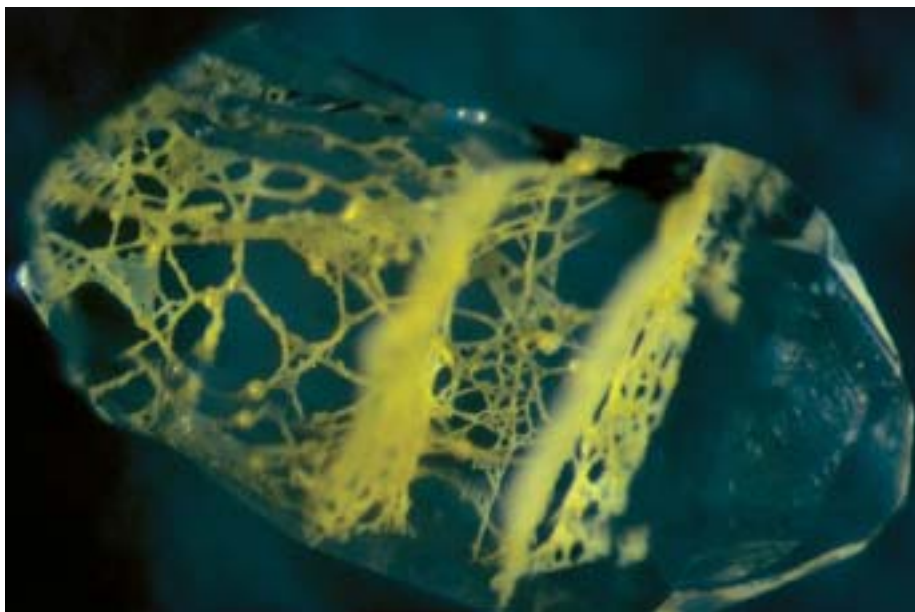


Fig. II.6.4.1/1. *Left*: Filaments of probable microbiological origin, Faeroe Islands. Micron-thick filaments, partly gravity-oriented, are encrusted with chalcedony to form up to 1 mm-thick ‘rods’. From a cavity in Tertiary basalt. Typical example of macroscopically visible microbial fossil of subsurface origin. Sample width 40 mm. *Right*: an enlargement (field of view 5.6 mm). Probable microbiological filaments encrusted by chalcedony. Sample immersed in glycerine, showing extremely fine filamentous cores of ‘chalcedony rods’.

been observed in other minerals, such as magnetite, haematite, limonite and magnesioferrite. Some of the latter might be oxidation products (magnetite, limonite) or precursors (greigite) of pyrite.

Based on terrestrial occurrences, pyrite formation can be purely inorganic as well as biologically catalysed. Prerequisites for the formation of pyrite framboids are the availability of iron and sulphur. While these conditions might be available in a whole range of environmental settings, the frequent presence of framboidal pyrite in organic-rich sediments suggests that organic matter plays an important role in its formation. Carbonaceous sediments appear to be an ideal host for pyrite formation. They contain abundant reactive iron (Canfield, 1989) and organic material that may serve as an energy source for sulphate-reducing bacteria producing  $\text{H}_2\text{S}$ . This biologically-produced  $\text{H}_2\text{S}$ , in turn, reacts with the iron to form (framboidal) pyrite. While, from a stoichiometrical point of view, organic carbon is unnecessary, experimental data indicate only limited formation and stability of framboidal pyrite without the presence of organic matter (for a review see Sawlowicz, 1993). The relationship between framboidal pyrite and organic matter (and as such evidence for biological activity) is two-fold. Apart from serving as a substrate for the sulphate reduction, framboidal pyrite frequently replaces fossil organic matter on a micro- or macroscale (Briggs et al., 1991; 1996).

In summary, sedimentary pyrite and, in particular, framboidal texture are frequently observed in recent and ancient sediments on Earth. Although examples exist for a purely inorganic origin of pyrite framboids (e.g. laboratory experiments: Sweeney & Kaplan, 1973), its presence in sedimentary systems is considered overall as evidence for a biological origin. Additional support for this conjecture can best be obtained from the sulphur isotopic composition, showing a characteristic signature (for a review see Strauss, 1997). In that respect, close examination of martian surface and, in particular, subsurface material for pyrite as a potential biogenic mineral might provide important insight as part of the search for life. Microscopic examination would have to provide a spatial resolution compatible with expected size ranges down to  $<10\ \mu\text{m}$ . Investigations could best be done on the freshly broken surface of a rock specimen. In addition, a freshly cut or, even better, polished surface might also yield insight into mineral textures. Finally, magnetic properties of possible precursor



**Fig. II.6.4.1/2.** Goethite filaments from Hohenlimburg, Germany. They are probable mineralised microbial remains, enclosed in quartz crystal. Field of view 2.8 mm. Sample from solution cavities in Devonian limestone. (Sample courtesy P. G. Penkert, Dormond)

minerals or oxidation products should also be assessed. As pointed out above, framboidal textures have also been observed in non-pyritic minerals.

#### II.6.4.1 Extinct Microbes in Rock

In existing concepts, the search for microbial fossils on Mars is strongly focused on the remains of former surface-bound microbial activity in sediment sand and hot spring environments. While it has become accepted that subsurface life is active on Earth to a depth of several km in some places (e.g. Stevens & McKinley, 1996; Stevens, 1997), hardly any fossil evidence of this has been reported so far, with the notable exception of a paper by Kretzschmar (1982). Recent work has shown that subsurface microbialites similar to those reported by Kretzschmar (1982) are common in terrestrial samples from numerous subsurface environments (Hofmann & Farmer, 1997). This work is in progress and about 100 sites have so far been identified.

Geological environments containing such suspected fossil remains include:

- sites of low-temperature hydrothermal alteration of volcanic rocks (dominant);
- oxidation of sulphide ore deposits (quite common);
- hydrothermal vein-type ore deposits (rare);
- silica deposits associated with serpentinisation of ultrabasic rocks (rare).

The depth of emplacement of filamentous microstructures of probable microbial origin is unknown in most cases, but reaches 400-800 m in at least three of the better-studied sites (Fig. II.6.4.1/3). There is a growing body of evidence that subsurface microbial activity commonly results in macroscopic structures similar to surface microbialites (stromatolites). Many of the hitherto recognised possibly microbial structures in such environments are enclosed in microcrystalline silica (chalcedony, agate).

The recent identification of agates as products of post-impact hydrous alteration in an impact melt of a Finnish crater (Kinnunen & Lindqvist, 1998) makes it likely that similar silica varieties do exist on Mars since cooling impact melts interacting with groundwater must have been common.

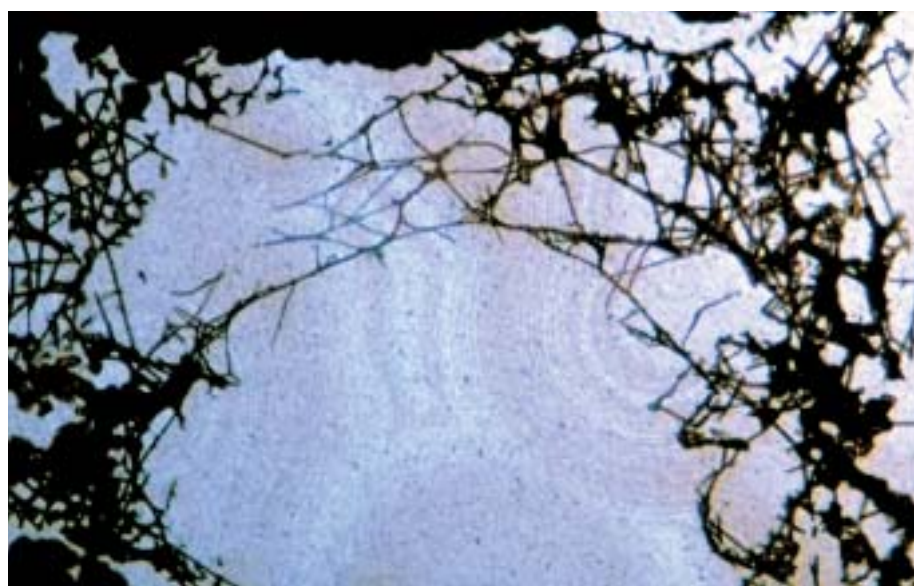
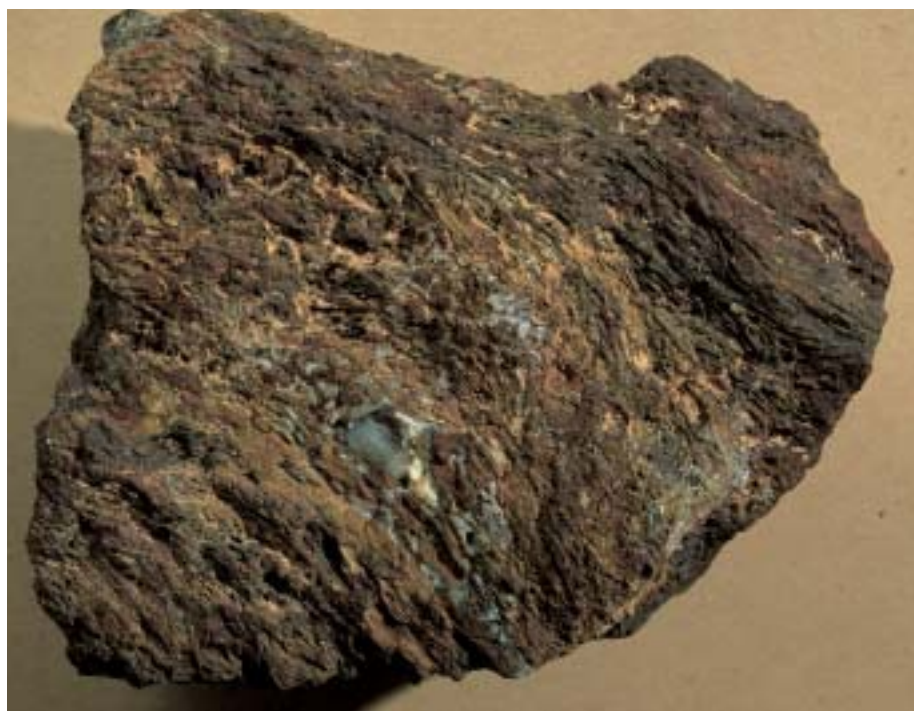
The chances of subsurface microbial fossils existing on Mars might be higher because a subsurface biosphere can be expected to be relatively more important than on Earth because of the inhospitability of the martian surface. Considering not only

### II.6.4 Investigation of the Rock Structure



Fig. II.6.4.1/3. Filamentous structure of probable microbiological origin, from the Cady Mountains, California. Mineralised filaments, up to 10 cm long, are from a goethite-chalcedony vein of clearly subsurface origin in Tertiary volcanic rocks. Sample size 13×15 cm.

*Bottom:* close-up view (field of view 0.9 mm), showing filamentous structures of goethite on chalcedony.



surface-bound but also subsurface microbial fossils in the evaluation of landing sites and target selection for sampling enlarges the selection of potentially interesting sites and materials. A fractured or vesicular basalt, for example, would be considered of low interest in existing strategies focused on sediments and thermal spring deposits, but may nevertheless harbour rich subsurface fossil remains.

#### II.6.4.2 Rock Formation and Composition

Even if microbial fossils are not found, investigation of rock structures will provide significant new information on the rock formation process. At the Mars Pathfinder site, there was evidence for a bimodal distribution of rock sizes. Rounded rocks, which may have been smoothed during the flood that produced Ares Vallis some 3 Gyr ago, may preserve some evidence of the differentiation process that occurred in Mars' early history.

A second group of rocks, however, may have been a later addition. These rocks could be the ejecta produced by an impact and as such would be from the upper layers of the present Mars. Some rocks may also have a volcanic origin and there is a pressing need to understand the range of magma chemistries that produced volcanic landforms. It is therefore important to understand the chemical and physical structure of rocks at the landing site.

One interpretation of the dust-like spectrum of rocks such as ‘Scooby Doo’ at the Mars Pathfinder landing site is that they are indurated soils produced by chemical processes. These rocks may differ substantially in composition and structure from the large rocks and boulders which appear to have been transported to the site by flow or impact. They may in many respects resemble sedimentary rock. This offers the prospect of investigating layering. The large-scale structures near Ares Vallis appear to show evidence for at least two directions of flow. It is not clear how soon after each other these flood events occurred or whether deposits from the initial event had time to settle before the second event. The study of subsurface layers could provide an answer.

Penetration of a sedimentary deposit may uncover an older layer produced at a time when conditions were more conducive to life. Hence, investigation and sampling of such a subsedimentary layer may be important. This will also allow us to inspect possible differences between the exposed surface and the matter underneath and get an impression on the physical behaviour of the soil.

Scooby Doo was also sufficiently robust that the Sojourner rover was unable to scratch the surface. Thus penetration of it may reveal unoxidised material at relatively shallow depths while at the same time being easier to drill through than a basalt.

How did the ubiquitous red soil on Mars originate? Did the soil form, once and for all, thousands of millions of ago when the planet was warm and wet? Or is the soil-forming process still going on today, through the interaction of an oxidising atmosphere with the surface rocks of the planet, perhaps aided by acidic volatiles supplied by volcanic eruptions?

Stated differently: what has been the role of liquid water in the formation of the soil of Mars? If liquid water has been essential for the formation of the martian soil, then liquid water may have played an even more decisive part in the history of the planet. The interplay of liquid water, the atmosphere and the surface rocks of Mars may have produced biology.

Through the investigations of iron compounds in the martian soil, it may be possible to contribute significantly to the study of the history of the planet’s surface and the evolutionary processes related to the interaction of water, air and rocks.

Why specifically iron compounds? Iron is the third most abundant element on Mars, following oxygen and silicon. The element iron interacts strongly with liquid water, especially with water in which gases such as carbon dioxide and oxygen are dissolved. The iron occurs often as  $\text{Fe}^{2+}$  as well as  $\text{Fe}^{3+}$ , thus reflecting the oxidative power of the environment in which a given iron-compound formed. The identification of the iron compounds in the soil will thus elucidate many aspects of the processes that formed the reddish soil of Mars and the history of liquid water on that planet. For example:

- The iron in the base rocks of Mars is present mainly as  $\text{Fe}^{2+}$ , while the iron in the soil is present as  $\text{Fe}^{3+}$ . It is thus certain that an oxidation has taken place. The existing combination of iron minerals present in the soil will, to some degree, tell how and how fast the oxidation occurred. Thus we will learn about processes of importance for the understanding of the evolution of a possible martian biosphere.
- Is some of the  $\text{Fe}^{3+}$  in the soil present in minerals commonly formed via precipitation (for example iron sulphates)?

## II.6.5 Investigation of Sedimentary Layering

## II.6.6 Investigation of the Soil



- Which type of iron containing clay minerals, if any, dominates?
- Does the magnetic phase present in the soil contain the element titanium, i.e. is the magnetic phase titanomagnetite ( $\text{Fe}_{3-x}\text{Ti}_x\text{O}_4$ )? If so, the magnetic phase has been inherited directly (intact) from the underlying bedrock, and the role of liquid water in the soil-forming processes has been limited. If the magnetic phase forms via precipitation of  $\text{Fe}^{3+}$  compounds in liquid water, the magnetic mineral will not contain titanium.

Mössbauer spectroscopy is, without doubt, the best method for investigating iron-containing compounds. As discussed in Section II.5.7.4, this technique yields information on the minerals present, their magnetic properties and, in case of superparamagnetism, particle-size distribution and, finally, the oxidation state of the iron.

## II.6.7 Investigation of Dust Particles

The dust particles suspended in the martian atmosphere play a key role in the atmospheric energy budget. This, in turn, controls the global circulation and the present climate. It is also suspected that the dust had a major influence on the evolution of the surface and the long-term evolution of the climate. The particle properties are inferred from measurements by ground-based observations and instruments on Mars orbiters and landers. For example, very good data were obtained by imaging the sky brightness with Mars Pathfinder's camera.

The deduction of material properties of the atmospheric particles involves solving a multi-parameter inverse problem of modelling the observed, diffuse light, produced by multiple scattering by the particles. The parameters are at least the atmosphere's optical depth and the dust's average single-scattering properties. These single-scattering properties can, in turn, be interpreted in terms of particle size distribution, composition and shape. Size distribution can be best constrained and it is known that martian aerosols have a mean radius of somewhat less than 2  $\mu\text{m}$ . The values of the imaginary index of refraction allows some constraint on the material composition. Combining the interpretation of data in the visible and thermal-IR, it can be said that the dust is composed of basalt and clay minerals such as montmorillonite, with a small fraction of iron oxide that gives martian dust the characteristic reddish tint.

The deduction of the shape of the particles – a very important parameter – is the most troublesome yet. Possible shapes include plate-like particles, round with smooth surface, more crystal-like with sharp edges or even agglomerates. Each category has different dynamical, thermal and radiative properties, and hence a different impact on any of the processes involving atmospheric dust. The difficulty in constraining the shape is two-fold. Firstly, to date, the solutions to the problem of calculating the scattering matrix for irregular particles either depend on a set of free parameters or are limited to relatively small particles. Secondly, it is not completely possible to separate out the influence the various parameters have in obtaining the solution to the inverse problem mentioned above. The variations in the modelled sky brightness, for example, are just as sensitive to the particle shape as they are to the index of refraction or optical depth. An estimate of the optical depth is often available independently, helping somewhat, but the remaining parameters are not easily separable.

The best solution to the above problem is to image the particles *in situ* on the martian surface. A great step in this direction will be made during NASA's Mars Polar Lander mission, which carries a variable-focus camera with 1:1 resolution of about 20  $\mu\text{m}$ . The MarsEnvironment Compatibility (MECA) package for NASA's Mars Surveyor 2001 Lander mission will finally deliver images at a resolution of about 1  $\mu\text{m}$ . Within the same instrument package will be an Atomic Force Microscope with a resolution of at least 10 nm. Combinations of results from these instruments should help greatly in constraining the shape of the martian aerosols.

## SCIENTIFIC METHOD AND REQUIREMENTS

The advent of new, lightweight, technology has made it possible to produce small highly integrated experiments which can be placed in many positions on landers and rovers. It is, however, essential that these systems form an integrated package. Achievement of the scientific objectives within a low mass can only be achieved if the objectives are focussed and the concept for the means by which the objectives are to be achieved are clear and straightforward. Here we discuss the scientific methods available and their requirements.

### II.6.8.1 Objectives

Initially, a panoramic survey of the site must be obtained. This survey must:

- identify the position of the landing site;
- provide an assessment of the rock density at the site;
- determine the rock distribution and its heterogeneity;
- determine the rock-size distribution and assess evidence for bimodal distributions caused by different processes;
- study the evidence for macroscopic influence on the site (flooding, impact, etc);
- investigate aeolian effects (such as wind tails and ventifacts) and allow comparisons with global circulation models;
- study the surface photometric function;
- investigate the bulk mineralogy of rocks and soils;
- search for spectral inhomogeneity of soils and compare this to the local morphology;
- search for spectral inhomogeneity of rocks and investigate evidence for sedimentation and in-flux of external material;
- search for potential targets for the rover, including identification of unusual morphology, differentiation between weathered and unweathered rock, study of significant colour differences, identification of potential dangers to navigation.

Assuming that some form of mobile device (such as a rover) is deployed, the survey must also:

- assess the morphology of disturbed soil and determine its consistency;
- compare the colour of rover tracks with undisturbed soil and assess the compaction parameters needed to produce this colour change;
- assess the rate at which the disturbed soil re-equilibrates with the surroundings.

In addition, the survey must make:

- an assessment of the damage to the site caused by the landing (including contamination resulting from the landing strategy employed);
- determine the orientation of the landing vehicle;
- determine the optical depth at the site for purposes of power management.

The system must also determine the properties of the diffuse illumination. The sky can be extremely bright and very red because of the high optical depths of atmospheric dust (Thomas et al., 1998). In order to provide a quantitative assessment of the colour and albedo of the surface, the properties of the airborne dust must be measured.

It should be noted that the spectra, derived from Mars Pathfinder imager measurements, showed little or no contrast in the 800 nm - 1  $\mu$ m range. This spectral region was specifically targeted to investigate absorption by Fe<sup>2+</sup>-bearing minerals. The only soil with any significant absorption (near the rock 'Lamb') showed a band of <5%. This implies that a high dynamic range is vital if one wishes to identify unusual surface properties in mineralogical spectra.

### II.6.8.2 Requirements

The panoramic camera will be the most sophisticated system. The Imager for Mars

## II.6.8 Initial Survey and Target Selection

Pathfinder (IMP) provided an excellent balance between resolution, colour information and data volume. We propose to follow a similar strategy. In order to define the shape of the spectral reflectance curve, a minimum of nine filters is required (440, 530, 600, 700, 800, 900, 930, 960 & 1000 nm). This will allow us to search for potentially interesting targets with absorption bands in the 900-1000 nm region of the spectrum. An additional set of three filters is required to provide information on the optical depth. Two other filters need to be set aside to provide red/blue stereo coverage. We note that attempting to acquire a stereo pair at different times may save a filter position. However, the change in the illumination between the acquired images would need to be carefully considered and modelled. The uncertainties in this process are probably too large and hence dedicated stereo pairs are required. Thus, the system needs a minimum of 14 positions.

In order to quantify both atmospheric phenomena and to provide a survey of the entire scene, the system needs to be articulated in both azimuth and elevation. Using current detector devices (1000×1000-pixel frame transfer CCDs) and a pixel scale of 1 mrad px<sup>-1</sup> (equivalent to the IMP), a 40×40° field of view can be acquired in one frame. An estimated daily telemetry requirement of 8 Mbit would be necessary. High-quality once-only products, such as surveys, require around 275 Mbit.

It needs to be emphasised that present developments in micro-integrated camera systems have concentrated on the CCD and accompanying readout electronics. These developments have driven the total mass of these elements to around 50 g. However, the panoramic survey camera requires a higher dynamic range, azimuth and elevation motors (covering almost 4 $\pi$  steradians), a mast and a significant filter wheel assembly. Acousto-optical tunable filters might be used to reduce the mass of the latter but they are currently not available in large enough dimensions to cover a 1000×1000-pixel focal plane. We therefore conservatively assume a mass of 2.5 kg for this system and a power requirement of 8 W.

The calibration of the panoramic camera requires additional hardware to be deployed on the lander. The Mars Pathfinder camera used a series of black and white and colour calibration targets to provide good cross-calibration between the image frames acquired at different times and to validate the pre-flight calibration. A similar approach should be adopted. Places on the lander, in view of the camera and seeing as large a solid angle of the sky as possible, should be set aside for a calibration target. The target should be large enough: 100 pixels across seen from the camera.

Consideration should be given to whether the rover can deploy a calibration target away from the lander in order to minimise scattered light contributions from the lander's body and to allow full sky illumination of the target.

## II.6.9 High-Resolution Studies

### II.6.9.1 Objectives

After specific areas of interest have been identified by the panoramic survey, a detailed investigation needs to be performed. This should be by a rover (or some other mobile device) moving to an area of interest and obtaining higher resolution data from the target's proximity. A primary requirement of this investigation is knowledge of the detailed optical appearance. This must determine the morphological structure of a target, its colour, the degree of weathering and the dust coverage. This will determine how much the material has been chemically or physically processed, allow a better assessment of its origin, and help to identify an even more detailed target for grinding and/or sampling and subsequent detailed investigation. This implies that a simplified camera system with colour capability needs to be on the mobile device.

It is generally accepted that microscopic investigation of an unprepared surface provides little useful additional information (see Part I). If a surface can be physically prepared (by grinding or polishing), however, a microscope with front side illumination can investigate the material's microstructure. This will allow an assessment of inclusions in the rock and place constraints on the processes that formed the rock. Colour capability is important since it will allow us to differentiate between individual minerals. We note that polarisation measurements are useful only in transmission.

The sample materials should be subjected to microscopy with the following aims, as discussed in Section II.5:

- determine the mineralogy;
- determine the texture of rock and soil fragments;
- search for possible biogenic structures.

Results will allow the definition of the geological framework for any found biogenic structures or biochemical signatures. The interpretation of images will largely rely on experience gained by imaging terrestrial materials with a system providing identical image quality. Exchange of images with unknown content should be performed between at least two groups well before landing on Mars. The search for possible biological structures will be supported by a collection of images of mineralised terrestrial analogues that is currently being built up.

As the nature of martian life is unknown, the search can only be based on knowledge of Earth materials. Within sedimentary material, evidence might be sought for layering, veins within the rocks, amugdules, botryoidal surfaces, mottling, bleaching, geopetal features and filamentary or ropy structures. Studies of microfossils on Earth show that evidence for past life can exist at all scales and, therefore, by probing increasingly smaller scales the chances of discovery increase. Examples of microfossils found on Earth at relatively low resolution were given in Figs. II.6.2/1, II.6.2.1/1 & II.6.2.1/2. The search for microbial remnants should be made with the highest spatial resolution that can be reasonably achieved on a small rover. We estimate this to be better than  $<0.01 \mu\text{m}$ .

### II.6.9.2 Requirements

It is necessary to cover, in some way, the intermediate resolution between the panoramic camera (resolution near 1 mm) and the resolution of a high-power device designed to reach our requirement of  $0.01 \mu\text{m}$ . The simplest approach is to use optical microscopy. The practical limit of optical microscopy is around 300 nm with relatively large instruments. Instruments resolving  $3 \mu\text{m}$  ( $1.5 \mu\text{m px}^{-1}$ ) within a very constricted mass (around 200 g) and volume ( $150 \text{ cm}^3$ ) have already been proposed for several missions and could be readily available. A data volume of  $3 \text{ Mbit day}^{-1}$  (every third day) and a power consumption of 4 W (principally for the focusing mechanism) has been estimated.

The three primary concerns with an optical microscope are the depth of field, the proximity of the front lens to the target, and the illumination of the target. The depth of field of such an instrument is typically  $<20 \mu\text{m}$ . This, then, drives the quality of the focusing mechanism that must accompany the device (and is included in the 200 g). The gear cannot be too long in order to limit the volume of the instrument. This drives the accuracy of the positioning of the rover with respect to the target: better than 1 cm. The illumination would normally be provided by the instrument. Super-bright light-emitting diodes need to be considered.

The microscope needs to be supported by imaging from a simple system at a resolution of  $<0.5 \text{ mm px}^{-1}$  in order to identify the structure of the material prior to sampling. This should have colour capability (to identify whether, for example, a rocky surface is dusty and/or weathered). This system might be incorporated in the microscope but may be more reasonably included as a separate device (around 2.5 W,  $200 \text{ cm}^3$  including 4-position filter wheel, 100 g). The microscope also needs to be accompanied by a grinding, polishing or an immersion device to prepare the sample prior to examination (see below).

The Atomic Force Microscope (AFM) measures atomic-scale forces between a surface and a probe tip by deflection of a microcantilever. AFMs produce topographic images up to sub-Å resolution. AFMs have the advantage of being able to produce images of non-conductive surfaces in air or liquid, whereas Scanning Electron Microscopes (SEMs) require conductive surfaces *in vacuo*. Resolutions of 1 nm will be achieved by the MECA experiment accepted for NASA's Mars Surveyor 2001

mission with a mass of <1 kg. Groups in Europe are already able to obtain similar results with masses <2 kg without significant mass reduction efforts at this stage. An instrument of this type (AFM or SEM) and the means to approach the sample are mandatory for an exobiology oriented mission.

## II.6.10 Subsurface Investigations

### II.6.10.1 Objectives

Sampling of unoxidised material can be performed either by penetrating rocks to a relatively shallow depth or by drilling through surface soils to a far greater depth. The two techniques, however, provide access to very different types of material. Drilling through the dusty layer may reveal sediments underneath. If this is the case, the sediments may show layering that will provide information on the nature of the deposition process. The resolution required is approximately 0.1 mm.

### II.6.10.2 Requirements

A borehole imager could be produced in two ways. Either the focal plane assembly remains above the surface and the image is transferred to it via a fibre optic cable, or the entire camera accompanies the drill down the hole with only power and telemetry cables connecting the system to the surface. In view of the complexity of fibre optics, the miniaturisation of focal planes and the relatively low angular resolution required (<10 mrad px<sup>-1</sup>), the latter seems preferable. The device needs to be oriented so that the field of view is orthogonal to the direction of tunnelling. It needs to be accompanied by light sources providing three different colours for better identification of strata.

A similar data volume to that of the microscope (3 Mbit day<sup>-1</sup> every third day) is envisaged. The mass should be around 100 g with a volume of <125 cm<sup>3</sup> (excluding cabling to the rover) but including light source. The power consumption should be around 2.5 W.

## II.6.11 Raman Spectroscopy

### II.6.11.1 Objectives

Raman spectroscopy is a useful tool on robotic missions to obtain information on the mineralogical structure and composition of planetary surfaces by allowing the identification of minerals (silicates, carbonates, phosphates, sulphates, nitrates) and organic molecules *in situ* on a micro scale and hence identifying mineral components and their chemical structures (Haskin et al., 1997). In combination with an optical imaging system (high-resolution camera, microscope, confocal microscope) the instrument is capable of providing spatially-resolved spectra from which the texture of rocks and soils can be related to mineralogical components or to local variations of crystalline phases on microscopic scale.

### II.6.11.2 Requirements

A Raman system can very easily fulfil spaceflight requirements (small size, low power, low sensitivity to mechanical vibration). A Raman spectrometer consists of three parts: energy analyser, detector and light source. In planetary missions, a diode laser source or a frequency-doubled diode-pumped solid-state laser is usually proposed. It is convenient to use an excitation wavelength between 400-500 nm with a maximum power output of 3-5 mW. The laser beam is focused on the sample at a working distance of several mm (spot size several microns). A CCD or an array of photo diodes can be used as a detector. A Raman analyser covering 200-4000 cm<sup>-1</sup> with 6 cm<sup>-1</sup> resolution is proposed for the Mars Surveyor 2001 rover. The mass requirement was 0.7 kg with a volume of 225 cm<sup>3</sup> and a peak power of 1.1 W. Commercial miniature spectrographs with high resolution are already available.

## II.6.12 Optical Spectroscopy

The Mars Pathfinder data showed that absorption bands in the optical are extremely weak (contrary to expectation) with shallow 5% absorption bands in the 800 nm - 1 µm region. The lack of significant variations over the surface were also somewhat



surprising. To achieve more than Pathfinder, an optical spectrograph must have an extremely high signal-to-noise and even in this case it is arguable whether an unambiguous identification of a mineral could be made. Other techniques (such as Mössbauer spectroscopy) should be investigated.

Mössbauer spectroscopy can provide a clear indication of the composition of iron compounds and FE-bearing minerals. It is discussed further within the Team II report in Section II.5.7.4.

## II.6.13 Mössbauer Spectroscopy

### II.6.14.1 Objectives

IR spectroscopy in the range 0.8-4.0  $\mu\text{m}$  can be used to determine the abundances of many types of minerals, including clays, hydrates and carbonates. Carbonate materials (such as magnesite, calcite and siderite) can easily be distinguished from one another. All have near-IR features in the 1.7-2.5  $\mu\text{m}$  range attributable to the  $\text{CO}_3^{2-}$  anion. Sulphates such as gypsum and anhydrite, have absorptions in the 1.0-1.5  $\mu\text{m}$  and 4.0-4.7  $\mu\text{m}$  ranges. Ferrous absorptions of pyroxenes are also evident in the 1.0-2.0  $\mu\text{m}$  region. The ratio of the 1  $\mu\text{m}$  to 2  $\mu\text{m}$  absorptions is diagnostic of the composition. Although olivine has only been found in one martian meteorite, the structure of the broad absorption feature is also a diagnostic of composition. The absorptions of C-H and C≡N bearing organics can be found throughout the 1.7-4.0  $\mu\text{m}$  range. A spectral resolution of  $>100 (\lambda/\Delta\lambda)$  would be sufficient. Good spatial resolution is needed to investigate variations within a sample and we estimate that a resolution of 200  $\mu\text{m}$  should be attainable.

A secondary objective for IR spectroscopy would be investigation of the atmosphere.  $\text{CO}_2$ ,  $\text{H}_2\text{O}$  and CO all have characteristic absorption bands in the IR. The variations of these gases together with the dust cycle are strongly coupled in the martian atmosphere and hence this could provide significant additional information on the time variability of the atmosphere. Local sources of these gases may be present, indicating hydrothermal activity.

It should be noted that the highest resolution near-IR spectroscopic measurements of Mars come from the Hubble Space Telescope, at around 200  $\text{km px}^{-1}$ . These data show variations in ferric alteration minerals and primary ferrous silicates but are limited by their coarse spatial resolution. It is not clear whether we will see low contrast in high spatial resolution data (as was seen in the optical with the Mars Pathfinder imager) or whether IR spectroscopy at the 200  $\mu\text{m}$  level will be extremely revealing.

### II.6.14.2 Requirements

Low-mass IR spectrometer systems have been proposed for several missions, including the RoLand lander on the Rosetta mission, and some systems are now commercially available. The system may require illumination devices (such as LEDs) in order to compensate for absorption lines in the solar continuum at the surface caused by dust in the atmosphere. The system should, however, be comparable in mass and power consumption to the microscope.

Because transmission IR spectroscopy may not be applicable (requiring preparation of thin sections), the less diagnostic reflection mode may have to be selected. Even in this mode, however, identification of mineral and organic compounds and detection of  $\text{H}_2\text{O}$  and (OH) in silicates should be relatively easy. On the other hand, a Raman microspectrometer should probably provide most of that information in a more unambiguous way.

## II.6.14 IR Spectroscopy

### II.6.15.1 Objectives

Thermal-IR spectroscopy in the 6-9.5  $\mu\text{m}$  region can be used to investigate specific absorptions/emissions of carbon-bearing molecules. Strong features are evident at 6.18  $\mu\text{m}$  resulting from the C=C and C=O bonds, 6.86  $\mu\text{m}$  (-CH<sub>2</sub>-, -CH<sub>3</sub> scissor),

## II.6.15 Thermal-IR Spectroscopy

7.26  $\mu\text{m}$  (C-H bend), and 7.90  $\mu\text{m}$  (O-H or C-H bend). This wavelength range could therefore provide a direct test for the presence of organics.

Although carbon-bearing molecules are most interesting from an exobiology viewpoint, a thermal-IR system can also provide significant information on salts, evaporites (e.g. phosphates, sulphates, sulphides, nitrites, chlorides) and the minerals that might be formed at hydrothermal springs. Carbonates are easily detected in thermal-IR spectra, and variations in anion composition can also be determined. Hydroxide-bearing minerals (e.g. clays) also have characteristic thermal-IR spectral features resulting from the fundamental bending modes of OH. The Al-O-H bending mode near 11  $\mu\text{m}$  in kaolinite is a good example. Clays show clear spectral variations directly related to differences in degree of hydration and leaching. Oxides, which may be important in sedimentary rocks, may be identified using their broad absorption features in the 7-8.5  $\mu\text{m}$  range. Silicates can also be detected using the broad absorption features around 10  $\mu\text{m}$  and 20  $\mu\text{m}$ . The exact wavelengths of the absorption depends upon the structure of the mineral matrix.

Thermal emission spectroscopy can also be used to determine whether geothermal or hydrothermal activity is occurring. The presence of warm regions on the surface would be extremely important in the search for extant or extinct martian biota (Walter & DesMarais, 1993). Is Mars geologically dead or is there sufficient heat produced to drive a subsurface hydrothermal circulation system? If this circulation is on a small scale, thermal emission spectroscopy may be a requirement in order to look in the optimum place for life.

#### **II.6.15.2 Instrumentation**

A thermal emission spectrometer will fly with the Athena rover on Mars Surveyor 2001. It is a miniature version of the instrument now flying on the Mars Global Surveyor mission. This device is mounted on a mast with the panchromatic camera to allow accurate boresighting, and covers a wavelength range of 5-28  $\mu\text{m}$  to look at water-deposited minerals. The highest angular resolution of the device is 7 mrad. In addition to mineralogy, the device can provide temperature profiles in the atmospheric boundary layer, and thermophysical properties of rocks and soils (see <http://astrosun.tn.cornell.edu/athena/minites.html>).

## **II.6.16 Chemical Inspection of Subsurface Material**

### **II.6.16.1 Objectives**

The chemistry of carbon and sulphur-bearing phases and particularly their isotopic composition provides redox-sensitive information about the environment as well as evidence for inorganic- and biologically-controlled processes within the geochemical cycle of sulphur. Strongly oxidising conditions prevail on the present surface. In order to obtain redox-sensitive information, in particular reduced organic or mineral phases, it is important to study subsurface material, which might be shielded against the hostile surface conditions. Depth-controlled analysis of drill-core material should reveal a redox-gradient. The presence of oxidised and reduced phases characterises environmental conditions and – as part of it – possible signs of extant or extinct biological activities.

### **II.6.16.2 Requirements**

Carbon can be present as organic matter or as carbonate minerals. Sulphur-bearing phases are likely part of a mineral matrix, a soil or a rock. Liberation for subsequent determination of chemical and isotopic properties can be performed via combustion at mineral-specific temperatures. Further details are discussed in the Team II report (Section II.5).

Before any analytical detection, it seems necessary to break/crush/pulverise the matrix in order to easily liberate carbon and/or sulphur-containing compounds. Small rock chips or, better, rock powder would be suitable material to guarantee a complete liberation/volatilisation.

The main experiments involved in the search for extinct life are:

<i>Experiment</i>	<i>Objective</i>
Panoramic camera	Survey of landing sites and target selection
Optical microscope	Detection of large ( $>3\ \mu\text{m}$ ) fossils
Atomic Force Microscope	Detection of small ( $>10\ \text{nm}$ ) fossils
Raman/IR spectroscopy	Detection of organic and sulphur-bearing compounds

## II.6.17 Summary of Possible Instrumentation

Secondary objectives associated with the investigation of martian geology and geochemistry are:

<i>Experiment</i>	<i>Objective</i>
Panoramic camera	Survey of landing sites and target selection
Optical microscope	Intermediate-resolution imaging of rock structure
Atomic Force Microscope	High-resolution imaging of target structure
Borehole imager	Investigation of layering of sediments
Mössbauer spectrometer	Investigation of chemical composition of silicates
Thermal emission/IR spectroscopy	Investigation of atmospheric gases Search for hydrothermal activity

The mass power and volume estimates are:

<i>Instrument</i>	<i>Mass (kg)</i>	<i>Power (W)</i>	<i>Volume (cm<sup>3</sup>)</i>	<i>Priority (1=high)</i>
Panoramic camera	2.5	8	1100 +mast/ e-box	1
Optical microscope	0.2	4	150	2
Colour camera	0.1	2.5	200	2
AFM/SEM	$<2.0$	5	500	3
Raman	0.7	1.1	225	4
IR/TIR spectrometer	0.2	4?	200?	6
Optical spectroscopy	4	6		10
Mössbauer	0.5	1.6	600	3

## SAMPLING REQUIREMENTS

The major problem with the investigation of biogenic material is not with the recognition of fossils at high resolution but with the chance of selecting the correct sample. If only a limited number of samples can be tested for microbial material, it is clear that some selection must be made prior to sampling. Thus the investigating experiments must either be mobile or the samples brought to a fixed analysis system. For example, a prime source of fossils should be the oldest sedimentary material available at the site. Without mobility, this cannot be investigated unless it is brought within reach of the sampling apparatus. At the Mars Pathfinder landing site, which was selected in part because it was expected to have 18% rock coverage, sand dunes were found of at least  $6\times 2\ \text{m}$  surface area. Furthermore, the rock distribution on the west side of the lander was very different from that seen on the east side. Thus, the inhomogeneity of the surface ensures that there is no guarantee of the best targets being within a few centimetres of the lander. Hence, either the high-resolution investigations must be mobile or a mobile sampling device must bring the samples

## II.6.18 Mobility Requirements

back to the lander. The former is clearly preferable if we wish to study large rocks.

Golombek et al. (1997) point out that the rock density at the Pathfinder site was similar to that predicted from Viking thermal emission spectroscopy measurements and Earth-based radar reflectance. Thus, although the rock density is inhomogeneous at small (metre) scales, it is probably homogeneous at scales comparable to or greater than the area visible to the Mars Pathfinder imager. Thus, we estimate that the landing system must have an effective mobile range of greater than 20 m from the landing site.

### **II.6.19 Grinding/ Polishing/Immersion**

A major problem that increases with higher optical magnifications is that detailed structures often are not recognisable in dirty, unprepared samples. Also, even with clean surfaces, roughness renders the microscopic inspection of the interior of minerals and mineral aggregates almost impossible. This problem may be avoided by polishing or grinding surfaces or by immersion into/covering by a liquid (which must be non-contaminating, non-freezing, non-evaporating etc). A polishing tool rather like a conventional sander is easy to envisage and should be capable of smoothing surfaces rough at the 1 mm level and smoothing them to a roughness at the 0.1  $\mu\text{m}$  (to be confirmed) level.

Immersion is obviously difficult to envisage. However, there may be some possibilities using epoxy-like substances that should not be ruled out at this stage.

### **II.6.20 Drilling and Digging**

The penetration of indurated or sedimentary soils (as ‘Scooby Doo’ at the Pathfinder landing site is inferred to be) provides important information. Investigation of the borehole may provide evidence of layering, while the sub-sedimentary layer may give us access to knowledge of the preflood surface of Mars. It does, however, require more force, particularly considering that the borehole needs to be wide enough to accommodate instrumentation for the investigation of layers.

Simple digging of trenches in the surface layer will probably not provide a substantial leap forward in our knowledge over that acquired by Viking, Pathfinder and future US Mars landers.

The advantage of boring into rock is that the depth required to reach unoxidised material is likely to be significantly less than for a loosely compacted regolith. Only centimetres may be required in order to fully expose the extent of atmosphere-rock interactions. A centimetre-sized core would then provide us with samples of a range of different oxidation states.

### **II.6.21 Sieving and Magnetic Separation**

Sieving may be useful for rejecting the fine dust hindering inspection of larger grains. This could provide a first cleaning of the sample by removing dust lightly adhering to the sample. Magnetic separation also provides an interesting alternative. The sample could be passed over a series of strong magnetics that remove magnetised dust (Hviid et al., 1997) from its surface.

### **II.6.22 Cutting and Sawing**

As the geological character of any landing site is difficult to predict with certainty, a lander must be able to cope with the sampling of a range of materials such as dust, loose and hard soil, and various small and large rocks. Not knowing the depth of oxidation, it would be risky to concentrate fully on drilling into soil without having the ability to sample the interior of small or large rocks. A hard-rock drill or a hard-rock saw are needed to perform this task. Ideally, it would be possible to saw smaller rocks picked up from the surface, as well as to drill into large, unmovable rocks. Tools must be available to sample all possible materials. The penetration of hard rocks is essential for petrographic characterisation (surfaces might be covered by ‘desert varnish’), unbiased chemical analysis (avoiding a surface layer of possibly anomalous composition) and the search for microbial fossils (both surface and subsurface type microfossils may now be in hard rocks).

Consideration of a diamond wire-saw or blade-saw should be included in the sample preparation scheme. A diamond wire-saw to cut rocks of about 1-5 cm diameter would basically consist of two wheels (5 cm and 1 cm diameters), a diamond wire about 40 cm long and a sample holder. Total weight could be held to as low as 300 g. The benefit of such an instrument would be enormous. It would allow imaging of fresh rock surfaces (essential for mineralogy and the detection of possible biogenic structures). In addition, the depth of oxidation could be determined in relatively impermeable rock, allowing extrapolation to depth of oxidation in the soil. Analyses of completely unweathered material with an alpha-proton-X-ray spectrometer would also be possible. However, it will be necessary to take account of contamination effects introduced by all sample preparation techniques and to choose materials and techniques appropriate for the subsequent observations and analyses. Thus, a diamond saw would be totally unacceptable for preparing a gas chromatograph-mass spectrometer sample intended for a ppm-level search for carbon. Alternatives such as boron nitride would have to be substituted, although trace contaminations would need to be considered.

A collection of microbial structures from Earth as a basis for comparison should be available. Such a database would be a very helpful tool in recognising possible microbial structures on Mars. This database would not only include images of fossil microbes, but of minerals the precipitation of which was initiated by microbial growth.

The recognition and identification of rocks and minerals based on the same type and quality of data that can realistically be expected from a Mars mission should be tested and exercised using data (mainly electronic images of contents unknown to interpreters) given to experienced geologists etc.

Imaged materials should include all types of geological materials with a focus on those most likely present on Mars. Samples should also include dirty, uncleaned field material. Based on such a study, the degree of confidence obtainable by Earth-based 'Mars field work' and the variations caused by different interpreters could be estimated.

## II.6.23 Data Analysis

- the investigation must have mobility to a distance of at least 20 m from the landing site;
- the preparation of surfaces and samples must guarantee access to unweathered material;
- contamination of samples during preparation must be carefully considered;
- optical microscopy (down to a resolution of around 1  $\mu\text{m}$ ) and either atomic force microscopy or scanning electron microscopy (down to a resolution of 0.01  $\mu\text{m}$ ) of the same sample are required, although fossils may be evident at many scales;
- successful microscopy will strongly depend on the samples and their preparation;
- methods of sample acquisition must be as versatile as possible, enabling deep-core sampling of sediments, core sampling of hard rocks and soil sampling;
- penetration of hard rocks is essential for petrographic characterisation, unbiased chemical analysis and searching for fossil microbes;
- a database with terrestrial microbial structures must be available for comparison;
- petrographic and biological interpretation of images must be exercised under realistic conditions;
- IR spectroscopy and thermal emission spectroscopy are interesting but, for goals focused on surface mineralogy, Raman and Mössbauer spectroscopy may be more appropriate. Optical spectroscopy is unlikely to produce useful results.

## II.6.24 Summary of Major Conclusions



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# II.7 Conclusions

Three fundamental requirements were identified as needing to be satisfied if a search for evidence of life on Mars is to be conducted satisfactorily. These are:

- i. the landing site must be selected as one of high exobiology potential. That has not been the case so far. Sites associated with sedimentary deposits, in areas relatively free from aeolian sand, would be prime targets.
- ii. sampling that site must occur in several locations and should result in samples free from the effects of the surface oxidation processes. Hence, mobility is needed, together with drilling systems that can reach well into the regolith and the surface rocks.
- iii. an integrated measurement set must be performed on those samples such as to reduce the potential for ambiguity. Hence, observational, spectroscopic and analytical techniques should be used, which together can provide a coherent description of the petrology, mineralogy and geochemistry of that site.

The preferred sites are generally located in large sedimentary terrains with low aeolian sand coverage. Hydrothermal sites may provide an alternative. Higher-resolution imaging of potential targets is needed, together with confirming mineralogical mapping. It was agreed that the choice of sites would be narrowed down to five, selected using the above criteria, among others. Those currently identified are listed in Table II.7.1/1. However, as new information becomes available these may change. The final site selection process obviously also has to take account of technical and mission constraints.

## II.7.1 Landing Sites for Exobiology

Table II.7.1/1. Landing Sites for Exobiology.

<i>Name</i>	<i>Location</i>	<i>Exobiological Potential</i>	<i>General Remarks</i>
Marca Crater	10.4°S / 158.2°W	Good: lake beds	Several craters with lacustrine deposits
Hydroates Chaos	0.02°N / 33.94°W	Good: lacustrine deposits	Complex basins rock debris
Apolinaris Patera	8.6°S / 187.5°W	Good: hydrothermal systems	Volcano + rock debris of various ages
Gusev Crater	14°S / 184.5°W	Good: lake beds	Lacustrine basin + large outflow channel
Capri Chasma	15°S / 47°W	Good: probable water-laid deposits	Chaotic terrain/ sediments

## II.7.2 The Sample Acquisition, Distribution and Preparation System

Samples must be free from the effects of the surface oxidation processes. That implies sampling from within rocks and from well below the surface. Since it cannot be precluded that the subsurface region has been subjected to churning over geological time, sequential oxidation analysis is needed.

### II.7.2.1 Subsurface Sample: Acquisition and Preparation

It was concluded that the percussive, unguided 'mole' drill is unsuited to these requirements, primarily because it is ineffective in rock materials which, it is hoped, will form part of the sedimentary regolith at the site.

Instead, subsurface sampling will be by percussive core drilling, using a core diameter <1 cm. Performing analysis of sequentially presented samples will provide the essential information on the variation of oxidation state with depth. That will be in addition to a morphological, elemental, isotopic, mineralogical and molecular analysis of the samples, as described below. Drilling and sampling is planned to a depth in the range 1-1.5 m. Owing to its size and other factors, this large drill will probably have to be located on the main Lander vehicle, although Rover-mounting would be preferred. The maximum drilling depth available will depend on the constraints imposed by the Lander configuration on the accommodation of the multiple drill bits, as well as other resource limitations and the nature of the regolith. The mounting of the drill on the Lander should, if possible, include the possibility of adjusting the position of drill entry over a limited range. Exactly how that is achieved will depend on the Lander's basic design.

Each successive core sample will need to be stored temporarily, since the long (10 h) time requirement for several analyses precludes a synchronised continuous procedure. Prior to such storage, the core sample will be inspected by the (low-) resolution colour camera system.

In preparation for the subsequent detailed analysis programme, the core sample will have to be cut, using a saw device. Three fractions would be produced: F1, F2, F3.

*Fraction F1 is used for:*

- optical and atomic force microscopy;
- Raman spectroscopy;
- APX spectroscopy;
- Mössbauer spectroscopy;
- ion probe analysis.

*Fraction F2 is subjected to grinding:*

Subsample F2.1 used for: pyrolysis/gas chromatography/mass spectrometry;

Subsample F2.2 used for: oxidant analysis.

*Fraction F3 will be stored.*

### II.7.2.2 Surface Rock Sample: Acquisition and Preparation

Drilling into rocks at the surface would also involve a sequential sampling process, but with the target penetration depth not exceeding 15 cm. Such a drill must be mounted on a Rover. The selection of the penetration point will be by close-up camera scanning areas of the rock. Again, the objective is to obtain a pristine sample of the rock by reaching below the weathered rind and the region affected by oxidation processes.

The sequential cores would first be inspected by a low-resolution colour camera. Selected subsamples would be transferred to the sample distribution system located in the Lander, for preparation for analysis in the instruments, as outlined above.

An alternative approach to rock sample acquisition was also considered. This involved simply the collection of small (cm-sized) surface 'rocks' by the Rover and their subsequent sectioning at the Lander, preparatory to microscopy and analysis. This avoids the complications of the Rover-based drilling operations, although at the cost of some biasing in the sampling process.

**Table II.7.3/1. Instrument Characteristics for an Exobiology Observation System.**

<i>Instrument</i>	<i>Performance</i>	<i>Exobiology Objectives</i>	<i>Other Objectives</i>	<i>Mass (kg)</i>	<i>Power (W)</i>	<i>Data (Mbit/d)</i>	<i>Vol (m<sup>3</sup>)</i>
Panoramic Stereo Camera	1000×1000px 0.3 mrad/px 14 filters	Study rock types/ gross bio-features. Rover targets	Landing-site survey. Direct the Rover	2.5	8	8	1100 +mast/ electronics
Low-Resolution Microscope	0.1mm/px 5 filters	Examine samples prior to high-resolution microscopy	Check samples prior to chemical analysis or storage	0.2	2.5		200
Optical Microscope	≤3 μm resolution. <20 μm depth of field	Detection of large fossil structures	Intermediate-resolution mineralogical studies	0.3	4	3	300
Atomic Force Microscope	1 nm Field 1×1 μm to 50×50 μm	Imaging of pre-selected fossils	High-resolution mineralogy	1.5	5	1	500

### II.7.2.3 Soil Samples

These will require a scoop system and subsequent sieving to provide samples for microscopy (on a metal tape) and chemical analysis.

Observational methods selected involve a macroscopic system, low- and high-resolution optical microscopes, and an Atomic Force Microscope to reach the nm-resolution level. On Earth, the earliest prokaryotic microbial ecosystems have left two main categories of morphological fossil evidence.

At the macroscopic level are the laminated biosedimentary structures (stromatolites). These microbiolite structures have a record extending back some 3.5 Gyr. If Mars had a comparable early period of abundant water, then it is possible that macroscopic microbiolite structures may be associated with the oldest of the martian sedimentary structures. Hence, degraded macroscopic structures of similar origin might be observed in sedimentary surface rocks and scarps by a panoramic camera with a resolution of about 1 mm. The camera will also be extensively used in a geological and mineralogical context.

The second main category of morphological fossil evidence left on Earth derives from the cellular relics of individual microorganisms. These microfossils, with a record also extending back to about 3.5 Gyr, are to be found ranging in size from <5 μm for some individual bacteria through to about 1 mm for filamentous cyanobacteria. Consequently, by analogy, low- and high-power optical microscopes will be required, together with an Atomic Force Microscope to reach the sub-μm resolution level.

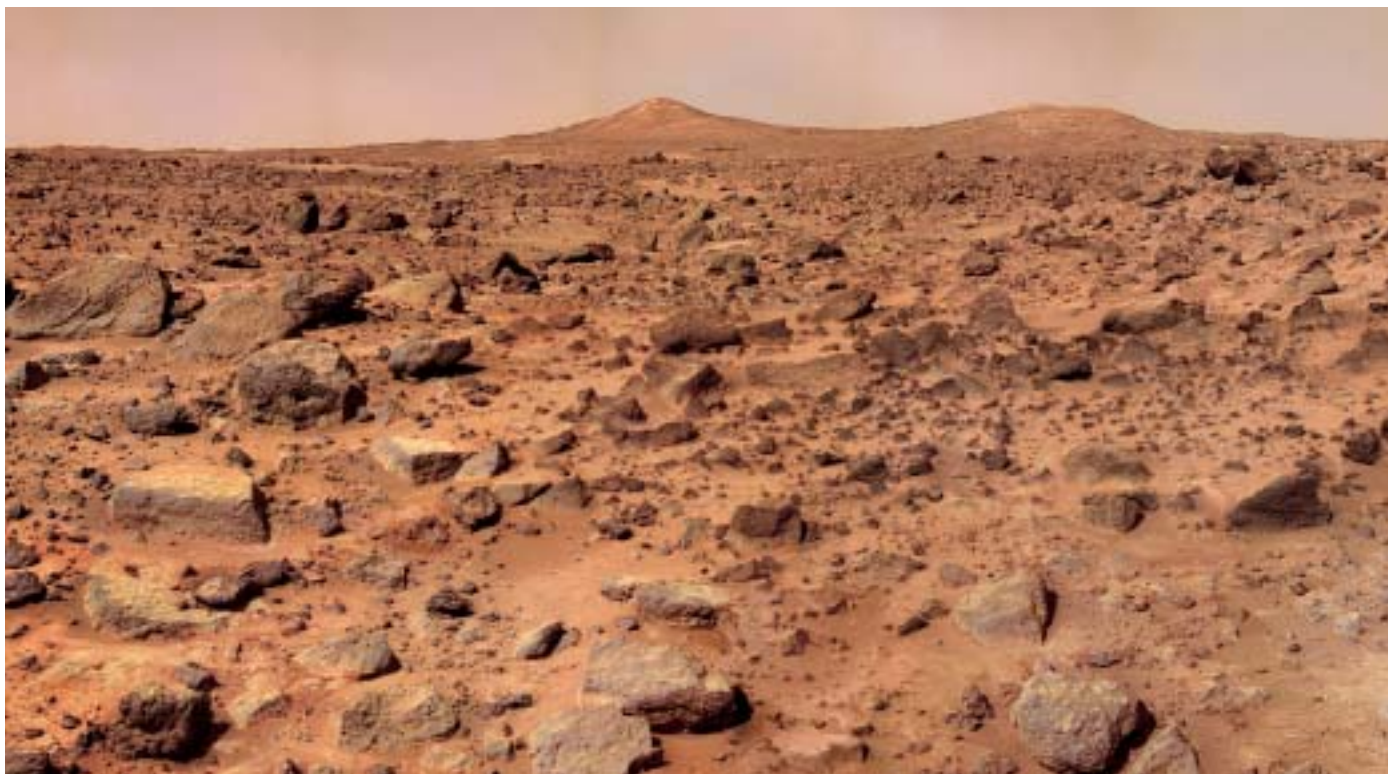
Table II.7.3/1 summarises the primary and secondary science objectives of these instruments and their basic characteristics.

The analytical methods selected include radiation and particle spectroscopic techniques, together with mass-discrimination instruments. Each of these may be applied to the samples derived from surface rocks and from the subsurface drilling. The resulting mineralogical and geochemical analyses, together with the petrological studies using the observational instruments, provide the essential basic information on the general planetological setting of the site, the local environment and on any traces of past or present biological activity.

## II.7.3 The Exobiology Observation System

### II.7.4 The Exobiology Analysis System





**Fig. II.7.4/1. Looking towards 'Twin Peaks' at the Mars Pathfinder landing site. (NASA)**

Mineralogical characterisation of the site samples comprises the following goals:

- mineralogy, texture and bulk chemistry of primary rocks;
- mineralogy and sedimentology of the soil, and wind/water-deposited sediments, regolith, etc (including grain size/shapes secondary minerals such as clays, carbonates, zeolites, hydrates, chlorites, etc);
- mineralogy of mobile phases and hard ground cements (halogenides, sulphates, nitrates, silica, carbonates, iron oxyhydrates, etc);
- search for biomarkers (framboidal sulphides or oxides, bio-phosphates, oxalates, silica, biogenic magnetite, barite, and the fossil structures, as discussed earlier).

Geochemical analysis of these same samples will establish their elemental bulk composition. Major, minor and trace element abundances will help to define the geological history of the site and an analysis of the oxidation state of certain elements (e.g. Fe, Mn, S, N) will provide information on the redox conditions there and their historical development. A knowledge of the relative abundances of the biologically significant elements C, H, N, O, S and P and their distributions between organic and inorganic matter is of particular interest. The abundance of nitrogen and its oxidation state in the martian soil will be of significance in determining what happened to the initial atmospheric nitrogen.

Isotope ratios provide a very valuable set of chemical biomarkers. Most notable is the C depletion in favour of  $^{12}\text{C}$  in photosynthesis. Similarly, substantial isotopic fractionation in favour of hydrogen against deuterium is found to occur through the activity of methanogenic microorganisms on Earth. In addition, a determination of  $^{15}\text{N}/^{14}\text{N}$  can provide important information on possible biological activity, as can the  $^{34}\text{S}/^{32}\text{S}$  isotopic composition change between sulphides and sulphates.

The determination of minerals such as phosphates, manganese oxides and certain carbonates that may result from biological processes are important analysis objectives, as is determining the nitrate and sulphate content. So too is the quantitative

**Table II.7.4/1. Recommended Instruments for Searching for Organics on Mars.**

<i>Instrument</i>	<i>Analysis</i>	<i>Mass (kg)</i>	<i>Power (W)</i>	<i>Volume (m<sup>3</sup>)</i>	<i>Data</i>
Alpha/Proton/ X-ray Spectrometer	Elemental analysis for all except H and He	0.5	0.4 (1.3 peak)	300	16 kbit/ sample
Mössbauer Spectrometer	Fe-bearing minerals Fe-oxidation state and ratios	0.5	1.6	600	1.2 Mbit/ sample
Laser Raman Spectrometer 200-3500 cm <sup>-1</sup> & 8 cm <sup>-1</sup> resolution	Molecular analysis of organics and minerals	1.5	1.1 (2.5 peak)	250	to be determined
Infrared Spectrometer 0.8-10 µm. Spectral resolution 100. Spatial resolution 200 µm	Molecular analysis of minerals and organics	1.0	3.5	800	to be determined
Pyrolytic Gas Chromatograph and Mass Spectrometer	Analyse inorganic/organic compounds; isotopic ratio and chirality determination	4.0	8 (15 peak)		2.5 Mbit max 0.6 Mbit min

determination of water, particularly as a function of depth. The depth profile of the abundance of oxidants in the regolith is another obvious requirement.

As yet, no organics have been found on Mars and their discovery and analysis would be of prime importance for exobiology. It will be necessary, however, to differentiate carefully between organics of an abiotic origin, especially those of meteoritic origin.

On Earth, the primary biopolymers undergo a complex process of degradation and condensation following the death of the organism. The result is a complex and chemically stable macromolecular material (kerogen). In addition, certain stable lipid-rich biopolymers survive this degradation process, to contribute directly to the constitution of the kerogen. Sediments may also contain other stable organic compounds that have survived, at least in part, the processes of alteration. Many can be classified as biomarkers on the basis of their structures and/or carbon isotopic compositions. Such biomarkers and kerogens of recognisable biological origin have been found in sediments on Earth dating back at least 0.5 Gyr. It is therefore conceivable that such materials might be detected on Mars, given appropriate sites.

Taking into account factors such as survivability, especially in an oxidising environment, has led to the following recommended priority order in searching for organics:

1. volatile low molecular weight compounds, including hydrocarbons (especially methane), alkanolic acids and peroxy acids;
2. medium molecular weight compounds, including hydrocarbons (straight- and branched-chain, isoprenoids, terpenoids, steroids and aromatics);
3. macromolecular components, which would be kerogen-like components, oligo- and polypeptides.

Table II.7.4/1 lists the analysis instruments that have been identified as suitable, in total, to cover the range of determinations, elemental, isotopic, and molecular, outlined above.

**II.7.5 A Possible  
Exobiology Experiment  
Package and Operating  
Arrangement**

The previously described equipment could be distributed between a large Lander and a Rover on the following basis:

<b>ROVER (option 1)</b>	
– robotic positioning arm	2.0 kg
– a rock surface grinder	0.4 kg
– low-power microscope	0.2 kg
– APX spectrometer	0.5 kg
– a small rock-coring drill	3.5 kg
– core sample containers	0.5 kg
<i>Total mass:</i>	<i>7.1 kg</i>

- ROVER (option 2)**
- small robotic arm equipped to collect cm-sized surface rocks, place in transfer containers, and subsequently pass to the Lander. (2 kg);
  - close-up colour camera (0.1 kg);
  - sample containers for the collected small rock samples (0.5 kg).

<b>LANDER</b>	
– subsurface drill system	6.5 kg
– sample handling/distribution	4.0 kg
– sample sectioning	0.3 kg
– sample grinding	0.4 kg
– low-resolution microscope	0.2 kg
– optical microscope	0.3 kg
– Atomic Force Microscope	1.5 kg
– microscopy transfer stage	1.0 kg
– Alpha/Proton/X-ray Spectrometer	0.5 kg
– Mössbauer Spectrometer	0.5 kg
– Laser Raman Spectrometer	1.5 kg
– IR Microspectrometer	1.0 kg
– Pyrolytic, Gas Chromatograph, Mass Spectrometer	5.5 kg
– Oxidant detector	0.4 kg
– Laser Ablation ICP mass spectrometer	2.5 kg
<i>Total mass:</i>	<i>26.1 kg</i>

In this configuration, the Lander is the centre for subsurface sampling and for the *in situ* sample preparation and analysis processes. The Rover then provides a selection of rock samples from nearby locations either as small core samples or as small (cm-size) rocks, for sawing in the Lander. In the latter case, the Rover may be of small dimensions and be equipped with a camera and close-up imaging system.

# **Annex I**

## **Team IV: A Manned Mars Station and Exobiology Research**

*Team IV was asked to examine the ways in which a manned station on Mars, operating over an extended period, might assist in further developing exobiology research on that planet a decade or so from now. It was also asked to consider whether such a station would pose significant risks to that research.*

The Team did not consider the question of whether the benefits from manned intervention in exobiology research on Mars constituted a reason to argue for a manned mission. On the contrary, it was assumed that the reasons for such a mission would be based upon a variety of considerations, most of which would not directly relate to exobiology. Hence, the discussions were narrowly focused upon the question ‘What could a manned presence do to aid exobiology research?’

Until there is a sustainable manned presence on Mars, all exobiology research will have to be done by *in situ* measurements using robotic systems, together with sample return missions. Consequently, human intervention is likely to be welcomed, just as it is on Earth in a research environment, to do those things that cannot readily be done by machines. These primarily involve the exercise of judgement, based upon extensive professional and personal experience, coupled with flexibility and ingenuity to adapt and to improvise.

To benefit fully from exobiology research and its associated fields of mineralogy and geochemistry, it is therefore important that some of the Mars crew have professional training in these fields. Without that background expertise, the value of their presence will be substantially diminished, just as it would be in a terrestrial laboratory.

The extent of their stay on Mars and, in particular, the time they will have for scientific activities, as distinct from life-support and base-systems maintenance activities, are very important factors in judging their potential value to science. If humans are to play a useful scientific role on Mars then it is essential they have the time, the means and the professional training to do so. Science support should not come as some late afterthought.

The Team considered a number of ways in general where human intervention could be beneficial. More difficult was the question of specific activities, since exobiology research on Mars is only two missions old. Organics have yet to be discovered and fossils are a distant dream. Consequently, the Team could extrapolate only a short distance. Nonetheless, there was a consensus that trained professionals could be of considerable value in the following types of activities on Mars:

- site identification by local analysis of areas identified from orbital surveys. This may be achieved by long-range rovers under the control of Mars-located specialists or, for shorter journeys, actually driven by specialists;
- sample acquisition at those sites would be aided by manned intervention. This would be especially valuable when deep (>1.5 m) subsurface drilling and core sampling is undertaken;
- the actual sampling process, in a geological field prospecting scenario, is greatly improved if undertaken by a qualified person, rather than by a remotely-controlled robot;
- there is inevitably an element of serendipity in even the most carefully planned search for interesting samples. If the search is performed by trained personnel, then benefits can accrue owing to the exercise of judgement and ‘intuition’;
- if a laboratory can be established on Mars, then manned supervision of preliminary sample analyses becomes a valuable facility that can significantly speed up the



(NASA/Pat Rawlings)



search rate. It is assumed that very detailed analyses, using large instruments, would still be performed on Earth, using carefully pre-selected returned samples;

- the search for water/ice will no doubt be a prime task for the establishment of a manned base on Mars. The results of such searches will be important also for exobiology research. The participation of trained exobiologists/geologists in that activity will be beneficial;
- if microbial life is discovered at sites on Mars, then human supervision of a protected culturing and bio-analysis facility will be essential. The return of Mars microbes to the Earth should be rigorously avoided;
- a manned base would be involved in processing very large quantities of the martian atmosphere. In so doing, it may be possible to carry out accurate long-term studies of the trace components and their variations. For gases such as methane, their detection and monitoring can have important implications in exobiology studies.

The Team pointed out that, in selecting the location of a human base on Mars, consideration needs to be given to the distance to interesting exobiology sites and the possible range of manned Rovers. Frequent access is likely to be needed to a diverse range of locations, so the base needs to be sited, if possible, near a region of exobiology interest.

The risks involved in a manned presence relate to the risk to the crew from Mars microbes (if any), the risk to life on Earth via returned Mars microbes (accidentally or deliberately) and the risk to Mars owing to imported microbes from Earth.

The Team concentrated upon the risk to Mars from imported microbes; in other words, the risk to exobiology research on Mars. The other issues were of much wider implication and are dealt with under agreed international protocols.

Since humans contain vast numbers of bacteria and other microbes, it is inevitable that Mars will become contaminated as soon as humans arrive on the surface. The surface of Mars is, as far as we know, inhospitable to microbial life. Therefore much of what is imported and reaches the Mars surface environment will die. But some may not. These may find sheltered ecological niches that allow them to survive, propagate and, eventually, mutate.

Whether dead or alive, the imported microbes will progressively contaminate the Mars environment, viewed from an exobiology viewpoint. Consequently, the Team stressed the vital importance of preceding the manned mission to Mars by a substantial series of robotic missions to carry out the essential exploratory search for life by *in situ* measurements at sites selected by high-resolution mineralogical and spectroscopic orbital surveys.