The Quarantine and Certification of Martian Samples

Committee on Planetary and Lunar Exploration, National Research Council

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Space Studies Board
Division on Engineering and Physical Sciences
National Research Council

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Cover: Rock and soil samples collected on the surface of Mars begin their journey back to Earth in this artist's impression. Courtesy of NASA/JPL.

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Preface

One of the highest-priority activities in the planetary sciences identified in published reports of the Space Studies Board's Committee on Planetary and Lunar Exploration (COMPLEX) and in reports of other advisory groups is the collection and return of extraterrestrial samples to Earth for study in terrestrial laboratories. In response to recommendations made in such studies, NASA has initiated a vigorous program that will, within the next decade, collect samples from a variety of solar system environments. In particular the Mars Exploration Program is expected to launch spacecraft that are designed to collect samples of martian soil, rocks, and atmosphere and return them to Earth, perhaps as early as 2015.

International treaty obligations mandate that NASA conduct such a program in a manner that avoids the cross-contamination of both Earth and Mars. The Space Studies Board's 1997 report *Mars Sample Return: Issues and Recommendations* examined many of the planetary-protection issues concerning the back contamination of Earth and concluded that, although the probability that martian samples will contain dangerous biota is small, it is not zero.[1] Steps must be taken to protect Earth against the remote possibility of contamination by life forms that may have evolved on Mars. Similarly, the samples, collected at great expense, must be protected against contamination by terrestrial biota and other matter. Almost certainly, meeting these requirements will entail opening the sample-return container in an appropriate facility on Earth—presumably a BSL-4 laboratory—where testing, biosafety certification, and quarantine of the samples will be carried out before aliquots are released to the scientific community for study in existing laboratory facilities. (BSL-4 is the most stringent level of biological containment defined; see Chapter 6.)

The 1997 report contains useful, but brief, observations about the nature and staffing of a "sample-receiving, containment, and research facility." The report concludes that "no facility meeting all the requirements currently exists" (p. 31). Although NASA's Ames Research Center and Johnson Spaceflight Center have staff with some of the requisite experience in biology, geochemistry, and sample curation, they lack experience in, and do not have facilities to maintain, high-level biological containment. The report notes that other organizations, such as the U.S. Army Medical Research Institute of Infectious Diseases and the Centers for Disease Control and Prevention, have expertise in biological containment, but they lack expertise in "the biology of nonpathogenic microbes,

[1]Space Studies Board, National Research Council. 1997. Mars Sample Return: Issues and Recommendations. National Academy Press, Washington, D.C.

microbial paleontology, and the relevant aspects of geology and geochemistry" (p. 31). Moreover, these containment facilities are not designed to protect against terrestrial contamination.

Given the high priority attached to Mars sample return missions by NASA and the scientific community, and the 1997 report's recommendation that the sample-receiving facility should be operational at least 2 years before the sample return mission is launched, further concrete planning is urgently needed. This planning should build on the recommendations contained in the 1997 report and the preliminary efforts undertaken by NASA (in particular, the 1997 Quarantine Protocol Workshop[2] and the report of the Mars Sample Handling and Requirements Panel[3]).

The nature of the required quarantine facility, and the decisions required for disposition of samples once they are in it, were regarded as issues of sufficient importance and complexity to warrant a study by the Committee on Planetary and Lunar Exploration (COMPLEX) in isolation from other topics. (Previous studies have been much broader, including also consideration of the mission that collects samples on Mars and brings them to Earth, atmospheric entry, sample recovery, and transport to the quarantine facility.) The charge to COMPLEX stated that the central question to be addressed in this study is the following:

- What are the criteria that must be satisfied before martian samples can be released from a quarantine facility?

In addressing this central question, a number of closely related issues were to be considered:

- What are the optimal techniques for isolating and handling martian materials, determining their content of biota (if any), and carrying out basic geochemical characterization studies in the facility?
- How much capability for scientific analysis, beyond that required for biosafety certification, should be incorporated into the facility, and what principles should govern the utilization of this scientific capability?
- To what extent can valuable lessons be learned from the experience with the Apollo lunar samples and from recent developments in the biotechnology and biomedical communities?

This project was formally initiated at COMPLEX's March 31 to April 2, 1999, meeting in Boulder, Colorado, with a series of presentations describing the scientific and technical aspects of Mars sample-return missions, planetary protection issues, and the work of NASA's Mars Sample Handling and Requirements Panel. Work continued at the July 26-28, 1999, meeting in Washington, D.C., with a series of presentations relating to biological containment facilities, the physical and biological effects of gamma-ray sterilization, and personal perspectives on quarantine and curation issues. Data gathering relating to this study concluded at the November 1-5, 1999, meeting in Irvine, California, with a series of presentations related to biomarkers and life-detection techniques.

A full draft of this report was completed in February 2000. The text was reviewed by the Space Studies Board in March 2000, sent to external reviewers in October 2000, and revised during the period from December 2000 to March 2001. An unedited prepublication text was released on May 29, 2001. That text is superseded by this final published report.

Although many COMPLEX members worked on this report, the bulk of the task of assembling their many individual contributions was performed by John Wood with the assistance of William Boynton, John Hayes, Peter Jahrling, William Schopf, and Everett Shock. Important contributions were also made by Anna-Louise Reysenbach and by Space Studies Board members Gary Olsen and Mitchell Sogin.

The work of the writing team was made easier thanks to the efforts of Carl Agee (Johnson Space Center), Carlton Allen (Lockheed Martin), Jeffrey Bada (University of California, San Diego), John Battista (Louisiana State University), Donald Bogard (Johnson Space Center), Michael Carr (U.S. Geological Survey), Andrew Chess

[2]NASA. 1999. Mars Sample Quarantine Protocol Workshop Report, D.L. DeVincenzi, J. Bagby, M. Race, and J. Rummel, eds. NASA/CP-1999-208772.

[3]NASA and the Jet Propulsion Laboratory. 1999. Mars Sample Handling and Requirements Panel (MSHARP) Final Report. NASA/TM-1999-209145.

(Whitehead Institute and Massachusetts Institute of Technology), John Cronin (Arizona State University), Michael Daly (Uniformed Services University of the Health Sciences), Donald DeVincenzi (Ames Research Center), Martin Favero (Johnson and Johnson), Steven Fontaine (MDS Nordion), Everett Gibson, Jr. (Johnson Space Center), Bill Green (Space Studies Board), John Kerridge (University of California, San Diego), Joseph Macquaker (Manchester University, U.K.), Philippe Masson (University of Paris Sud), Michael Moldowan (Stanford University), Kenneth Nealson (Jet Propulsion Laboratory), Dimitri Papanastassiou (California Institute of Technology and Jet Propulsion Laboratory), C.J. Peters (National Center for Infectious Diseases), Margaret Race (SETI Institute), Jonathan Richmond (Centers for Disease Control and Prevention), John D. Rummel (NASA Headquarters), Bernd Simoneit (Oregon State University), Steven Squyres (Cornell University), and Robert Walker (Washington University).

This report has been reviewed by individuals chosen for their diverse perspectives and technical expertise, in accordance with procedures approved by the National Research Council's Report Review Committee. The purpose of this independent review is to provide candid and critical comments that will assist the institution in making the published report as sound as possible and to ensure that the report meets institutional standards for objectivity, evidence, and responsiveness to the study charge. The contents of the review comments and draft manuscripts remain confidential to protect the integrity of the deliberative process. COMPLEX thanks the following individuals for their review of this report: John Bagby (Colorado State University, retired), Michael H. Carr (U.S. Geological Survey), Benton C. Clark (Lockheed Martin Astronautics), George W. Clark (Massachusetts Institute of Technology), Jack Farmer (Arizona State University), Edward L. Korwek (Hogan & Hartson, LLP), and James Henry Scott (Carnegie Institution of Washington).

Although the reviewers listed above have provided many constructive comments and suggestions, they were not asked to endorse the conclusions or recommendations, nor did they see the final draft of the report before its release. The review of this report was overseen by Wesley T. Huntress, Jr., Carnegie Institution of Washington. Appointed by the National Research Council, he was responsible for making certain that an independent examination of this report was carried out in accordance with institutional procedures and that all review comments were carefully considered. Responsibility for the final content of this report rests solely with the authoring committee and the institution.

Contents

Executive Summary

Samples of the planet Mars are expected to be collected by robotic spacecraft and returned to Earth for scientific study early in this century, perhaps as soon as 2015. There is a possibility, although it is acknowledged to be remote, that the samples will contain specimens of microorganisms that have lived on Mars, perhaps even in a viable state. The samples must be collected and handled in a way that will protect the terrestrial environment from contamination by these hypothetical organisms and also protect the samples from contamination by terrestrial organisms and other contaminants.

An essential element of the plan to handle martian samples responsibly is a quarantine facility, into which the samples will be received as soon as they arrive on Earth. Such a facility will perform the dual role of protecting the terrestrial environment and safeguarding the scientific integrity of the returned samples. In other words, it must combine the functions of a biological containment laboratory and a clean room.

Initial examination of the samples, including testing for potential biohazards, will be carried out in this facility, and the samples will be held there until conditions are met that permit release of aliquots[1] of the samples to the laboratories of investigators elsewhere in the United States and abroad who are qualified to carry out specialized studies of them, and who have been formally approved to do so.

COMPLEX has studied the time required to plan, build, and staff an adequate quarantine facility. The time needed is surprisingly long, 7 years (see Table 6.3 in Chapter 6). If Mars sample return and quarantine are to be taken seriously, this need must be addressed. It dictates the most important recommendation of this report:

> ***Recommendation.*** It is imperative that planning and construction of the Mars Quarantine Facility be begun at least 7 years in advance of the anticipated return of Mars samples. This responsibility cannot be deferred without compromising the quarantine and study of the Mars samples. [Chapter 6]

OPERATIONS IN THE QUARANTINE FACILITY

COMPLEX considers that only the most basic operations should be conducted in the quarantine facility: unpacking, preliminary examination, baseline characterization, weighing, photography, splitting, repackaging,

[1] An aliquot is a representative subsample of some entity.

and storage. Another important operation will be the preparation of heat- and/or radiation-treated samples for distribution to the scientific community. In addition, certain life-detection studies that cannot be made on sterilized[2] samples—such as testing for biohazards—will have to be carried out in the quarantine facility. To try to bring other scientific studies with bulky, complex instrumentation into the containment facility, along with the personnel who conduct the studies, would unacceptably increase the complexity, cost, and potential for failure of the facility.

Recommendation

- The Mars Quarantine Facility should be designed to the smallest and simplest possible scale consistent with its role as a biological containment and clean room facility. No scientific investigations should be carried out in the quarantine facility that can be executed on sterilized samples outside the facility. [Chapter 6]
- Protocols should be developed that specify in detail the steps and procedures to be followed for handling Mars samples in the quarantine facility. Necessary protocols include those for inventorying and preliminary analyses of the samples, searching for evidence of biological activity, testing for biohazards, and preparing sterilized aliquots of the samples for distribution to the scientific community. [Chapter 6; see also Chapter 4.]
- Because it cannot be carried out on sterilized samples, biohazard assessment should be performed in the quarantine facility prior to any release of samples from the facility. Elements of these studies might include culturing experiments; attempts to infect animals, plants, and cell cultures; and genome detection via the polymerase chain reaction or similar techniques. [Chapter 6; see also Chapter 4.]

In addition to studying the optimal properties of a quarantine facility, COMPLEX has also considered life-detection techniques to be employed within (and outside) it, and the means of sterilizing samples within the facility so they can be removed from it. One important approach to life detection involves the extraction of key organic compounds (*biomarkers*, diagnostic of life processes) from samples for analysis at specialized laboratories outside the quarantine facility. Removal of these extracts from the facility will be contingent upon demonstration that the effects of the extraction process would be more than adequate to kill any known terrestrial organism.

Recommendation. It is important that a program of research be conducted to determine the efficacy of supercritical fluids and commonly used organic solvents in killing organisms. It is highly desirable to be able to remove solvent extracts from quarantine without the damage to dissolved biomarker compounds that would be caused by heat or ionizing radiation. Sterilization probably is systematically achieved by the supercritical fluids used in making extracts, but this needs to be verified before extracts can be removed from the Mars Quarantine Facility. [Chapter 4]

Much of the program of life detection will depend on studies of organic compounds in samples that were sterilized so they could be removed from the quarantine facility. Unfortunately, COMPLEX's recommended techniques for sterilization—treatment by heat and or gamma radiation to such a level as to kill any known terrestrial organism—damage organic compounds to some extent. The vulnerability of organic compounds to heat and gamma-ray treatment is only imperfectly known. It is important that studies be carried out to enlarge knowledge in this area.

Recommendation. A program of research should be initiated to determine the effects on organic compounds in rocky matrices, and also on microscopic morphological evidence of life, of varying degrees of application of heat and gamma irradiation. This research should be started well in advance of the return of the Mars samples, so that treatment protocols can be designed intelligently and data obtained from analyses of treated samples can be interpreted with minimal ambiguity. [Chapter 5]

[2]Throughout this report, COMPLEX uses the words "sterilized" and "sterilization" as being synonymous with treatment by heat and/or gamma radiation to such a level as to kill any known terrestrial organism.

The nature of the quarantine facility, which must satisfy dual and partly conflicting requirements, is an important topic of study.

Recommendation. A major obstacle to design of the Mars Quarantine Facility is the problem of combining biological containment with clean-room conditions. It is essential that work on the solution of this problem be started immediately, to include mockups of containment/clean-room combinations whose efficacy can be tested, so that the design of a quarantine facility can proceed. [Chapter 6]

COMPLEX considers that affiliation of the quarantine facility with an ongoing containment facility (e.g., the U.S. Army Medical Research Institute of Infectious Diseases, in Ft. Detrick, Maryland; the Centers for Disease Control and Prevention, in Atlanta, Georgia; or the Medical Branch of the University of Texas at Galveston, where a BSL-4 facility is being constructed) is preferable to independent construction, for several reasons. These include:

1. *Institutional support*. A collaborative agreement with a host institution would mean that the Mars Quarantine Facility could draw on that institution for personnel, training, experience, security, and specialized utilities.
2. *Economy*. Sharing the resources named under 1 above should effect a large economy in operation of the Mars Quarantine Facility.
3. *Environmental impact*. Clearing an environmental impact statement for a BSL-4 facility can take years. Ideally, the Mars Quarantine Facility would operate under the environmental impact statement of its host institution.

Recommendation. The Mars Quarantine Facility should be affiliated with an ongoing containment facility that has BSL-4 capability and should be physically part of it or proximate to it, but control of the Mars Quarantine Facility should be under the jurisdiction of NASA. [Chapter 6]

Several initiatives cited above should be begun *prior to* design of the quarantine facility and planning of quarantine protocols, i.e., immediately: research on the efficacy of supercritical fluids and commonly used organic solvents in killing organisms; on the effects of varying degrees of treatment by heat and by gamma irradiation on organic compounds; and on ways of combining biological containment with clean-room conditions. An oversight committee should be formed to monitor these activities (see below, "The Apollo Experience").

It is possible that the Mars sample return program will be an international venture, with other nations playing an important role in flight operations. The role of international partners in a sample return program should be carefully defined. The potentially sizeable contribution of another nation to the Mars program raises questions of how the earliest access to and ultimate curation of the samples will be shared. It is beyond the scope of COMPLEX's charge to comment on the ultimate curation of the samples, but the committee believes strongly that, for practical reasons, their preliminary examination, baseline description, cataloguing, and packaging should be carried out at a single quarantine facility in the United States.

Recommendation. All samples in the initial collection returned from Mars should be placed in a quarantine facility in the United States, at least until the preliminary examination of the samples has been completed. Management and operation of the Mars Quarantine Facility should be shared between the United States and major international partners that participated in the collection of martian samples. [Chapter 6]

RELEASE OF SAMPLES FROM THE QUARANTINE FACILITY

COMPLEX considered the possible results of initial searches for evidence of life[3] in the martian samples, especially analyses of the samples for total organic carbon. The committee recommends the following:

[3]In this report the word "life," when used in the context of martian life, should always be understood to mean "life as we know it," to allow for the possibility of life forms distinctly outside our terrestrial experience.

Recommendation

• If the samples returned from Mars contain evidence of life, or if evidence of life is equivocal (e.g., organic matter is present), aliquots that have been treated by the application of heat and/or gamma radiation to levels more than adequate to kill any known terrestrial organism (Chapter 5) should be certified for release from the Mars Quarantine Facility. [Chapter 4]

• If the samples contain evidence of life, or if evidence of life is equivocal, removal of untreated aliquots from the Mars Quarantine Facility for transfer to approved containment laboratories elsewhere should not be excluded, on the condition that containers and transfer procedures conform to protocols established by a panel of experts (e.g., from the Centers for Disease Control and Prevention) in containment.

Here "approved containment facilities elsewhere" refers principally to the case where a major international partner in the Mars sample return program wishes to establish an independent BSL-4 facility in which to study untreated samples (see Chapter 6). [Chapter 4]

• If the samples are shown to be altogether barren of organic matter, to contain no detectable organic carbon compounds and no other evidence of past or present biological activity, untreated aliquots of the samples should be released for study beyond the confines of the Mars Quarantine Facility. [Chapter 4]

The possibility that the martian samples will contain unequivocal evidence of life is very remote, and for this reason COMPLEX's response is based on the far more likely contingency that evidence of life will be equivocal or absent altogether. Unequivocal evidence of life would dictate a very elaborate plan of handling, curation, and study, which COMPLEX has not attempted to develop.

Recommendation. If unmistakable evidence of life as we know it is found in the Mars samples, they should be dedicated to biological studies. Studies of the biosignatures in them should be minimal until an optimal study plan has been developed and an appropriate research facility set up and staffed. In the interim, no aliquots of the samples should be released from the confines of the Mars Quarantine Facility unless warranted by ongoing biological studies, and the samples are sterilized. [Chapter 4]

Historically (e.g., with Apollo and the Viking Landers) there has been a degree of competition between biological and physical scientists for access to planetary research materials, and COMPLEX anticipates that this competition may be particularly intense where the martian samples are concerned. The above recommendation concedes that discovery of life in the samples would be of such supreme importance that the wishes of physical scientists should be subordinated to biological studies of the samples if this happens.

However, the discovery of life in the martian samples is unlikely, and in the far more probable case that only equivocal evidence of life is found, COMPLEX recommends that (sterilized) aliquots of the samples be made available to both biological and physical scientists for study. (Potential life-detection studies are not wholly compromised by sterilization.) COMPLEX is concerned that distribution of these samples not wait on resolution of uncertainties in the evidence, which can take a very long time (years) to occur. Scientists who have prepared their laboratories and staffs to study the samples should be allowed to begin work on them, and the results of their studies will provide important feedback for the planning of later Mars missions. Moreover, the public would find it difficult to understand why the study of samples from a much-publicized mission would be deferred for a period of years.

Recommendation. In the likely event that initial examination of the Mars samples can neither prove nor definitively rule out evidence of life in them, plans should be in place to promptly sterilize aliquots of the samples and remove them from the Mars Quarantine Facility for biological and geochemical studies in specialized laboratories elsewhere. This action should not be deferred pending resolution of the question of whether the samples contain life or artifacts of life. [Chapter 4]

THE APOLLO EXPERIENCE

COMPLEX reviewed details of the Lunar Receiving Laboratory, a quarantine facility constructed and used during the Apollo program, and attempted to profit from its triumphs and failures. Lessons learned in the review (Chapter 7; see also Appendix B) led COMPLEX to make these recommendations:

Recommendation. It is essential that the design for the Mars Quarantine Facility be kept as simple as possible, consistent with the facility's mission of protecting Earth's environment and the samples. Although it may be feasible to store the samples at low temperatures, an effort to try to maintain a Mars environment (temperature, pressure) during sample handling would complicate the design and operation of the facility to a very large degree, probably unnecessarily, and it should not be attempted for the first Mars sample return. [Chapter 7]

Recommendation. A continuing committee of senior biologists and geochemists that includes appropriate international representation should be formed and charged with reviewing every step of the planning, construction, and employment of the Mars Quarantine Facility. The committee should be formed during the earliest stages of planning for a Mars sample-return mission. Members of the committee should also participate in the design of the spacecraft and those portions of the mission profile where biological contamination is a threat. [Chapter 6]

1

Introduction

A basic goal of NASA's Mars Exploration Program, begun in 1996, is to collect and return to Earth carefully chosen samples of Mars surface material. The interest in Mars and Mars sample return is driven in large part by the quest for evidence of life on that planet, either extant life or life in past ages, although sample return will also answer other important questions about the climatic, geologic, and geochemical history of Mars. Sample return is essential to the pursuit of these questions because sample research in terrestrial laboratories is a vastly more sensitive and informative scientific tool than remote analysis on the martian surface by even the most sophisticated robotic instruments.

The phrase "follow the water" describes a unifying theme that has been adopted for the present stage of Mars research, derived from the recognition that water is an essential ingredient of life on Earth and probably on Mars. It has long been known, from orbital photographs of river channels on the surface of Mars, that liquid water was once present on the martian surface, although it is not now (the vapor pressure of water in the martian atmosphere is far too low to permit the stable coexistence of liquid water).[1-3] Most recently, the surprising discovery has been made of small gullies on Mars that appear to be very recent, eroded by a liquid that issued from remarkably shallow depths (e.g., Figure 1.1).[4] When samples are collected on Mars for return to Earth, a high priority will be given to sampling materials from these previously aqueous environments to maximize the chances of the materials including relicts of martian life.[5] These materials can be expected to include loose soil, rock fragments, and possibly rock cores, including cores of sedimentary rocks (Figure 1.2) containing information on a microscopic scale that can be accessed only in terrestrial laboratories.

The amount of solid sample material expected to be returned by a mission to Mars will be small, probably less than 500 g. The possibility that life exists, or has existed, on Mars greatly complicates the collection and study of samples because it dictates avoidance of the biological contamination of Mars and Earth by one another, and this

[1]Sagan, C., Toon, O.B., and Gierasch, P.J. 1973. Climatic change on Mars. Science 181: 1045-1049.
[2]Carr, M.H. 1987. Water on Mars. Nature 326: 30-35.
[3]Head, J.W. III, Hiesinger, H., Ivanov, M.A., Kreslavsky, M.A., Pratt, S., and Thompson, B.J. 1999. Possible ancient oceans on Mars: Evidence from Mars Orbiter laser altimeter data. Science 286: 2134-2137.
[4]Malin, M.C., and Edgett, K.S. 2000. Evidence for recent groundwater seepage and surface runoff on Mars. Science 288: 2330-2335.
[5]Mars Program Architecture: Recommendations of the NASA Astrobiology Institute, January 10, 2000.

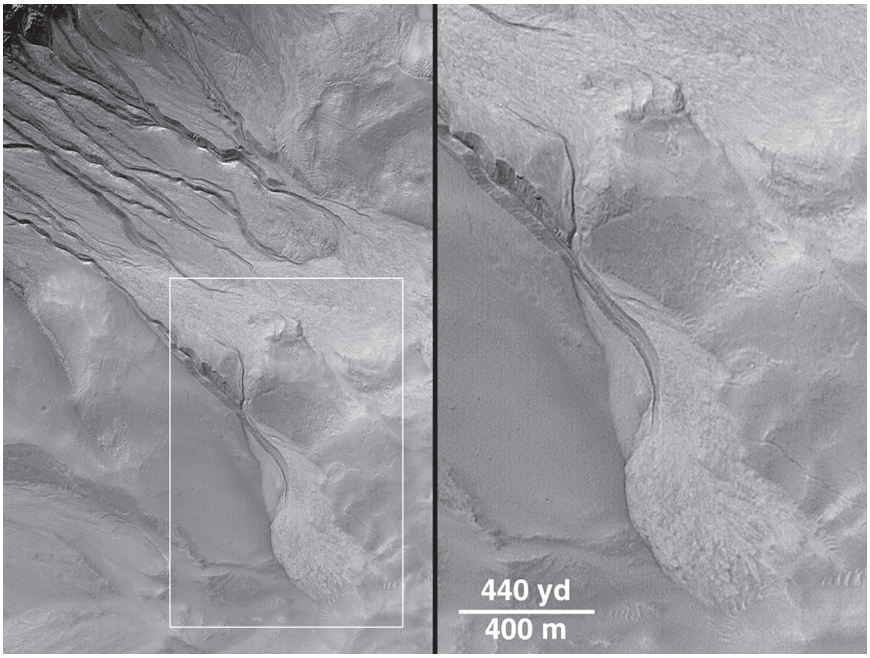

FIGURE 1.1 Drainage gullies and apron deposits on the north wall of a 12-km crater on Mars, apparently eroded by water that has seeped from the crater wall, as photographed by the Mars Orbiter Camera system on the Mars Global Surveyor spacecraft. The crater is near 37.4°S latitude, 168.0°W longitude. The sharpness of the channels and the absence of an overlay of small craters suggest that the drainage has been very recent. The right-hand image is an enlargement of the area outlined in the left-hand image. SOURCE: MGS MOC Release No. MOC2-241, June 22, 2000, courtesy of NASA/Jet Propulsion Laboratory/ Malin Space Science Systems.

is difficult to ensure. Mars must be protected from contamination by Earth organisms (*forward contamination*) to preserve its indigenous martian life forms, if there are any, for future study, and also because terrestrial contaminants accidentally brought back from Mars in the Mars samples would be detected in terrestrial laboratories and might be mistaken for Mars life. Earth must be protected against *back contamination* by hypothetical Mars organisms because of the possibility, acknowledged to be extremely remote,[6,7] that if they exist, they might be harmful to terrestrial life or the terrestrial environment.

Avoidance of possible back contamination requires that the Mars samples be placed in a quarantine facility when they arrive on Earth, and that they not be released from this facility until it has been determined (*certified*) that they are intrinsically devoid of extraterrestrial life or have been made safe for distribution (e.g., by sterilization). It also dictates design of a return spacecraft and a mission profile that isolates the samples from the terrestrial environment from the time the spacecraft enters the atmosphere until the sample canister is opened in the quarantine facility.

A sample-return mission (Mars Surveyor 2003) had been scheduled to return martian material to Earth in

[6]Space Studies Board, National Research Council. 1997. Mars Sample Return: Issues and Recommendations. National Academy Press, Washington, D.C.

[7]NASA and the Jet Propulsion Laboratory. 1999. Mars Sample Handling and Requirements Panel (MSHARP) Final Report. NASA/TM-1999-209145.

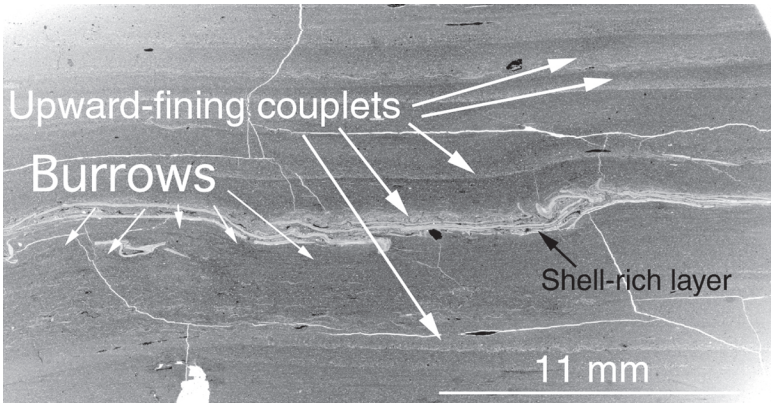

FIGURE 1.2 A thin section* of a terrestrial sedimentary rock, cut perpendicular to the plane in which sedimentation deposited layers of mud and silt. The rock is a sample of the Kildonnan Member Mudstone from the middle Jurassic period in Scotland. Each upward-fining couplet in the section records a particular episode on Earth—a storm—in which a batch of muddy water entered the volume where this rock originated and the mud settled, as coarser grains while the flow was fast and finer grains as it slowed. One depositional episode left a thin layer of shell fragments, and it can be seen that during quiescent periods bottom-dwelling organisms burrowed into the sediment surface. SOURCE: Photograph courtesy of J. Macquaker, Manchester University, United Kingdom.

*Thin sections are routinely prepared for the microscopic study of rocky material. The sections, ~30 μm thick and supported on glass slides, are made by a process of sawing, grinding, and polishing. At this thickness most minerals are relatively transparent, and the section can be illuminated by transmitted light (as the above section is).

2008, but failures of the Mars Climate Orbiter and Mars Polar Lander missions in 1999 dictated a reassessment of the Mars Exploration Program that inevitably will result in delaying the date for sample return to Earth until 2015 or later. This may seem to put sample return comfortably far in the future, from a planning perspective, but the present report concludes that the order of a decade of time will be needed to adequately plan and prepare a quarantine facility to receive the samples.

The nature of the required quarantine facility and of quarantine procedures has been considered by the National Research Council's (NRC's) Task Group on Issues in Sample Return[8] and several other groups in the last 5 years.[9,10] During 1999, the Committee on Planetary and Lunar Exploration (COMPLEX), a standing committee of the Space Studies Board of the NRC, carried out the present study focusing on the problems connected with quarantine of the Mars samples.

Although the NRC's earlier study of Mars sample return dealt only briefly with the quarantine issue,[11] it made a series of broad-based recommendations on the topic, several of which are reproduced in Box 1.1. COMPLEX endorses these recommendations, and the present study is built upon them.

[8]Space Studies Board, National Research Council. 1997. Mars Sample Return: Issues and Recommendations. National Academy Press, Washington, D.C.

[9]NASA and the Jet Propulsion Laboratory. 1999. Mars Sample Handling and Requirements Panel (MSHARP) Final Report. NASA/TM-1999-209145.

[10]NASA. 1999. Mars Sample Quarantine Protocol Workshop Report, D.L. DeVincenzi, J. Bagby, M. Race, and J. Rummel, eds. NASA/CP-1999-208772.

[11]Space Studies Board, National Research Council. 1997. Mars Sample Return: Issues and Recommendations. National Academy Press, Washington, D.C.

Box 1.1
Earlier Recommendations on the Quarantine of Mars Samples

- Samples returned from Mars by spacecraft should be contained and treated as though potentially hazardous until proven otherwise. No uncontained martian materials, including spacecraft surfaces that have been exposed to the martian environment, should be returned to Earth unless sterilized.
- Controlled distribution of unsterilized materials returned from Mars should occur only if rigorous analyses determine that the materials do not contain a biological hazard. If any portion of the sample is removed from containment prior to completion of these analyses, it should first be sterilized.
- The planetary protection measures adopted for the first Mars sample-return missions should not be relaxed for subsequent missions without thorough scientific review and concurrence by an appropriate independent body.
- A research facility for receiving, containing, and processing returned samples should be established as soon as possible once serious planning for a Mars sample-return mission has begun. At a minimum the facility should be operational at least two years prior to launch. The facility should be staffed by a multidisciplinary team of scientists responsible for the development and validation of procedures for detection, preliminary characterization, and containment of organisms (living, dead, or fossil) in returned samples and for sample sterilization. An advisory panel of scientists should be constituted with oversight responsibilities for the facility.
- A panel of experts, including representatives of relevant governmental and scientific bodies, should be established as soon as possible once serious planning for a Mars sample-return mission has begun, to coordinate regulatory responsibilities and to advise NASA on the implementation of planetary protection measures for sample-return missions. The panel should be in place at least one year prior to the establishment of the sample-receiving facility (at least three years prior to launch).
- An administrative structure should be established within NASA to verify and certify adherence to planetary protection requirements at each critical stage of a sample-return mission, including launch, reentry, and sample distribution.
- Throughout any sample-return program, the public should be openly informed of plans, activities, results, and associated issues.

SOURCE: Space Studies Board, National Research Council. 1997. Mars Sample Return: Issues and Recommendations. National Academy Press, Washington D.C., pp. 3-6.

2

Detection of Potential Biohazards

Central to the topic of quarantining samples and certifying them for release from quarantine is the problem of detecting life in them, that is, determining if life is present or has recently been present. The problem is compounded by the possibility of life forms that function and reproduce in a manner outside the range of our experience with terrestrial life. Detection of evidence of past life in returned martian samples is discussed in Chapter 3; discovery of fossilized life in the samples would profoundly influence the search for extant life.

BASIC ASSUMPTIONS AND MEASUREMENTS

A search for evidence of life, assumed to be carbon-based and microbial,[1] can take advantage of many recent advances in molecular biology and organic geochemistry. Analytical and investigative techniques used in these areas of research are advancing rapidly, and many new methods are likely to be in place by the time samples are returned from Mars. A series of tests can be envisioned that will provide evidence of viable or recently dead organisms, detect chemical fossils or probable biological molecules (biomarkers), and, at the same time, quantify contamination by terrestrial microbes and organic compounds. To avoid misinterpretation of "false positives," it will be essential to detect and identify terrestrial contaminants, both microbial and organic, on the outbound spacecraft (particularly on surfaces that may come into contact with the returned samples) as well as in all areas within the quarantine facility in which returned samples are exposed to the environment (where the nature and amount of contamination should be carefully monitored during sample processing). However, although these techniques are effective for identifying terrestrial life or its products, signals arising from martian organisms or their products might differ substantially from those of their terrestrial counterparts.

Measurement of the total concentration of organic carbon[2] in each martian subsample will provide an invaluable baseline. Samples in which organic carbon is below 10^{-12} gC/g are unlikely to contain microorganisms. Samples containing detectable amounts of organic carbon will be worthy of intensive—and cautious—study.

[1]Pace, N.R. 2002. The nature of biochemistry in the universe. In Space Studies Board, National Research Council, Signs of Life: A Report Based on the April 2000 Workshop on Life Detection Techniques, National Academy Press, Washington, D.C. (in press).

[2]"Organic carbon" is carbon that is commonly bound to hydrogen, typically as molecules of some complexity. Terrestrial organisms consist mostly of organic molecules. However, "organic" does not necessarily mean "formed by life processes." Organic compounds can be created by nonbiological as well as biological processes.

Some analytical techniques can utilize samples as small as 100 micrograms and have carbon-detection limits of about 10 nanograms (roughly equivalent to 3 million bacterial cells of minimum size).[3] With more specific information about the material in the samples, lower detection limits are possible.[4] Improvements can be confidently foreseen.[5,6] Plausibly, the amount of sample required might be reduced to 10 micrograms while preserving sensitivity at the parts-per-million level. The resulting ability to detect 10^{-11} gC would probably represent the ultimate attainable sensitivity for organic carbon. Thus purely chemical tests could not equal the sensitivity of an optimally targeted bioassay, which might have the capability of detecting only a few viable cells containing in total 10^{-13} gC or less. However, the chemical analysis is general. It does not require provision of the specific nutrients and conditions a given organism might require for growth. Moreover, its efficacy would be enhanced because it would detect not only viable cells but also organic substrates being utilized by cells or debris resulting from the accumulation of dead cells. In spite of its limitations, analysis for organic carbon is probably the best available technique for screening and surveying samples.

DETECTING LIFE

The attempt to detect actual life, extant or recent, is clearly different from a search for the chemical traces left by life or for identifiable fossils (the topic of Chapter 3). Whether by molecular biological techniques or by cultivation in the laboratory, detection of terrestrial microorganisms is more difficult than is commonly appreciated. For example, the polymerase chain reaction (PCR—see the section "Detection by Analysis of Chemical and Isotopic Structures" below), a powerful molecular biological technique, is capable of detecting minute amounts of genetic material but, because of its extreme sensitivity, is also easily subject to error-causing contamination. Moreover, although positive results from techniques such as PCR sometimes provide evidence that biological activity may have occurred in a sample, cultivation is still the only means of demonstrating viability unequivocally. Even so, cultivation unfortunately is not a comprehensive solution to the problem of detection, because many terrestrial organisms that are clearly "alive" have never been successfully cultivated in the laboratory.[7,8]

Further, although it can be argued that existing technology (and likely future developments) will allow the detection of organic compounds and their identification even if they should be novel compounds unique to Mars, existing life detection methods are not so easily adapted to finding Mars life if the latter employs novel and unique biomolecules. Nevertheless, the principles of genetic and metabolic analysis of geological and environmental samples will be extremely useful in efforts to identify and quantify the magnitude and sources of any terrestrial contamination in martian samples, and they may serve for detecting Mars life if it is sufficiently similar to life on Earth.

Potential Biohazards: Organisms, Viruses, Viroids, Prions

Before discussing techniques for life detection, it is important to explain why researchers will pay essentially all their attention to organisms, largely ignoring viruses, viroids, prions, or other possible biohazards. As the following paragraphs make clear, only organisms are sufficiently self-reliant to proliferate in a completely alien world: The other agents require host organisms and also have extremely specific requirements with respect to their hosts.

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[3]Space Studies Board, National Research Council. 1999. Size Limits of Very Small Microorganisms: Proceedings of a Workshop. National Academy Press, Washington, D.C.

[4]Personal communication, Mitchell Sogin, Marine Biological Laboratory, October 2001.

[5]Wong, W.W., Clarke, L.L., Johnson, G.A., Llaurador, M., and Klein, P.D. 1992. Comparison of two elemental-analyzer gas-isotope-ratio mass spectrometer systems in the simultaneous measurement of 13C/12C ratios and carbon content in organic samples. Anal. Chem. 64: 354-358.

[6]Perks, H.M., and Keeling, R.F. 1998. A 400 kyr record of combustion oxygen demand in the western equatorial Pacific: Evidence for a precessionally forced climate response. Paleoceanography 13: 63-69.

[7]Hugenholtz, P., and Pace, N.R. 1996. Identifying microbial diversity in the natural environment: A molecular phylogenetic approach. Trends Biotechnol. 14: 190-197.

[8]Head, I.M., Saunders, J.R., and Pickup, R.W. 1998. Microbial evolution, diversity, and ecology: A decade of ribosomal RNA analysis of uncultivated microorganisms. Microb. Ecol. 35: 1-21.

As used here, the term *organisms* refers to all forms of cellular life, including bacteria, archaea, eukaryotic microorganisms, plants, and animals. All organisms possess their own genetic material, deoxyribonucleic acid (DNA). In addition, all organisms possess (1) machinery for duplicating the DNA, the process called DNA replication; (2) machinery for expressing genes by copying parts of the DNA into a related molecule called ribonucleic acid (RNA), the process called transcription; and (3) machinery for reading some RNA molecules as instructions for the synthesis of specific proteins, the process called translation. On Earth, all organisms perform these processes, and, in the case of transcription and translation, the machineries are so highly conserved that they are commonly used to detect and identify organisms. Parts of the translational machinery, the small subunit ribosomal RNA (SSU rRNA), or its gene (frequently called the rDNA), are particularly easily detected in all characterized organisms and have been examined for more than 30,000 different cases.[9]

Some organisms can synthesize everything they need from a relative handful of inorganic compounds (e.g., water, carbon dioxide, ammonia, sulfate, and some trace minerals).[10] Plants and cyanobacteria are examples of such primary producers (autotrophs). Other organisms (heterotrophs) cannot make new organic matter and so must consume organic compounds. The nutritional requirements of these organisms can range from the very simple (e.g., only one organic compound needed) to the very complex (perhaps 100 different compounds needed).[11] Such fastidious organisms are very specialized, and each type is usually restricted to a very few specialized environments.

Because most biologically important compounds are available only when produced by organisms, inevitably the organisms with the most complex nutritional requirements require an association with a host organism or community of organisms than can supply their needs. Some are pathogenic or predatory, while others are symbiotic or scavengers. If appropriate hosts (alive or dead) are not available, such organisms will eventually die.

By contrast, an organism that is nutritionally more flexible can often be found in a variety of different environments. These organisms can pose at least two threats. First, some can act as opportunistic pathogens, taking advantage of the nutritional richness of a host organism when it is available and thereby endangering the host, but also being capable of living independent of a host as well. Second, some as alien organisms might irreversibly damage, or even take over, an ecosystem, as has occurred when species of plants or animals have accidentally or intentionally been released into new environments. Although many die out naturally, others overwhelm the new ecosystem in which they lack natural enemies, or might otherwise destroy a system that is not adapted to their effects.

Viruses are obligate parasites, deriving from their host organisms not just important chemical compounds but also protein-synthesizing machinery. Although viruses possess their own genetic material (DNA or RNA), they always rely on their host for its translational machinery, and sometimes also for its genome-replicating and genome-transcribing apparatus. Thus, no matter what raw molecules are provided to a virus, it cannot, in the absence of an appropriate host organism, make any new protein and hence cannot make more viruses. This absolute need for co-opting cellular machinery leads to substantial specialization of viruses in terms of the hosts that they can successfully infect. Thus, although viruses can be very successful among and destructive of the terrestrial organisms with which they have coevolved, it is extremely unlikely that viruses for any truly novel form of life—one that is not based in the same genetic materials and exactly the same relationships between gene sequence and amino acid sequence in proteins (the translation table that we call the genetic code)—would be able to replicate on Earth.[12] This means that we need not speculate about a particularly resistant strain of virus that defies the biological principles of terrestrial life.

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[9]Woese, C.R. 1987. Bacterial evolution. Microbiol. Rev. 51: 221-271; available online at <http://www.cme.msu.edu/RDP/html/index.html>. See also Maidak, B.L., Cole, J.R., Lilburn, T.G., Parker, C.T., Jr., Saxman, P.R., Stredwick, J.M., Garrity, G.M., Li, B., Olsen, G.J., Pramanik, S., Schmidt, T.M., and Tiedje, J.M. 2000. The RDP (Ribosomal Database Project) continues. Nucleic Acids Res. 28: 173-174; available online at <http://www.cme.msu.edu/RDP/html/index.html>.

[10]Brock, T.D., and Madigan, M.T. 1991. Biology of Microorganisms, 6th Edition. Prentice-Hall, Englewood Cliffs, N.J., pp. 121-127.

[11]Brock and Madigan, 1991; see footnote 10 above.

[12]Space Studies Board, National Research Council. 1998. Evaluating the Biological Potential in Samples Returned from Planetary Satellites and Small Solar System Bodies. National Academy Press, Washington, D.C.

Viroids are in many respects akin to viruses, but they are simpler: They are nothing but naked genetic material (RNA), relying entirely on host machinery for replication, transcription, and translation.[13] Viroids have been found only in plants, and any given type of viroid has a very limited range of hosts. Also, because viroids do not possess any molecules or structures to facilitate their entry into a host, they depend on wounding of a plant, for example by an insect, to gain entry. All the considerations that limit speculation about exotic viruses apply also to viroids. With respect to inactivation, the most significant differences between viruses and viroids are that viroids as naked RNA are more susceptible to chemical degradation but, because they have less genetic material, viroids also present a smaller target for inactivation by gamma irradiation.

Prions are infectious proteins.[14] They have no genetic material. Instead they reflect an inappropriate folding of a normal cellular protein, or part of one. In essence, a prion causes the misfolding of a normal protein, making that protein behave as a prion, creating a chain reaction of misfolding. Because a prion has no genetic material, the host organism must already produce a protein that can be directed into the incorrect configuration by the prion. In all known cases, the range of organisms that prions can infect is limited, because corresponding proteins must exist in each host and must be sufficiently conserved in structure to allow for both normal folding and prion-induced folding. Transmission of prions between individuals appears to be very limited, with the exception of extreme cases such as those in which diseased brains have been added to animal feed, or extracts of diseased brains from one animal have been injected into the cranium of another animal. Overall, although the possibility cannot be excluded that a molecule of extraterrestrial origin could act as a prion, leading to the pathological refolding of a terrestrial protein, there is no reason to consider such a possibility as more likely than a random terrestrial molecule acting as a prion. These two factors, low contagiousness and the low likelihood that an alien molecule would specifically interact with a terrestrial protein, make prions of little concern.

In summary, two categories of biological threat could be posed by samples returned from Mars. The first is that the samples might contain biological materials similar to those found on Earth. In this case, all four types of infectious agents—organisms, viruses, viroids, and prions—would be of concern, but then the means of sterilizing the samples are also well understood. The second is that the samples might possibly contain a life form significantly different from terrestrial life. In this case, returned viruses and viroids could not replicate in terrestrial organisms, because such agents would rely on a gene expression system different from that of organisms on Earth. Alien organisms might, however, find a niche in which they could proliferate. At the present state of technology, it would be difficult or impossible to fully evaluate the potential impacts of alien life forms on complex terrestrial ecosystems. Researchers anticipate technological advances, in particular advances associated with the monitoring of complex gene expression patterns, that may provide tools for detecting environmental perturbations. However, the necessary first step toward evaluation of how possible alien life forms might affect complex terrestrial ecosystems or human health will be the ability to detect the presence of microbial life in samples returned to Earth.

There are two additional scientifically pragmatic reasons for focusing life detection efforts on organisms rather than viral parasites or infectious proteins. First, because they constitute the vast bulk of the biomass in terrestrial ecosystems (since all of the other agents must be produced by organisms), organisms or their traces might be easier to detect than other agents. Second, demonstrating that one has found an alien virus, viroid, or prion without a known host organism on which to grow it is an essentially futile exercise.

Detection by Cultivation

The traditional method for detecting viable organisms is cultivation. If growth on an otherwise sterile medium is observed, the result is definitive. No known abiological inoculum will produce increasing numbers of cells (a result that can be traced back to Pasteur's refutation of spontaneous generation). Verification of the occurrence of growth, not chemical precipitation, is frequently carried out initially by optical microscopy, and it can be confirmed by electron microscopic examination.[15] Growing samples can be repeatedly diluted and regrown (although

[13]Diener, T.O. 1999. Viroids and the nature of viroid diseases. Arch. Virol. Suppl. 15: 203-220.
[14]Pergami, P., Poloni, T.E., Corato, M., Camisa, B., and Ceroni, M. 1999. Prions and prion diseases. Funct. Neurol. 14: 241-252.
[15]Brock, T.D., and Madigan, M.T. 1991. Biology of Microorganisms, 6th Edition. Prentice-Hall, Englewood Cliffs, N.J., pp. 45-46.

there are caveats). Despite published reports of "growth" that was almost certainly chemical rather than biological,[16] such results are extremely rare and are generally negated by the use of appropriate experimental controls.[17] In addition to uninoculated cultures, inocula subjected to sterilizing treatments can be used as controls in experiments designed to check for positive growth.[18] By far the most significant source of false positive indications of growth is contaminated cultures. Contamination is a substantial concern for all aspects of sample analysis, but contamination with viable organisms presents the added complication that the organisms can replicate and their significance thereby increase.[19]

The more significant problem with the use of culturing for life detection is that of false negatives, leading to failure to detect the existence of viable organisms that defy cultivation.[20] In spite of great advances in available methods, several lines of evidence indicate that the majority of organisms on Earth (whether measured by cell count, cell mass, or genetic diversity) have not been successfully cultivated in the laboratory. In the worst case, there is a complete lack of recordable growth; less extreme is the case in which organisms must be incubated for months before there is an observable increase in cell density. Studies of some organisms in their natural environment suggest that slow growth is intrinsic to the organism, and not just a result of failure to optimize the culture conditions.[21,22]

Thus cultivation-based studies will not provide high confidence that a sample is devoid of viable organisms. Nevertheless, the intrinsic sensitivity of the method for organisms that do grow successfully, and the historical role of cultivation in detecting contamination (by screening for common, easy-to-grow terrestrial organisms), mean that culture-based studies are important and cannot be bypassed.

Efforts to test the martian samples by cultivation will be limited primarily by logistics. Attempts at cultivation must be performed with unsterilized samples and hence must be carried out within the quarantine facility. Although a variety of growth conditions can be tested without specialized equipment, testing becomes more difficult when the samples require special environmental conditions (temperature, pressure, unusual atmospheric composition, and so on). Cultivation can provide very sensitive assays for terrestrial contamination and conceivably for some forms of alien life but cannot prove the absence of extraterrestrial life. The possibility of false negatives suggests that this approach should be limited to a circumscribed set of carefully chosen growth conditions.

Detection by Microscopic Examination

In surveys of terrestrial samples, some of the commonest methods of screening for cells are direct microscopic examination, epifluorescence microscopy, and electron microscopy.[23] In direct microscopy, many types of large or complex cells are quite evident, but small simple cells cannot be unambiguously identified. If putative cells are organized spatially in a "microcolony," there is increased reason to believe that they are of biological origin. If samples are stained with a DNA-binding fluorescent dye, cells become easier to see. However, a test that relies on extraterrestrial life being DNA-based is less robust than one that tests merely for cellular organization. For assessing cellular structure (as opposed to abiotic structures), electron microscopy provides additional resolution and hence more information. Although having a capability for basic optical microscopy within the quarantine facility is important, electron microscopy and most other detailed examinations should be carried out on sterilized samples outside the facility (Chapter 6)—assuming, of course, that the sterilization procedure used does not

[16]Baross, J.A., and Deming, J.W. 1983. Growth of "black smoker" bacteria at temperatures of at least 250° C. Nature 303: 423-426.
[17]Trent, J.D., Chastain, R.A., and Yavanos, A.A. 1984. Possible artefactual basis for apparent bacterial growth at 250° C. Nature 307: 737-740.
[18]Levin, G.V., and Straat, P.A. 1976. Labeled release—An experiment in radiorespirometry. Origins Life 7: 293-311.
[19]Monod, J. 1949. The growth of bacterial cultures. Ann. Rev. Microbiol. 3: 371-394.
[20]Pace, N.R. 1997. A molecular view of microbial diversity and the biosphere. Science 276: 734-740.
[21]Strous, M., Heijnen, J.J., Kuenen, J.G., and Jetten, M.S.M. 1998. The sequencing batch reactor as a powerful tool for the study of slowly growing anaerobic ammonium-oxidizing microorganisms. Appl. Microbiol. Biotechnol. 50: 589-596.
[22]Strous, M., Fuerst, J.A., Kramer, E.H.M., Logemann, S., Muyzer, G., van de Pas-Schoonen, K.T., Webb, R., Kuenen, J.G., and Jetten, M.S.M. 1999. Missing lithotroph identified as new planctomycete. Nature 400: 446-449.
[23]Brock, T.D., and Madigan, M.T. 1991. Biology of Microorganisms, 6th Edition. Prentice-Hall, Englewood Cliffs, N.J.

destroy morphological evidence of life. How various methods of sterilization affect microscopic morphological evidence of life needs to be established.

Viability cannot be assessed by the methods mentioned above. Most staining procedures and methods of preparing samples for electron microscopy kill any cells that were alive. Although there are some indirect tests of viability (or, more correctly, metabolic activity) based on dye exclusion, or dye uptake,[24] none are generally applicable to diverse (let alone extraterrestrial) samples, and interpretation of the results may be confused by staining of abiological particles.

Detection by Analysis of Chemical and Isotopic Structures

Some of the most general and robust methods for detecting extant or recent life are based on detecting the presence of specific molecules, or chirality[25] or isotopic anomalies in molecules. In all of these cases, viability, or even cellular structure, is not required. These criteria are also discussed below in the context of fossil evidence of long-dead organisms.

Molecules whose presence can be taken as evidence of life are proteins, DNA, RNA, straight-chain fatty acids, and a variety of other lipids. One common and sensitive method for detection of terrestrial life (viable or recently dead) is the use of the polymerase chain reaction to amplify fragments of DNA encoding ribosomal RNA (rRNA). The process is described in Box 2.1 and is sketched in Figure 2.1.1. Ribosomal RNA is a common target of PCR because it is universal among terrestrial organisms, and there is an extensive database of the diverse sequences that have been obtained.[26] Although, as noted above, it is not realistic to depend on DNA and rRNA genes being present in extraterrestrial life, their presence does provide a sensitive test of terrestrial contamination (even more sensitive than growth, in that it is not limited to organisms that researchers know how to cultivate).

The analysis of relatively large or complex molecules (whole genes) is motivated by attempts to avoid false positives caused by abiotic organic molecules. However, this approach is limited because it is modeled on the detection of molecules produced by terrestrial organisms. Alternatively, even without prior knowledge of the specific molecules involved, evidence for the biological origin of organic compounds can be based on the chiral enrichment of the molecules present. Although it is possible for abiotic processes to create anomalies, naturally occurring departures from random chirality in demonstrably abiotic samples are rare at best. If there are gross enantiomeric excesses, then net chirality can be measured with little preconceived notion of the identities of the molecules present. However, prior knowledge of the molecules to be screened can help optimize the sensitivity of the assays.

Stable isotopes are almost inevitably fractionated by biological systems. The compounds affected include the cellular constituents and the products of the cellular energy-producing reactions. Bulk samples can be analyzed for average composition, or an ion microprobe can probe localized features (resulting in greater sensitivity and much lower throughput, or speed of analysis, since many such features must be analyzed). The test is not definitive, however (see Box 3.2 on carbon isotopes in Chapter 3).

Terrestrial life also has an elemental composition that is far from equilibrated with its environment. The degree of disequilibrium can be assessed either by probing the bulk composition of a sample, or by using tomographic techniques to produce a spatially resolved map of abundances, with a much decreased analytical throughput.

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[24]Mason, D.J., Lopez-Amoros, R., Allman, R., Stark, J.M., and Lloyd, D. 1995. The ability of membrane potential dyes and calcafluor white to distinguish between viable and non-viable bacteria. J. Appl. Bacteriol. 78: 309-315.

[25]*Chirality* refers to the fact that asymmetric molecules can occur as right- and left-handed variants. Biological processes tend to favor one variant or the other, while abiotic processes produce them in equal numbers.

[26]Maidak, B.L., Cole, J.R., Lilburn, T.G., Parker, C.T., Jr., Saxman, P.R., Stredwick, J.M., Garrity, G.M., Li, B., Olsen, G.J., Pramanik, S., Schmidt, T.M., and Tiedje, J.M. 2000. The RDP (Ribosomal Database Project) continues. Nucleic Acids Res. 28: 173-174; available online at <http://www.cme.msu.edu/RDP/html/index.html>.

Box 2.1
The Polymerase Chain Reaction

Before a cell can divide, it must make a precise copy of all of its genes, so that each daughter cell inherits all of the original genes. Called DNA replication (DNA is the material of which genes are made), this process is initiated by an enzyme called DNA polymerase. The polymerase chain reaction (PCR) is a technique for amplifying (making many copies of) a defined portion of a DNA molecule by using the same biochemical reactions that cells use to copy their genes before they divide.[1] With DNA polymerase as the catalyst, it is possible to convert one copy of a DNA molecule into two copies identical to the first. If this process is repeated, the two copies become four copies. A third cycle results in eight copies, and the pattern can be rapidly continued until there are billions of copies of the original, making the DNA relatively easy to detect in the laboratory. The process is called a chain reaction because the product of each reaction is more molecules that can serve as the substrates (templates) from which more reactions can occur.

How PCR works is outlined below and diagrammed in Figure 2.1.1.

1. Genes are composed of double-stranded DNA, which has two distinct complementary strands. The strands have a directionality, and the two strands have opposite orientations. Each strand can be thought of as a mold for producing the other, which is why they are called complementary.
2. Before double-stranded DNA can be replicated, the two strands must be separated from one another, often by heating.
3. Given a single strand of DNA, DNA polymerase catalyzes the synthesis of a new complementary strand, so that the product is a double-stranded DNA identical to the original gene.
4. However, DNA polymerase cannot by itself synthesize the entire second strand; it must start at a "primer," a short single strand of DNA corresponding to the beginning of the "missing" strand. By supplying two specific primers for the reaction (one for each of the two strands) it is possible to define the precise part of the DNA that will be replicated.
5. The process can be repeated many times to produce million- or billion-fold amplification of the desired DNA region (*e-g* of Figure 2.1.1). However, heating the double-stranded DNA molecules for the next cycle of replication (Figure 2.1.1*e*) can destroy the DNA polymerase. The discovery of heat-stable DNA polymerases has permitted the process to be repeated without adding any new components.

[1]Mullis, K.B., and Faloona, F. 1987. Specific analysis of DNA in vitro via a polymerase-catalysed chain reaction. Methods Enzymol. 155: 335-350.

Relative Sensitivity and Throughput of Detection Techniques

The sensitivities of the various techniques outlined above are not discussed in detail here. To facilitate comparison, Table 2.1 indicates some of the properties of the techniques, characterizing them qualitatively or in terms of broad ranges, since it is frequently possible to improve one parameter at the expense of another.

Analyses based on cultivation and observation of growth can take advantage of amplification to detect as little as a single viable organism (of the right type) in a sample. In addition, this method can screen a sample of virtually any mass without an increase in the complexity of or time required for the test, so it has a high throughput. The

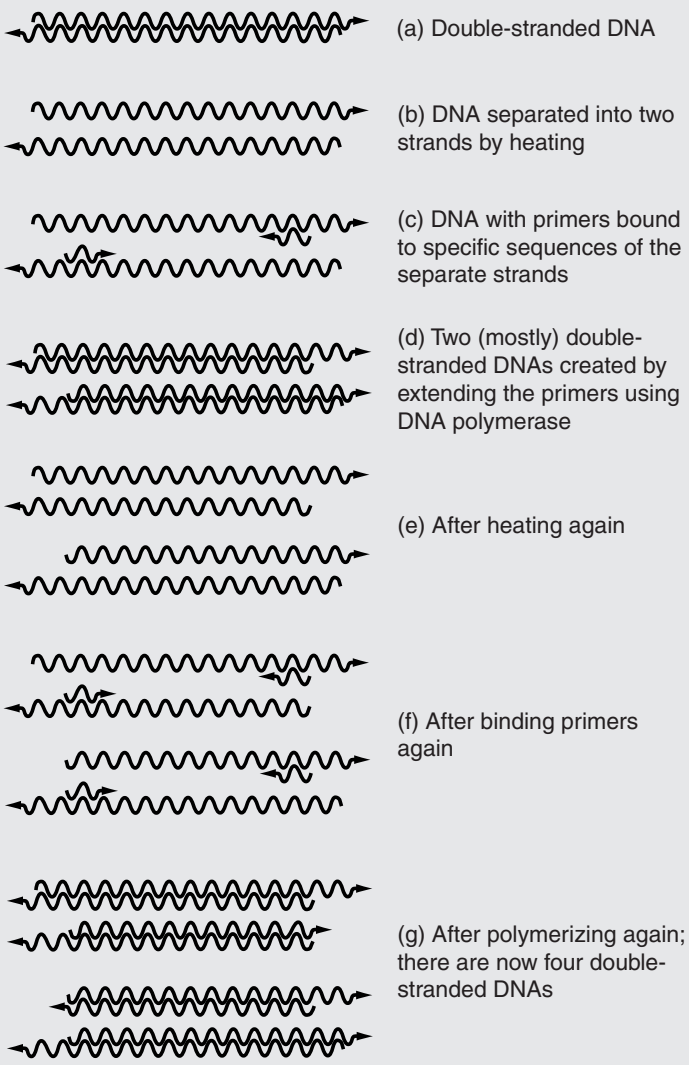

FIGURE 2.1.1 Schematic representation of the successive splitting and copying of nucleic acid molecules that constitute the polymerase chain reaction.

disadvantage of this technique is that growth is often slow and there are a limited number of organisms that can be detected.

Analyses based on PCR can also take advantage of amplification to detect as little as a single intact rRNA gene in a sample. In addition, PCR can be used to screen samples whose mass ranges from nanograms to a gram without an increase in the complexity of or time required for the test, so it also has a high throughput. Although the use of PCR with rRNA genes will enable detection of virtually any terrestrial organism, the disadvantage of this technique is that it is unlikely to detect novel life forms that originated independently of terrestrial life. In spite

TABLE 2.1 Properties of Some Techniques for Detecting Viable and Recently Dead Cells

Technique	Sensitivity	Specificity for Life Detection	Generality[a]	Time per Sample	Time per Gram[b]
Cultivation to test for growth[c]	Very high: 1-10 cells	Very high	Requires cell type that grows in culture	Days to months	Days to months
Test for metabolic activity	Moderate: 10^3-10^6 cells	Moderate to high	Specific metabolism, but any biology	Minutes to days	Minutes to days
Microscopic examination[d]	Very high: 1-100 cells	Moderate	High	Minutes to hours	Days to months
PCR[e]	Very high: 1-10 cells	Very high	Requires rRNA gene[f]	Minutes to hours	Minutes to hours
Chemical structure analysis[g]	Moderate to low: 10^6-10^{15} molecules; 10^3-10^9 cells	High	Moderate	Minutes to days	Minutes to days
Chirality analysis	Low: $>10^{10}$ molecules; 10^6-10^9 cells	Very high	Very high	Minutes to days	Minutes to days
Isotope stability analysis	Very high to moderate: 10^5-10^8 cells	High	Very high	Minutes to hours	Hours to months, depending on sensitivity
Elemental composition analysis	High to low: 100-10^8 cells	Moderate	Very high	Minutes to hours	Hours to months, depending on sensitivity

[a]Based on sensitivity for detection of extraterrestrial life.
[b]Time per gram exceeds time per sample in the cases where studying a gram of material requires analysis of many samples within the gram.
[c]That is, an increase in biomass.
[d]Observation at the micrometer level.
[e]Values assigned assume high DNA-extraction efficiencies and optimized PCR conditions.
[f]Other highly conserved genes might also be targeted.
[g]Biomolecular structures, e.g., lipids, macromolecules.

of these limitations, the potentially high sensitivity and high throughput of both cultivation and PCR make them uniquely valuable for testing returned martian samples.

Although the ability to detect as little as one cell by culturing or amplification by PCR (albeit with significantly less than 100% reliability) is appealing, this virtue is tempered by two facts. First, lack of detection is not a reliable indicator that the sample tested contained no viable organisms. Under any conditions chosen, most known organisms will not respond to culturing.[27] And although PCR is a more general method of detecting viable or recently dead terrestrial organisms than culturing, it too is capable of producing false negatives.

Another limitation of both culturing and PCR is that only a relatively small portion of a returned sample can be subjected to these detection techniques because doing so greatly compromises the samples for all subsequent

[27]Pace, N.R. 1997. A molecular view of microbial diversity and the biosphere. Science 276: 734-740.

TABLE 2.2 Time Scales[a] for Optical Scanning

Grain Diameter (mm)	Grain Volume (mm^3)	Grains per Gram	Surface Area per Grain (mm^2)	Surface Area per Gram (mm^2)	Time to Scan 1 Gram (days)
0.10	0.000524	707,000	0.0314	22,200	257
0.20	0.00419	88,400	0.126	11,100	129
0.30	0.0141	26,200	0.283	7,410	86
0.40	0.0335	11,100	0.503	5,560	64
0.60	0.113	3,270	1.13	3,700	43
0.80	0.268	1,380	2.01	2,780	32
1.00	0.524	707	3.14	2,220	26

[a]Time to microscopically examine the surface of 1 gram of silica sand grains for visible life, by surveying at a rate of 1,000 μm^2 per second, 24 hours a day.

scientific analyses, especially nonbiological analyses. The absence of detectable life in a small subsample can only place bounds on the abundance of life in the remaining material. If the density of organisms were, for example, 300 per complete sample, then the expected number in a 1% sample would be 3 and the probability of detecting none would be approximately 0.05, according to Poisson statistics. That is, if organisms detectable only at a low level were present, there would be a significant chance of missing them. If the density of organisms were 30 per complete sample, then the probability of detecting none in a 1% sample would be about 0.74, i.e., quite high. In essence, it would be possible to say only that viable organisms were not abundant in the sample, not that the rest of the sample was free of life.

Although it might be possible to achieve comparable sensitivity with microscopic searches for cell morphology, studies of stable isotopes, or analyses of elemental distributions, and although such studies might have the advantage of being able to detect novel life forms, none of these methods has a very high throughput. If the surfaces of sand-sized grains were scanned at the rate of 1,000 μm^2 per second, then to cover the surfaces of 1 gram of grains would require more than 3 weeks for 1-mm-diameter spherical sand particles, and more than 8 months for 0.1-mm particles (Table 2.2). Although it might be possible to examine samples more rapidly than this, a price would be paid in increased errors.

Life: Indigenous Versus Contaminant

A critical part of life detection in martian samples will be the ability to assess whether the source is extraterrestrial or terrestrial. In the case of extant or recent life, researchers expect to find intact molecules, and possibly cell growth. Short of cultured growth, almost certainly the best test for terrestrial contamination is the use of PCR amplification of rRNA genes. For reliability, more than one portion of the molecule (and possibly more than one gene) should be targeted for amplification. If there is more than a trace of terrestrial or terrestrial-like life, this approach should detect it.

If PCR were to detect rRNA genes in a sample, it would mean that the life would be traceable to a common origin with terrestrial life (based on the assumption that independent origin of exactly the same genetic material and machinery for gene expression is unimaginably improbable). Such a finding could indicate either of two things: (1) recent contamination of the sample or (2) an ancient common heritage of life on Mars and Earth. Because of the broad rRNA-based sampling of life that has been conducted on Earth,[28] it is likely that recent

[28]Maidak, B.L., Cole, J.R., Lilburn, T.G., Parker, C.T., Jr., Saxman, P.R., Stredwick, J.M., Garrity, G.M., Li, B., Olsen, G.J., Pramanik, S., Schmidt, T.M., and Tiedje, J.M. 2000. The RDP (Ribosomal Database Project) continues. Nucleic Acids Res. 28: 173-174; available online at <http://www.cme.msu.edu/RDP/html/index.html>.

contamination could be quickly recognized because it would yield rRNA sequences similar to specific, present-day lineages on Earth. In addition, if a novel rRNA gene sequence (one that is not represented in current databases) were found, it would be possible to use primer sequences directed for this novel sequence in the PCR protocol to screen rapidly and systematically for the same sequence in other terrestrial environments. Specific attention could be focused on screening sources of materials brought into the quarantine facility. If the recovered sequences were only distantly related to known terrestrial sequences, then the possibility would have to be taken seriously that they were linked by an ancient transfer event between the planets (i.e., panspermia). More complex histories would obviously be possible for an amplified sample that produced multiple sequences, but phylogenetic analyses could be used to check consistency with alternative hypotheses regarding origins and transport.

Life detected from the presence of life-associated chemical reactions would be very difficult to assess in terms of terrestrial or extraterrestrial origin. Depending on the reaction tested, the signal might also be of abiotic origin.[29,30] In the event of suggestive reactions, it would be important to seek other cellular or molecular evidence.

If chemical signatures of life were found, they could be compared to terrestrial biomolecules. A match would again raise questions as to whether the chemical compounds were similar because of recent contamination or because they had an ancient common origin. However, because the use of specific chemical compounds to detect life is a relatively insensitive technique, only abundant contamination would be detectable—an abundance that would also facilitate the identification of its source.

Other types of tests for evidence for life can be even more difficult to evaluate in this regard. Many combinations of observations are possible, so it is not productive to attempt an exhaustive analysis.

[29]Levin, G.V., and Straat, P.A. 1976. Labeled release—An experiment in radiorespirometry. Origins Life 7: 293-311.
[30]Levin, G.V., and Straat, P.A. 1977. Recent results from the Viking labeled release experiment on Mars. J. Geophys. Res. 82: 4663-4667.

3

Detection of Evidence of Earlier Life

The search for evidence of former life, assumed to be carbon-based and microbial, seems certain to be a time-consuming, needle-in-a-haystack hunt. The first task should be to identify the haystack: to detect and profile the distribution of carbonaceous ("organic") matter in a representative subset of the total sample. Organic matter is a signature general to known living systems whether they be viable, recently dead, or fossil. The failure of the Viking gas chromatography-mass spectrometry experiment (detection limits of 1 part in 10^6 to 10^9) to detect organic material in martian surface and subsurface materials at two landing sites dealt a severe blow to the prospect of life on Mars.[1] Because the infall of carbonaceous chondrite meteorites, if nothing else, should build up a finite level of organic compounds in the martian soil, it seems likely that such compounds are systematically destroyed in the martian environment, perhaps by ultraviolet radiation and/or an oxidizing agent such as hydrogen peroxide or hydroxyl radicals in the atmosphere,[2] although they may survive inside sufficiently large, impermeable rock fragments.

The search for organic carbon should be accomplished early during sample processing, by surveying a sterilized representative subset of the sample outside of the quarantine facility. Such a survey will require use of extensive laboratory facilities that are stringently clean[3] both of biological and organic terrestrial contaminants. Similarly specialized facilities and appropriate precautions to mitigate contamination will be required for all other paleobiological studies. Because of these requirements, both the initial assay (of a sterilized subset of the sample) and all subsequent searches for evidence of fossil life should be carried out in laboratories designed for and experienced in such studies.

Concurrent experiments to detect viable (including potentially biohazardous) and recently dead organisms, using the techniques discussed in Chapter 2, will depend critically on the abundance and distribution of organic

[1]Biemann, K., Oro, J., Toulmin, P. III., Orgel, L.E., Nier, A.O., Anderson, D.M., Simmonds, P.G., Flory, D., Diaz, A.V., Rushneck, D.R., Biller, J.E., and Lafleur, A.L. 1977. The search for organic substances and inorganic volatile compounds in the surface of Mars. J. Geophys. Res. 82: 4641-4658.

[2]Biemann et al., 1977; see footnote 1 above.

[3]"Clean," in the biological sense, refers to bioloads less than levels set by background measurements made in a facility or on a spacecraft. Other criteria can be used to define clean in the chemical sense.

carbon in the samples. These data will also support all other paleobiological investigations as well as the design of follow-on sample return missions.[4] If carbonaceous material is shown to be present, subsequent detailed studies will be required to establish whether it is indigenous to the sample (as opposed to being terrestrial contamination, introduced during sample collection and/or processing) and whether it is biogenic (rather than being of non-biological origin, implanted by impacting meteoroids or synthesized in the martian environment). Like the initial assay, these and all other paleobiological studies should be carried out in existing laboratories equipped for and experienced in such research.

Evidence of earlier life can take the form of characteristic patterns of organic compounds and isotopes or morphological fossils (both individual microorganisms and fragments of finely laminated biosedimentary structures, like stromatolites). Although these categories of evidence are very different, both must meet the following tests to constitute strong evidence.[5,6]

1. *Indigenousness*. To avoid the mistake of identifying contaminating materials introduced during sample collecting and/or terrestrial processing as martian life or evidence of it, the property studied must be shown to be indigenous to the martian sample. Materials present in high abundance in the sample, and millimeter-sized or larger structures (perhaps such as grains that contain stromatolithic laminae and cellular microfossils), should pose little problem in this regard; establishment of the indigenity of materials detected in trace amounts and of minute structures, especially if rare (including microbe-sized bodies), may be difficult. Stringent precautions should be taken to avoid both biological and organic contamination of the sample during both collection and processing.

2. *Biogenicity*. The supposed evidence must be demonstrably biological in origin. Criteria currently used in studies of Precambrian paleobiology[7] should prove particularly applicable to this purpose. To obtain data that will permit use of these criteria, the analyses should be designed to document multiple properties that are uniquely biological, depending on the nature of the evidence and its fidelity of preservation, ranging from biochemical properties (such as the presence of limited suites of biologically selected classes of stereoisomers, monomers, polymers, and organic-inorganic isotopic compositions), to organismal (cellularity, reproduction, heritable morphologic variability) and ecological properties (behavioral interactions and populational and community structures).

BIOGEOCHEMICAL PATTERNS

On Earth, early life left its geochemical mark in several ways.[8-10] The chemically variable, macromolecular carbon-rich *kerogen* of ancient sediments was derived principally from the "coalified," geochemically altered

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[4]Schopf, J.W. 1999. Fossils and pseudofossils: Lessons from the hunt for early life on Earth, p. 88 in Space Studies Board, National Research Council, Size Limits of Very Small Microorganisms: Proceedings of a Workshop. National Academy Press, Washington, D.C.

[5]Schopf, 1999; see footnote 4 above.

[6]Schopf, J.W., and Walter, M.R. 1983. Archean microfossils: New evidence of ancient microbes, p. 214 in Earth's Earliest Biosphere, Its Origin and Evolution, J.W. Schopf, ed. Princeton University Press, Princeton, N.J.

[7]Examples from the terrestrial geologic record:

- Limited suites of biologically selected classes of stereoisomers (e.g., Quaternary and Tertiary amino acids), monomers (e.g., Quaternary and Tertiary amino acids, pyrroles, purines, and pyrimidines), and polymers (e.g., Quaternary, Tertiary, and possibly Cretaceous nucleic acids and polysaccharides, Cenozoic and Mesozoic lignins and tetrapyrroles, and various types of Phanerozoic and Precambrian hydrocarbons). Organic-inorganic isotopic data include carbon, sulfur, and nitrogen isotopic distributions in Phanerozoic and Precambrian rocks.
- Examples of organismal properties include cellularity and cell division states (reproduction) seen in optical microscopic studies of Precambrian and Phanerozoic fossils, and the heritable morphologic variability routinely established by optical microscopic study of populations of Precambrian and Phanerozoic microscopic fossils of a single taxon.
- Among ecological properties, an example of behavioral interactions can be found in microscopic studies of populations of particular taxa of Precambrian and Phanerozoic microscopic fossils, such as mat-building (stromatolite-forming) filamentous cyanobacteria and endolithic (substrate-inhabiting) cyanobacteria. Populational and community structures are observed in optical microscopic studies of multicomponent populations of Precambrian and Phanerozoic microfossils that together make up stromatolitic biocoenoses (distinctive, more or less self-contained, microbial biological communities).

[8]Hayes, J.M., DesMarais, D.J., Lambert, I.B., Strauss, H., and Summons, R.E. 1992. Proterozoic biochemistry, p. 81 in The Proterozoic Biosphere, A Multidisciplinary Study, J.W. Schopf and C. Klein, eds. Cambridge University Press, New York.

Box 3.1
Gas Chromatography–Mass Spectrometry

Organic materials usually are mixtures of compounds. Gas chromatography-mass spectrometry (GC-MS) is a technique that allows certain of the compounds in organic mixtures (relatively volatile, nonpolar compounds such as biological lipids) to be separated and identified. It employs an instrument that combines a gas chromatograph with a mass spectrometer. The gas chromatograph separates compounds and sends them in sequence to the mass spectrometer. The mass spectrometer provides information that can either identify the compound outright or at least provide definitive information on its composition.

The gas chromatograph includes a carrier-gas supply, an inlet system, and a chromatographic column. The carrier gas, usually helium, flows continuously through the inlet system and the chromatographic column. The unknown sample is flash-vaporized in the inlet system and carried into the column. The column is a long, thin tube; its inner wall is coated with an organic gum in which the vaporized compounds tend to dissolve. Thus they travel less rapidly than the carrier gas and become separated according to their differing tendencies to dissolve in the gum. Individual compounds emerge from the end of the column spaced in time, in pulses 3 to 10 seconds wide. The temperature of the chromatographic column is adjusted so that a single chromatographic run can last 1 hour or more. Hundreds of compounds can be separated.

As gas containing each compound exits the gas chromatograph, that compound is separated from the carrier gas and introduced into the mass spectrometer. There an electron beam ionizes the gas molecules, and electrostatic fields inject a beam of the ions into an oscillating electric field. The properties of that field are varied so that molecules with different masses are sequentially directed to a measurement system. This process produces a mass spectrum, a plot that displays the relative abundances of molecules having a range of masses.

Interpretation of these spectra in terms of the composition of the organic mixture introduced into the GC-MS is not completely straightforward, because compounds in the mixture are to some degree damaged (fragmented) by the process of ionization in the mass spectrometer and because the spectra of compounds can overlap, creating mixed signals that cannot be separated without further evidence.

remains of cell walls of living systems. Enzyme-driven metabolic processes can create characteristic patterns of isotopic abundances, affecting in particular the ranges of compositions of the stable isotopes of carbon, sulfur, nitrogen, hydrogen, and possibly iron. Additionally, life leaves *biomarkers* (from "biological marker compounds") in the rocks, distinctive geochemically altered biomolecules such as various hydrocarbons, fatty acids, amino acids, and porphyrins that by their presence provide evidence of the existence of particular biosynthetic pathways and the biological groups in which they occur.

Martian samples that contain detectable levels of organic carbon may contain enough material to permit further analysis of individual organic compounds. This is generally accomplished by gas chromatography-mass spectroscopy (GC-MS; see Box 3.1) techniques. The products of such analyses are both chromatograms showing the relative abundances of organic compounds and mass spectra that can aid in the identification of the compounds. The shape of a chromatogram with peaks for many compounds can be used to judge whether the organic compounds were generated abiotically or through known biological synthesis pathways. Abiotic synthesis of

[9]Hayes, J.M., Kaplan, I.R., and Wedeking, K.M. 1983. Precambrian organic geochemistry, preservation of the record, p. 93 in Earth's Earliest Biosphere, Its Origin and Evolution, J.W. Schopf, ed. Princeton University Press, Princeton, N.J.

[10]Schidlowski, M., Hayes, J.M., and Kaplan, I.R. 1983. Isotopic inferences of ancient biochemistries: Carbon, sulfur, hydrogen, and nitrogen, p. 149 in Earth's Earliest Biosphere, Its Origin and Evolution, J.W. Schopf, ed. Princeton University Press, Princeton, N.J.

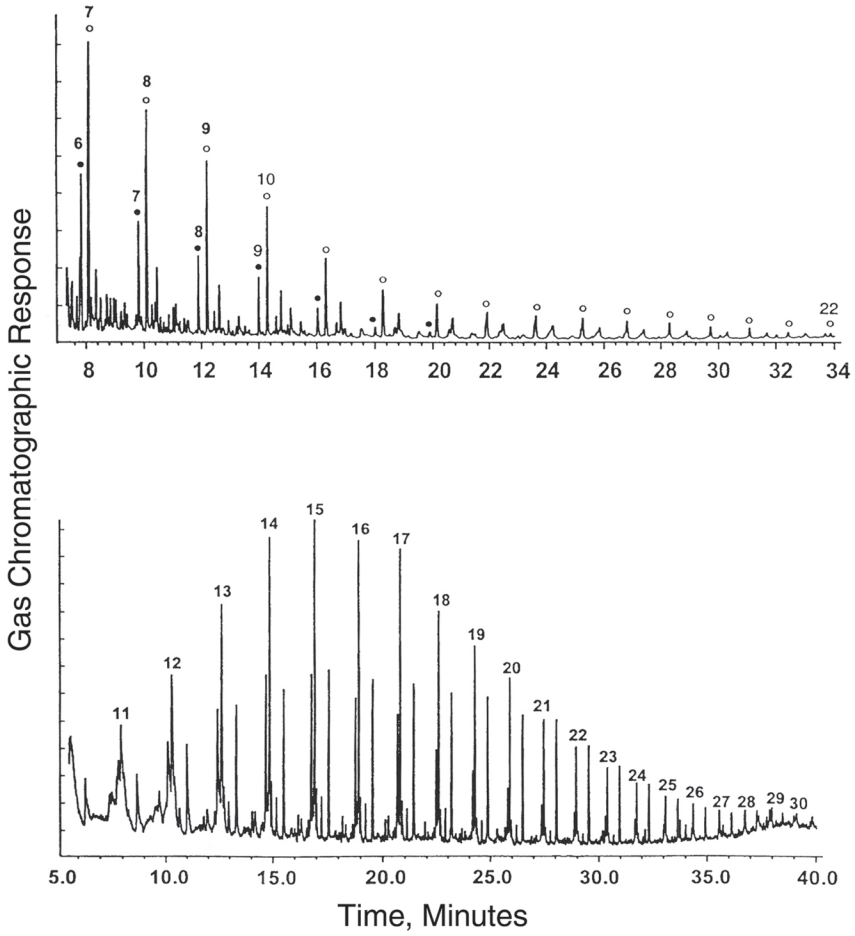

FIGURE 3.1 Gas chromatograms of compounds formed by an abiotic process, Fischer-Tropsch-type synthesis from formic acid solutions. *Top*, total ion chromatogram for the CH_2Cl_2 extract after derivitization of acids and alcohols (acids form the peaks under filled circles; alcohols, under open circles). *Bottom,* underivitized extract showing dominant peaks for straight-chain alkanes and alkenes. Numbers show the quantity of carbon atoms in each compound. The distribution of peaks forms a smooth envelope in both cases. SOURCE: Reprinted with permission from McCollom, T.M., Ritter, G., and Simoneit, B.R.T. 1999. Lipid synthesis under hydrothermal conditions by Fischer-Tropsch-type reactions. Origins of Life and Evolution of the Biosphere 29: 153.

organic compounds tends to generate very wide varieties of products leading to smooth envelopes of peaks in a chromatogram, as shown by the examples in Figure 3.1. These chromatograms show the distribution of hydrocarbons, alcohols, organic acids, and other compounds generated from formic acid in hydrothermal synthesis experiments.[11] The peaks are labeled with the number of carbon atoms in each molecule.

It can be seen in Figure 3.1 that the distribution of peaks with mass is extremely smooth, showing no particular preference for any individual molecules. In contrast, chromatograms of biological debris are not nearly as smooth,

[11]McCollom, T.M., Ritter, G., and Simoneit, B.R.T. 1999. Lipid synthesis under hydrothermal conditions by Fischer-Tropsch-type reactions. Origins of Life and Evolution of the Biosphere 29: 153.

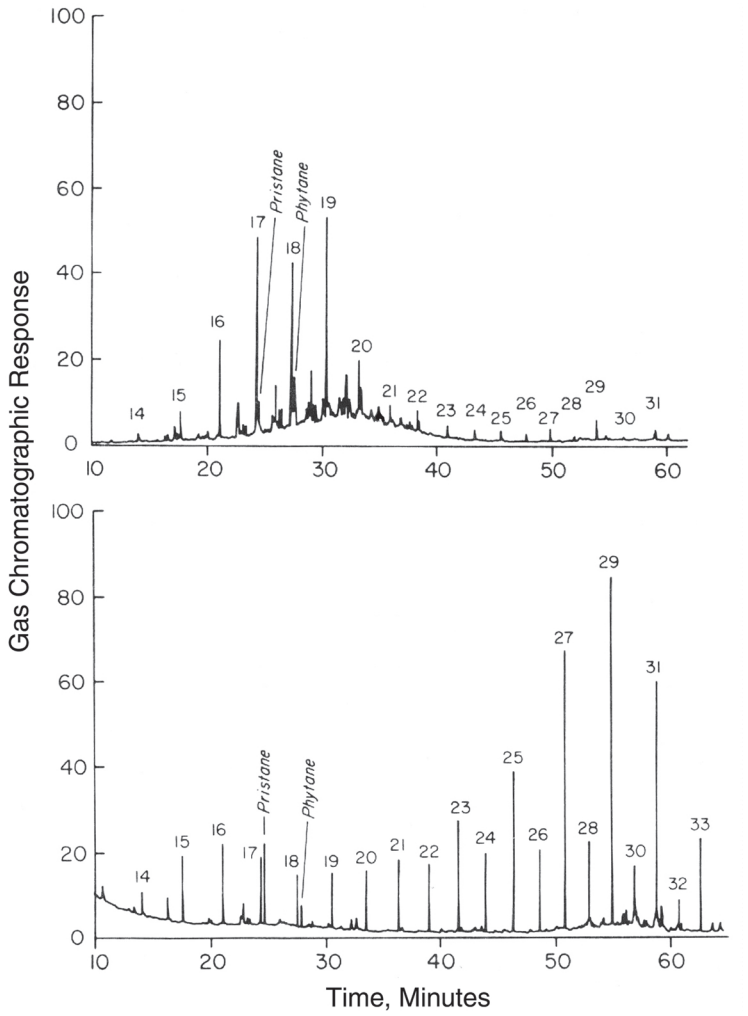

FIGURE 3.2 Gas chromatograms of compounds formed by biological processes; shown are total hydrocarbon extracts from surface marine sediments in the Guaymas Basin (above) and the Escanaba Trough (below). Peaks representing alkanes are identified by numbers representing the number of carbon atoms they contain. In contrast to the chromatograms of Figure 3.1, the distribution of peak heights is uneven. SOURCE: Reprinted with permission from Simoneit, B.R.T. 1993. Hydrothermal alteration of organic matter in marine and terrestrial systems, pp. 397-418 in Organic Geochemistry—Principles and Applications, M.H. Engel and S.A. Macko, eds. Plenum Press, New York.

and they generally contain individual peaks that stand out above the rest. Examples are shown in Figure 3.2, two seafloor sediment hydrocarbon samples that yielded more selective distributions. Compounds with odd numbers of carbon atoms often are more abundant than adjacent compounds with even numbers of carbon atoms. Enhancement in the concentration of individual compounds is characteristic of enzymatic processes that generate certain compounds preferentially.

Patterns must be assessed and interpreted very carefully. Abiotic processes can alter biological products so that their biological characteristics are practically invisible, and a succession of differing abiotic processes might produce a mixture of organic compounds with some "biological" characteristics. As a means of examining mixtures of organic compounds, however, the approach has several fundamental strengths. It is general and can be

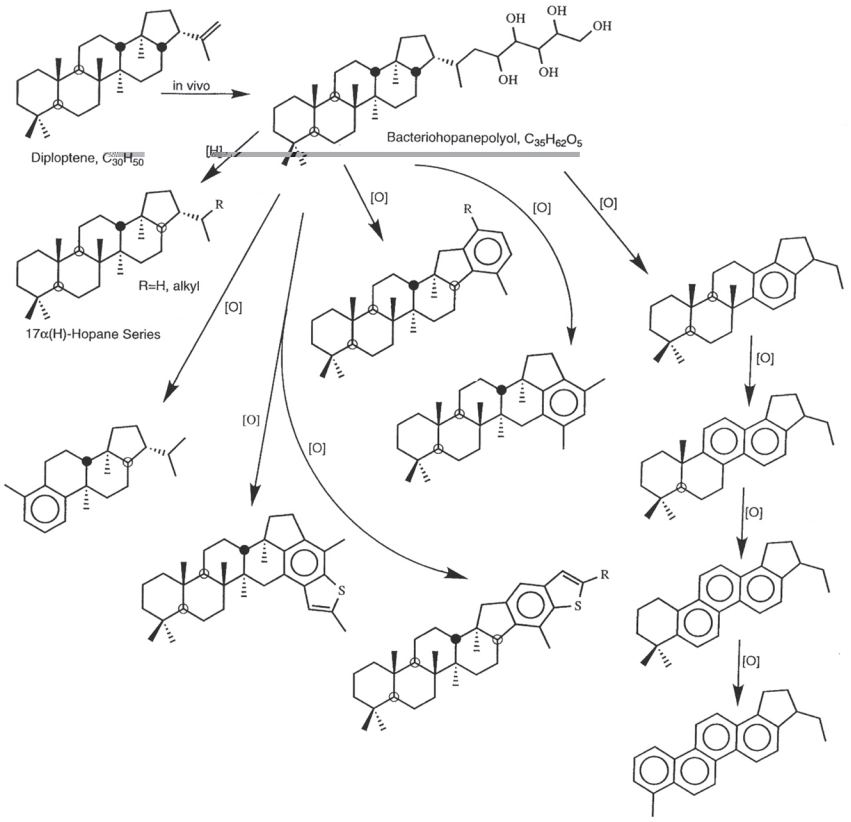

FIGURE 3.3 Alteration scheme for the hopanoid family of compounds, which are found in geologic samples. Hopanoids are the most extensively documented biomarkers for bacteria. The two molecules shown at the top, diploptene and bacteriohopanepolyol, are produced in bacterial cells. Reduction of bacteriohopanepolyol (arrow labeled [H]) produces a series of hopanes, and oxidation (arrows labeled [O]) leads to the series of partially aromatic compounds shown, all of which can be found in extracts of geologic material. SOURCE: Reprinted with permission from Simoneit, B.R.T., Summons, R.E., and Jahnke, L.L. 1998. Biomarkers as tracers for life on early Earth and Mars. Origins of Life and Evolution of the Biosphere 28: 475-483.

applied to any collection of molecular products. It can be extended or deepened in proportion to the detail available and has the potential to use all of the information provided by the analytical data. And it can be made multidimensional, with patterns of order being searched for in isotopic distributions, in relative abundances of structurally related compounds, and in distributions of structural types.

Biomarker compounds are derived from biochemical precursor molecules through alteration reactions that include oxidation, reduction, and hydrolysis. An example of biomarkers indicative of a bacterial source is shown in Figure 3.3. Note that some parts of the biological molecule are easily (and rapidly) transformed or lost, while other parts exhibit remarkable resistance to geochemical alteration. As an example, the compound in the lower right corner of Figure 3.3 maintains several major structural features of its biological precursor despite the loss of most of a major side chain, several methyl groups, and more than half of the hydrogen atoms originally attached to the multi-ring structure. Although the presence of this individual compound might be enough to deduce a biological origin, that deduction is strengthened if a series of related compounds is found in a sample. This is often

the case for biomarkers in terrestrial samples, and the presence and distribution of sets of biomarkers compounds are being exploited to greatly expand upon the fossil record of life on Earth.[12-14]

This point can be illustrated with some recent examples of the use of biomarkers to expand on the fossil record. Fossil diatoms are found in rocks of Jurassic age (144 to 208 million years old) and younger, and there is a strong correlation between ratios of certain cholestane biomarkers, rocks of the appropriate age, and paleolatitude, as documented by Holba et al.[15] These ratios can be used in samples where microscopic identification of diatoms is impossible because the biological opaline silica that is characteristic of diatoms has been converted to cristobalite. However, the link between diatoms and these biomarkers is largely circumstantial, and the authors report traces of these compounds in samples that predate the Jurassic. Stronger links are established between oleonane and flowering plants[16] and between 24-isopropyl cholestane and sponges,[17] and both of these compounds have been used to expand upon the fossil record. Similarly, the association of certain dinosteroids with dinoflagellates not only correlates closely with the presence of dinoflagellate fossils in Mesozoic rocks, but also has raised intriguing questions about the ancestry of these organisms, as these compounds have been extracted from Paleozoic and Precambrian rocks that are much older than any known dinoflagellate fossils.[18,19] More recent work has confirmed the presence of dinoflagellate ancestors as early as the Early Cambrian (~570 million years ago), which nearly doubles the known period in which these eukarya have existed.[20]

In another recent breakthrough driven by biomarker analysis, Brocks and co-workers report evidence of steranes,[21] which are indicative of eukaryotic organisms, in 2.7 billion-year-old shales from Australia. Not only does this study demonstrate that biomarkers can survive for great lengths of time under geologically gentle conditions, but this evidence also strongly suggests that eukaryotic organisms were present 500 million to 1 billion years earlier than the fossil record indicates. These same samples contain hopanes that are characteristic of cyanobacteria, which shows that bacterial oxygenic photosynthesis evolved well before there is evidence in the rock record for a highly oxidized atmosphere. The connection between cyanobacteria and fossil hopanoids has been thoroughly examined by Summons and co-workers,[22] who extended the record of these compounds to ~2.5 billion years ago with samples of the Mt. McRae shale from the Hammersly basin of Australia. These studies have focused on samples that are well preserved and of low metamorphic grade, with the hope of more easily finding biomarkers that might otherwise be altered or destroyed by high temperatures. Another recent development by

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[12]Brassell, S.C. 1992. Biomarkers in recent and ancient sediments: The importance of the diagenetic continuum, pp. 339-367 in Organic Matter: Productivity, Accumulation, and Preservation in Recent and Ancient Sediments, J.K. Whelan and J.W. Farrington, eds. Columbia University Press, New York.

[13]Peters, K.E., and Moldowan, J.M. 1993. The Biomarker Guide—Interpreting Molecular Fossils in Petroleum and Ancient Sediments. Prentice-Hall, Englewood Cliffs, N.J.

[14]de Leeuw, J.W., and Largeau, C. 1993. A review of macromolecular organic compounds that comprise living organisms and their role in kerogen, coal and petroleum formation, pp. 23-72 in Organic Geochemistry—Principles and Applications, M.H. Engel and S.A. Macko, eds. Plenum Press, New York.

[15]Holba, A.G., Tegelaar, E.W., Huizinga, B.J., Moldowan, J.M, Singletary, M.S., McCaffrey, M.A., and Dzou, L.I.P. 1998. 24-norcholestanes as age-sensitive molecular fossils. Geology 26: 783-786.

[16]Moldowan, J.M., Dahl, J., Huizinga, B.J., Fago, F.J., Hickey, L.J., Peakman, T.M., and Taylor, D.W. 1994. The molecular fossil record of oleonane and its relation to angiosperms. Science 265: 768-771.

[17]McCaffrey, M.A., Moldowan, J.M., Lipton, P.A., Summons, R.E., Peters, K.E., Jegnathan, A., and Watt, D.S. 1994. Paleoenvironmental implications of novel C^{30} steranes in Precambrian to Cenozoic age petroleum and bitumen. Geochim. Cosmochim. Acta 58: 529-532.

[18]Summons, R.E., Thomas, J., Maxwell, J.R., and Boreham, C.J. 1992. Secular and environmental constraints on the occurrence of dinosterane in sediments. Geochim. Cosmochim. Acta 56: 2437-2444.

[19]Moldowan, J.M., Dahl, J., Jacobson, S.R., Huizinga, B.J., Fago, F.J., Shetty, R., Watt, D.S., and Peters, K.E. 1996. Chemostratigraphic reconstruction of biofacies: Molecular evidence linking cyst forming dinoflagellates with pre-Triassic ancestors. Geology 24: 159-162.

[20]Moldowan, J.M., and Talyzina, N.M. 1998. Biogeochemical evidence for dinoflagellate ancestors in the early Cambrian. Science 281: 1168-1170.

[21]Brocks, J.J., Logan, G.A., Buick, R., and Summons, R.E. 1999. Archean molecular fossils and the early rise of Eukarya. Science 285: 1033-1036.

[22]Summons, R.E., Jahnke, L.L., Hope, J.M., and Logan, G.A. 1999. 2-Methylhopanoids as biomarkers for cyanobacterial oxygenic photosynthesis. Nature 400: 554-557.

Box 3.2
Carbon Isotopes

Carbon occurs as three isotopes: ^{12}C (98.9% abundance), ^{13}C (1.1%), and ^{14}C (a transient radioactive isotope created by cosmic rays). These are only average abundances; natural processes tend to fractionate ^{13}C from ^{12}C in forming particular organisms and minerals from their surroundings.

The fractionation arises because atoms of ^{13}C tend to react slightly more slowly than those of ^{12}C. Analysis of a succession of reactants and products thus commonly reveals declining relative abundances of ^{13}C. The magnitude of the decline, or "fractionation," can vary widely. The conversion of carbon dioxide to organic material by plants provides a notable example of isotopic fractionation. Plant biomass in Earth's oceans is depleted in ^{13}C by 2 to 3% relative to carbonate minerals that form there. Land plants are depleted in ^{13}C by about 2% relative to CO_2 in the terrestrial atmosphere. These small differences are easily detected in samples by mass spectrometric analysis. Isotope effects can be associated with both biological and nonbiological processes. Isotopic fractionations are, therefore, not a definitive indicator of life. Systematic interpretation of isotopic variations can, however, indicate relationships between reactants and products and provide evidence for or against biological processes.

Price and co-workers holds hope for even highly metamorphosed sediments.[23] These authors used a new extraction protocol to obtain trace amounts of organic compounds and report the extraction of cholestanes, tricyclic terpanes, hopanes, and steroids from anthracites, meta-anthracites, black shales, and carbonaceous schists that have reached temperatures from 200 to 450 °C during hydrothermal alteration and metamorphism. These compounds resemble many found in hydrothermally generated petroleum from sedimented ridge systems.[24]

Studies of terrestrial biomarkers in petroleum, rock, sediment, water, plant, animal, microbe, and atmospheric particulate samples have identified the diverse sources of organic compounds that can be present in a sample from Earth's surface. As a consequence, it is increasingly possible to identify terrestrial organic contaminants, and to fingerprint their sources. For example, chromatograms of organic compounds extracted from atmospheric particulates filtered from air in diverse locations reveal the presence of compounds derived from the burning of fossil fuels, biomass burning, cigarette smoke, cooking, and releases from industrial processes and natural vegetation. Individual peaks in chromatograms can be identified in catalogues of peaks of known compounds from known sources. These advances will greatly increase the probability of differentiating terrestrial contamination from the signal of indigenous organic compounds in samples from Mars.[25]

If organic carbon is found in martian samples, its isotopic composition can be informative (see Box 3.2). Dissimilarity between the isotopic compositions of martian and terrestrial organic carbon would demonstrate that the martian carbon analyzed is indigenous to that planet, possibly the product of an extraterrestrial life process. Similarity between the isotopic compositions of organic carbon from the two planets could mean that the martian carbon analyzed is actually terrestrial contamination, or it could mean that fractionation processes operate in the same way on both planets.

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[23]Price, L.C., and Dewitt, E. 2001. Evidence and characteristics of the hydrolytic disproportionation of organic matter in metasomatic processes. Geochim. Cosmochim. Acta 65(21): 3791-3826.

[24]Simoneit, B.R.T. 1993. Hydrothermal alteration of organic matter in marine and terrestrial systems, pp. 397-418 in Organic Geochemistry—Principles and Applications, M.H. Engel and S.A. Macko, eds. Plenum Press, New York.

[25]Simoneit, B.R.T., Summons, R.E., and Jahnke, L.L. 1998. Biomarkers as tracers for life on early Earth and Mars. Origins of Life and Evolution of the Biosphere 28: 475-483.

MORPHOLOGICAL FOSSILS

Fossils of ancient life are found in terrestrial rocks, as individual organisms or in colonies (Figure 3.4).[26-28] They may be cellular and three-dimensional in form (as in the case of microbes preserved by petrifaction), or they may be preserved as flattened compressions. Some are carbonaceous in character, whereas others are mineralic (e.g., calcareous or siliceous shells, or forms in which new minerals have replaced the original organisms). Intact whole bodies may be present (as for many fossil microbes), or parts thereof (such as skeletal fragments, spicules, and disarticulated cells). A chronology of the geologic record, and the positions of important fossils in it, are reviewed in Figure 3.5.

Two techniques have been important in the detection of terrestrial microfossils: the use of acid macerations and petrographic thin sections (see caption for Figure 1.2 in Chapter 1). Transmission and scanning electron microscopy (TEM, SEM) have been used to characterize microfossils previously detected in macerations or thin sections, but they have not proven to be reliable detection techniques.

Maceration, the easier and faster of the two detection techniques, refers to the dissolution of a rock in mineral acid (for example, hydrochloric acid for limestones, hydrofluoric acid for siliceous cherts and siltstones). Because of their coaly composition, organic-walled microfossils survive, apparently unscathed. Abundant fossils are concentrated in the resulting sludge-like acid-resistant residue, which can be slurried onto a microscope slide for study. Unfortunately, this technique is vulnerable to error-causing contamination.

In petrographic thin sections (Figure 3.6), fossils are detected encased within the rock, so indigenity can be demonstrated and the possibility of laboratory contamination can be ruled out.[29] Disadvantages of thin-sectioning are that special equipment is needed for preparation and the study of sections is time-consuming. However, the fact that fragile fossils are spared chemical maceration or mechanical crushing, and the assurance that external contamination has been avoided, justify use of the technique.

Transmission electron microscopic studies of terrestrial microfossils have involved examinations either of organic-walled fossils freed from their rock matrix by acid maceration, embedded in epoxy resin, and sectioned using a diamond knife; or of fossil-like objects detected in plastic (*Formvar*) surface replicas of polished and etched petrographic thin sections. Transmission electron microscopy of macerated microfossils has been used to elucidate the structure of cell walls, membranes, and internal organic contents.[30] But because macerations are susceptible to contamination, such studies are useful only for fossils previously detected in petrographic thin sections. Similarly, contamination by nonindigenous particles presents a problem for studies of surface replicas, as does the introduction of nonbiogenic artifacts of a variety of types (blisters, bubbles, strands of Formvar, and so forth) that in the 1960s and early 1970s were repeatedly identified mistakenly as "ancient fossils."[31]

Preparation of samples for study by SEM is simpler than for TEM and the results obtained are generally easier to interpret. But, as in TEM studies, establishment of the indigenity of the objects detected and their syngeneticity with a primary mineral phase is not straightforward. And, also as in TEM studies, fossil-like artifacts have been misinterpreted by SEM, especially in rock samples where mineralic morphology has been altered and smoothed to "biologic-like" shapes by acid-etching.[32]

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[26]Schopf, J.W., and Walter, M.R. 1983. Archean microfossils: New evidence of ancient microbes, p. 214 in Earth's Earliest Biosphere, Its Origin and Evolution, J.W. Schopf, ed. Princeton University Press, Princeton, N.J.

[27]Mendelson, C.V., and Schopf, J.W. 1992. Proterozoic and selected early Cambrian microfossils and microfossil-like objects, p. 865 in The Proterozoic Biosphere, a Multidisciplinary Study, J.W. Schopf and C. Klein, eds. Cambridge University Press, New York.

[28]Schopf, J.W. 1992. Paleobiology of the Archean, p. 25 in The Proterozoic Biosphere, a Multidisciplinary Study, J.W. Schopf and C. Klein, eds. Cambridge University Press, New York.

[29]Knoll, A.H. 1985. Exceptional preservation of photosynthetic organisms in silicified carbonates and silicified peats. Phil. Trans. Royal Soc. London, Part B, 311: 111-122.

[30]Schopf, J.W., and Oehler, D.Z. 1976. How old are the eukaryotes? Science 193: 47.

[31]Schopf, and Walter, 1983; see footnote 26 above.

[32]Schopf, and Walter, 1983; see footnote 26 above.

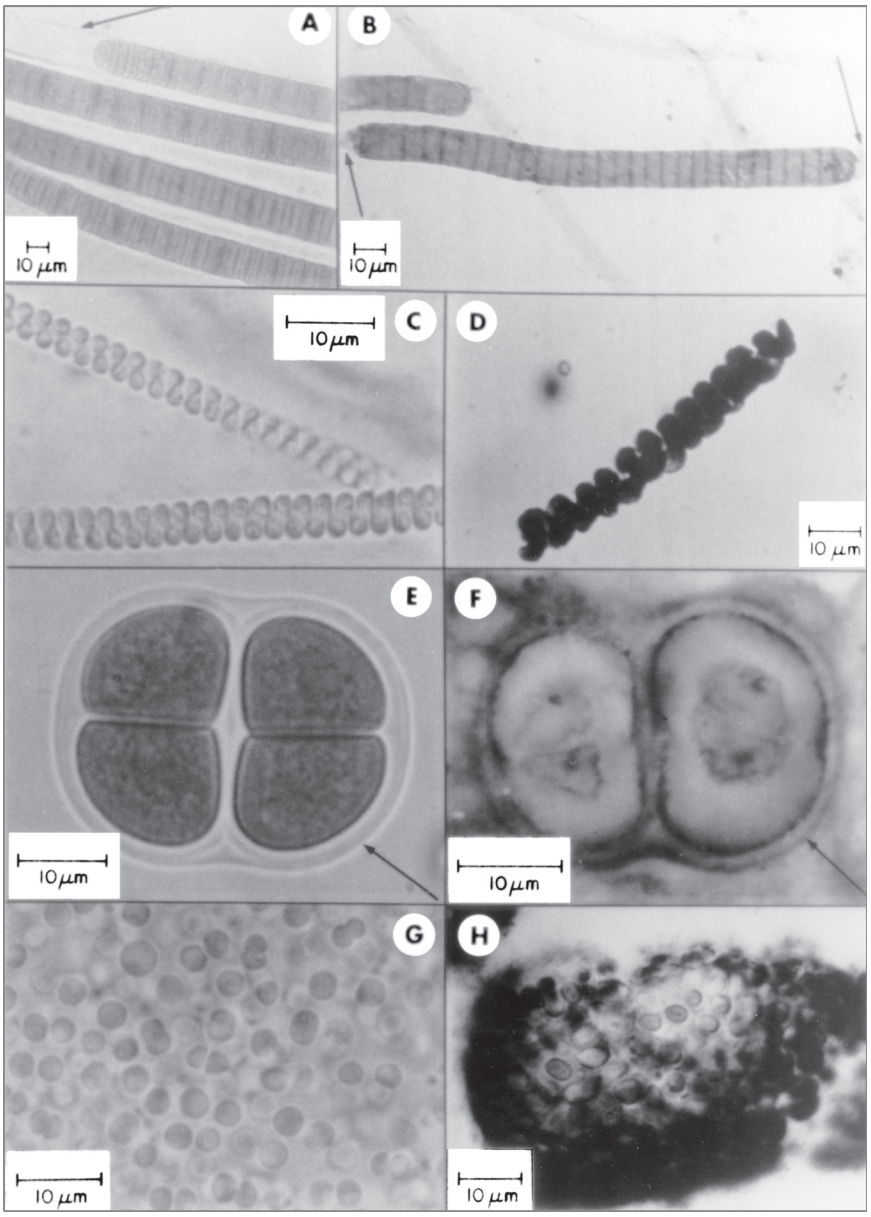

FIGURE 3.4 A comparison of living cyanobacteria with their fossil counterparts. A, C, E, and G show living bacteria from stromatolitic microbial mats in Baja California, Mexico. B, D, F, and H show fossils of comparable bacteria from terrestrial rocks of Precambrian age, 0.8 to 2.1 billion years old. SOURCE: Figure from Schopf, J.W. 1984. Disparate rates, differing fates: Tempo and mode of evolution changed from the Precambrian to the Phanerozoic. Proc. Natl. Acad. Sci. USA 91: 6735.

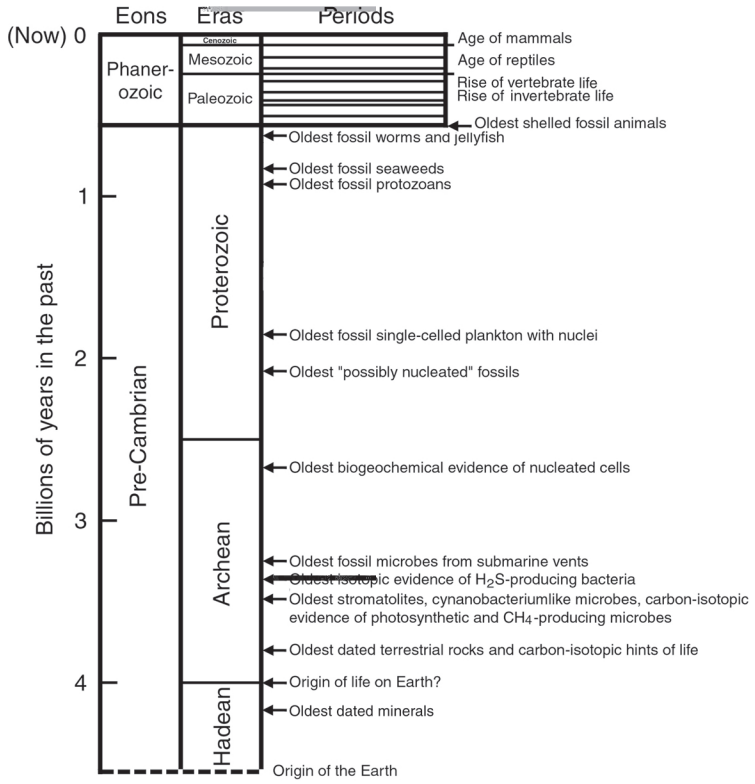

FIGURE 3.5 A summary of the names given to various intervals of geologic time on Earth, and important landmarks in the evolution of life on our planet. (Different time intervals and names are used in characterizing Mars and other planets.) Only the Phanerozoic Eon has been subdivided into periods. These are ~50 million years in duration; their names are not given in this figure.

Macroscopic *trace fossils* are also studied in terrestrial settings. These include stromatolites (lithified layered organosedimentary structures produced by the activities of mat-building microbial communities[33]) and also such structures as tracks, trails, burrows, and carbonate microborings that evidence the presence of living systems but are themselves not organismal remnants;[34-36] see Figure 1.2. Fossil evidence on this scale is unlikely to be found in the martian samples, although the possibility cannot be ruled out that a fragment of an ancient stromatolite will be found.

[33]Knoll, A.H., and Golubic, S. 1979. Anatomy and taphonomy of a Precambrian algal stromatolite. Precambrian Res. 10: 115-151.

[34]Schopf, J.W. 1992. Paleobiology of the Archean, p. 25 in The Proterozoic Biosphere, a Multidisciplinary Study, J.W. Schopf and C. Klein, eds. Cambridge University Press, New York.

[35]Walter, M.R. 1983. Archean stromatolites: Evidence of the earth's earliest benthos, p. 187 in Earth's Earliest Biosphere, Its Origin and Evolution, J.W. Schopf, ed. Princeton University Press, Princeton, N.J.

[36]Walter, M.R., Grotzinger, J.P., and Schopf, J.W. 1992. Proterozoic stromatolites, p. 253 in The Proterozoic Biosphere, a Multidisciplinary Study, J.W. Schopf and C. Klein, eds. Cambridge University Press, New York.

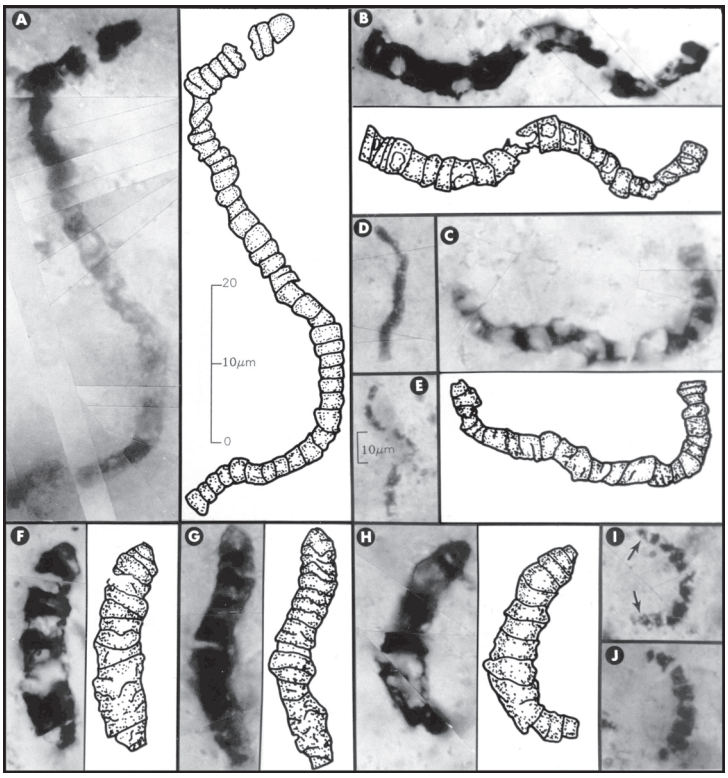

FIGURE 3.6 Carbonaceous prokaryotic fossil microorganisms, with interpretive drawings, in 3.46-billion-year-old siliceous rocks (chert) from Western Australia. All these fossils are completely encased in chert, which has been made into petrographic thin sections, rendering them visible under the microscope. The 10-μm scale bar in E is also applicable to D, I, and J; all other images are to the scale shown in A. SOURCE: Reprinted with permission from Schopf, J.W. 1993. Microfossils of the early Archean Apex chert. Science 260: 640-646. Copyright 1993 by the American Association for the Advancement of Science.

CLOSING OBSERVATIONS

In summary, there are significant limits on the ability to detect life, even in samples that are in-hand, on Earth. Methods based on molecular biology and PCR provide some of the best tools for detecting terrestrial contamination of samples, or Mars life that had an ancient common origin with terrestrial life. A variety of chemical, physical, and visual examinations provide some chance of detecting novel forms of life, but they all suffer from limitations in sensitivity, generality, and/or throughput. Due to the limited amount of any sample that can be devoted to most of the forms of testing (recognizing that the tests will irreversibly alter the sample), there will be major limitations on the statements that can be made about the absence of life in any unsterilized sample.

Further, while the techniques described in this chapter can demonstrate or suggest the presence of life in martian samples, none of them can conclusively prove the *absence* of life, in light of the unknown character of hypothetical Mars life.[37]

[37]Knoll, A.H. 1999. Overview, pp. 1-3 in Space Studies Board, National Research Council, Size Limits of Very Small Microorganisms: Proceedings of a Workshop. National Academy Press, Washington, D.C.

4

Quarantine Strategy

While the likelihood is small that any of the returned martian samples will contain evidence of past or present martian life, if such evidence *were* to be found, the intellectual rewards would be profound. For this reason the search for evidence of biological activity in the returned samples must be given a high priority, along with biohazard assessment if life is detected. Yet sample processing during quarantine must achieve other goals as well, primary among them a preliminary analysis and inventory of the soil, rocks, and drill-core specimens returned. To meet all these needs, sample-processing protocols (see Box 4.1) must be developed, to be carried out in the quarantine facility, which will (1) yield a properly documented inventory of the returned samples; (2) assure biological containment of these materials and prevent their contamination with terrestrial microorganisms and other matter, organic or inorganic; and (3) facilitate a rigorous search for evidence of biological activity.

An important part of the protocols will define the manner in which returned martian material is sampled for life-detection experiments. The initial inventory of martian materials should place them in a finite number of sensibly different categories (e.g., individual rock cores and soil samples taken from different locations in the sampling area); an aliquot of material from each of these categories should be tested for evidence of biological activity. The amount of material to be sacrificed for this purpose from each sample category will be specified by the life-detection protocol. Testing should proceed from the largest sample category, of which the smallest fraction of material needs to be devoted to life detection, to the smallest category; if results are consistently negative for the larger sample categories, justification may be found for reducing the amounts of the smaller sample categories used for life detection.

TRIAGE FOR THE MARS SAMPLES

The first step in searching for evidence of biological activity in each category of martian material should be analysis for its content of organic carbon (Chapter 2). Because the laboratory facilities required for precise analysis for total and organic carbon are too large to be included in a minimal BSL-4 quarantine facility, sample aliquots should be sterilized,[1] certified, and removed from the quarantine facility for analysis elsewhere. Prelimi-

[1]Throughout this report, COMPLEX uses the words "sterilized" and "sterilization" as being synonymous with treatment by heat and/or gamma radiation to such a level as to kill any known terrestrial organism.

Box 4.1
Protocol

Among other meanings of the word, a protocol is a written detailed plan formally specifying each step in a multistep scientific or medical procedure. For samples handled in the Mars Quarantine Facility, protocols must be written for procedures to:

1. Sterilize and cleanse of organic contamination the quarantine facility, prior to introduction of the Mars samples;
2. Place samples in the facility;
3. Inventory and carry out preliminary analyses of the samples;
4. Search for evidence of biological activity;
5. Assess whether the samples contain biohazardous material;
6. Sterilize aliquots of the samples in preparation for their removal from the facility;
7. Remove samples from the facility; and
8. Store samples within the facility.

Prior to use of such procedures on martian samples, the efficacy of each protocol must be firmly established by appropriate testing and then formally certified as ensuring the capability to accomplish the intended task.

nary visual examination of the martian samples, using optical microscopes, can begin within the quarantine facility. In addition, certain life detection studies that cannot be done on sterilized samples, such as biohazard assessment, will have to be conducted in the quarantine facility.

Three possible answers can be foreseen to the question of whether each category of martian samples contains evidence of biological activity: *yes*, *no*, or *uncertain*.

Unequivocal Evidence of Biological Activity (Considered the Least Likely Case)

It is possible, although COMPLEX considers it very unlikely, that the samples returned from Mars will be found to contain an unmistakable biological signature. This could take the form of copious amounts of organic matter and/or abundant evidence of viable or recently dead microbial life. If this turns out to be the case, the prospect of studying a whole new manifestation of life that developed on a planet other than Earth will be so overarchingly important that COMPLEX considers the samples should be dedicated in their entirety to biological studies. COMPLEX is confident that this response will also be supported by the broad scientific community and the public.

The unequivocal discovery of life in Mars samples would demand a far larger research effort than could be carried out by the quarantine personnel projected in Chapter 6, in a much more elaborately constructed physical facility than the kind that chapter recommends. COMPLEX has little doubt that funding would be made available for such a facility and such an effort, to study life on Mars. The present study does not attempt to deal with an eventuality of this sort. COMPLEX considers that planning of such a facility would be premature at this time.

Recommendation. If unmistakable evidence of life as we know it is found in the Mars samples, they should be dedicated to biological studies. Studies of the biosignatures in them should be minimal until an optimal study plan has been developed and an appropriate research facility set up and staffed. In the interim, no

aliquots of the samples should be released from the confines of the Mars Quarantine Facility unless warranted by ongoing biological studies, and the samples are sterilized.

COMPLEX anticipates that even if the martian samples are found to contain viable life, certain types of studies in specialized laboratories outside the quarantine facility will be desirable. Samples should be removed from the quarantine facility for this purpose only after they have been sterilized and effective sterilization has been certified (Chapter 5).

No Evidence of Biological Activity

The samples returned from Mars may be shown to be altogether barren of organic matter, containing no detectable organic carbon compounds and displaying no evidence of metabolic-like activities or potential infectivity, or any other evidence of past or present biologic activity. In this event, certification of unsterilized aliquots of the samples for release and study beyond the confines of the quarantine facility would be justified.

Uncertain Evidence (Considered the Most Likely Case)

The samples returned from Mars may be shown to contain small amounts of organic matter and/or evidence suggestive of viable or recently dead microbial life. Given the difficulties inherent in achieving and maintaining a stringently sterile and organically clean environment, such evidence of biological activity might represent terrestrial contamination, introduced during sample collection and/or processing. But because the nature of martian life (if any) is completely unknown, the source of the biological signal would have to be rigorously ascertained. Until the possibility of martian life is ruled out, unsterilized aliquots of the samples should not be released for study outside the quarantine facility.

This is by far the most likely outcome of the preliminary examination of the Mars samples. In the climate of desire to find life in the samples, researchers examining them will be reluctant to declare them unmistakably barren of viable entities, organic matter, or structures that might be fossils. The samples will be sufficiently complex to contain equivocal evidence of life, even if it is spurious. There will be ample latitude for disagreement among workers as to the biological significance of observations made. It is quite possible that a time will never come when everyone knowledgeable about the samples is satisfied that they do not contain evidence of life. An example of this depressing state of uncertainty is seen in the recent history of study of (Antarctic) Mars meteorite ALH84001: More than 5 years after possible evidence of life in the meteorite was published,[2] agreement still has not been reached on the significance of the observations.

The remainder of this chapter describes a strategy designed for the anticipated "uncertain" case: The samples do not contain unequivocal evidence of life, but the possibility of life also cannot be firmly ruled out.

A STRATEGY FOR QUARANTINE AND DISTRIBUTION OF THE MARS SAMPLES

This strategy for quarantine and distribution assumes that the Mars samples are found to be neither manifestly barren of organic matter nor obviously the bearers of live or recently dead organisms. The condition researchers are most likely to find is ambiguity about the evidence or lack of it for life in the samples. Because ambiguity of this sort can persist for a very long time (e.g., the debate about the significance of putative artifacts of life in martian meteorite ALH84001, just referred to), COMPLEX considers it undesirable to delay the release of returned samples in any form from quarantine for a period that will be indefinite but measured in years. Scientists who have prepared their laboratories and staffs to study the samples should be allowed to begin work on them, and the results of their studies will provide important feedback for the planning of later Mars missions. Studies in specialized laboratories outside the quarantine facility will also be essential to the continuing search for evidence

[2]McKay, D.S., Gibson, Jr., E.K., Thomas-Keprta, K.L., Vali, H., Romanek, C.S., Clemett, S.J., Chiller, X.D.F., Maechling, C.R., and Zare, R.N. 1996. Search for past life on Mars: Possible relic biogenic activity in martian meteorite ALH84001. Science 273: 924.

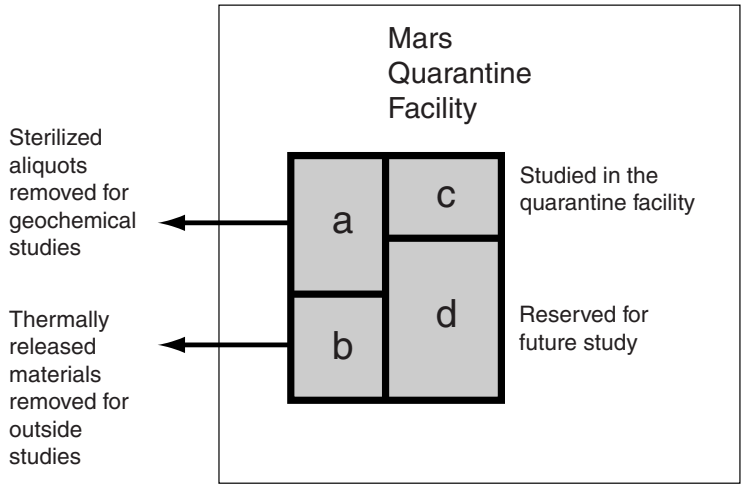

FIGURE 4.1 Recommended uses of Mars samples containing only equivocal evidence of life. *Schematic diagram*, including the box sizes.

of life in the samples. Suitably sterilized samples should be released promptly from the quarantine facility; these can be used for many types of research, including life-detection studies that are not wholly compromised by sterilization.

There is a conservative and prudent strategy that will permit studies of the samples to begin as soon as their preliminary examination in the quarantine facility has been completed, without danger of terrestrial back contamination or biological contamination of the samples, and without the expense and difficulty of trying to carry out the full range of needed sample studies within a BSL-4 containment facility (see Chapter 6). This strategy is summarized in Figure 4.1. Aliquots of the samples follow several paths, according to plans and following a schedule developed by an oversight committee of highly qualified scientists (Chapter 6). One set of aliquots (*a*) is sterilized so they can be removed promptly from the quarantine facility and used for geochemical studies. Another set of aliquots (*b*) is crushed, and any organic compounds they contain are extracted either by thermal desorption or by supercritical fluids.[3] These (presumably sterile) extracts are passed out of the quarantine facility for life detection/biomarker studies in outside laboratories (see the section below, "The Study of Extracts"). The crushed residue, which is useful for some geochemical studies, is further sterilized (to address the possibility that life might be harbored inside the crushed mineral grains) and also removed from the quarantine facility.

Some properties of the Mars samples that would be compromised by any sterilization procedure, such as their biohazard potential, will have to be studied in the quarantine facility (*c*). Finally, a portion of the samples will be reserved in the quarantine facility for undefined future studies (*d*).

Recommendation. In the likely event that initial examination of the Mars samples can neither prove nor definitively rule out evidence of life in them, plans should be in place to promptly sterilize aliquots of the samples and remove them from the Mars Quarantine Facility for biological and geochemical studies in specialized laboratories elsewhere. This action should not be deferred pending resolution of the question of whether the samples contain life or artifacts of life.

[3]In a pressure-temperature diagram, the phase boundary separating gaseous and liquid states of a substance extends only to the substance's critical pressure and critical temperature. At higher pressures and temperatures than these the substance is said to be a *supercritical* fluid, and no formal distinction can be drawn between the gaseous and liquid states. The fluid most commonly used for extracting organic compounds is carbon dioxide, whose critical temperature and pressure are 304.1 kelvins and 73.9 bars, respectively.

The Study of Extracts

Organic molecules in rocks and sediments are commonly studied after they have been physically separated from their host inorganic matrices. Typically, the rocks or sediments are washed or "extracted" with organic solvents that dissolve the organic molecules. Recovery and evaporation of the solvent leaves the organic molecules as a residue. Further separations of that residue, followed by spectroscopic analyses, can reveal the types and amounts of organic molecules that are present.[4] However, these techniques are not appropriate for preliminary analyses of the martian samples. There is a chance that the solvents will react with the inorganic matrix, especially if strong oxidants are present. In addition, the purification of solvents is notoriously difficult, the efficiency with which materials can be recovered is imperfect, and, for precious materials, the process is unacceptably wasteful. Nevertheless, obtaining the best possible information about any organic molecules that are present is clearly required as part of the preliminary analyses.

A significant portion of the organic molecules that would be recovered by extraction with organic solvents can instead be released from milligram-sized aliquots of martian samples by thermal desorption. Samples are placed in a small chamber swept with helium and heated, stepwise, to (for example) 150, 250, and 350 °C. Compounds volatilized in this way can be readily analyzed by gas chromatography-mass spectrometry (GC-MS; see Box 3.1 in Chapter 3). This procedure would recapitulate the GC-MS experiment used by the Viking landers on Mars. On Earth, 30 years later, the experiment will be far easier and still exceptionally informative. Samples are likely to be sterilized by this analytical procedure but not highly altered. The inorganic residues can be released for geochemical analyses.

More-polar organic compounds, those typically soluble in water and resembling simple cellular metabolites, proteins, and nucleic acids, would not be well characterized by the thermal-desorption plus GC-MS treatment. They can be extracted from samples in aqueous solution, but extremely pure water would be required to rule out very-low-level contamination, and water extraction cannot be expected to sterilize the material leached. Polar organic compounds should be extractable using supercritical fluids, such as liquid carbon dioxide or halocarbons. Such media can be evaporated far more readily than water, and supercritical fluid extraction is probably miniaturized more easily than water extraction. It is even possible that supercritical fluid extracts are demonstrably sterile, and research needs to be done to systematically and critically analyze the effectiveness of these solvents as sterilizing agents. Study of these techniques should be given a high priority in preparation for the preliminary analyses.

A protocol for these procedures is needed that defines how Mars samples are to be ground, prepared, and extracted. With such a protocol in place it will be possible to remove extracted organic compounds from quarantine, which will permit a wide variety of analytical studies of any organic compounds found. These studies can test for terrestrial contamination, differentiate between abiotic and biotic signatures in sets of compounds, search for biomarkers, and provide feedback to life detection experiments. In addition, isotopic studies of bulk extracts, as well as individual organic compounds, will provide evidence to test whether the organic compounds are indigenous and whether they contain evidence of possible biological isotopic fractionation.

> ***Recommendation.*** It is important that a program of research be conducted to determine the efficacy of supercritical fluids and commonly used organic solvents in killing organisms. It is highly desirable to be able to remove solvent extracts from quarantine without the damage to dissolved biomarker compounds that would be caused by heat or ionizing radiation. Sterilization probably is systematically achieved by the supercritical fluids used in making extracts, but this needs to be verified before extracts can be removed from the Mars Quarantine Facility.

Studies to Be Conducted in the Quarantine Facility

Certain studies of the Mars samples, including biohazard testing, cannot be carried out on sterilized samples. These studies will thus have to be performed within the quarantine facility (category *c* in Figure 4.1), and it will be

[4]Moldowan, J.W., Albrecht, P., and Philp, R.P. 1992. Biological Markers in Sediments and Petroleum. Prentice-Hall, Englewood Cliffs, N.J. 411 pp.

necessary to include the needed equipment and personnel in a design for the facility. Most particularly, these studies will include experiments that seek to demonstrate the presence of microbial life by methods that are conducive to microbial replication with resulting expansion of the numbers of viable organisms, or their genomes (Chapter 2). Examples of these experiments are as follows:

1. Attempts to isolate infectious microbes by providing environmental conditions and substrates for growth, either conditions that resemble those on Mars or terrestrial conditions that the new microbe might find hospitable;
2. Attempts to infect animals, plants, cell cultures derived from terrestrial life forms, or bacteria in which a martian genome could replicate; and
3. Attempts to detect genomes (either RNA or DNA) by polymerase chain reaction (PCR) or similar techniques in which all or part of the genome is amplified by several orders of magnitude.

Because of constraints both on the amount of sample to be returned and on the range of equipment that can be available in the quarantine facility, the experimental protocol should focus on molecular biological techniques (e.g., PCR) and minimize to the extent feasible experiments involving whole organisms.

Use of PCR within the quarantine facility will require special precautions. Because the technique is extremely sensitive it is vulnerable to false positives caused by contamination. PCR experimentation should be carried out by specially certified technicians in a dedicated space within the quarantine facility which is effectively isolated from activities elsewhere in the facility.

In the event that Mars samples contain organic carbon yet are found to be incapable of infecting the limited range of species or ecosystems that can be tested in the BSL-4 quarantine facility, unqualified release of the samples is not justified. Such samples should remain in the "uncertain" category of life detection. However, it may be desirable to downgrade the required level of biocontainment for those samples from BSL-4 to BSL-3 (see Chapter 6), because the less-stringent standards of BSL-3 permit a larger facility in which more complex testing can be conducted (e.g., examination of the impact of martian material on a model of the marine microbial ecosystem). A sufficiently broad range of testing at this level could conceivably justify the release of unsterilized material containing martian organic compounds from quarantine, if any remains after the program of testing.

Release of Samples from the Quarantine Facility

The primary charge to COMPLEX in defining the present study was to consider the question, *What are the criteria that must be satisfied before martian samples can be released from the facility?*

Summarizing the content of this chapter, COMPLEX recommends the following:

Recommendation

- If the samples returned from Mars contain evidence of life, or if evidence of life is equivocal (e.g., organic matter is present), aliquots that have been treated by the application of heat and/or gamma radiation to levels more than adequate to kill any known terrestrial organism (Chapter 5) should be certified for release from the Mars Quarantine Facility.
- If the samples contain evidence of life, or if evidence of life is equivocal, removal of untreated aliquots from the Mars Quarantine Facility for transfer to approved containment laboratories elsewhere should not be excluded, on the condition that containers and transfer procedures conform to protocols established by a panel of experts (e.g., from the Centers for Disease Control and Prevention) in containment.

Here "approved containment facilities elsewhere" refers principally to the case where a major international partner in the Mars sample return program wishes to establish an independent BSL-4 facility in which to study untreated samples (see Chapter 6).

- If the samples are shown to be altogether barren of organic matter, to contain no detectable organic carbon compounds and no other evidence of past or present biological activity, untreated aliquots of the samples should be released for study beyond the confines of the Mars Quarantine Facility.

5

The Sterilization of Samples from Mars

The array of instrumentation and the number of personnel needed to carry out a definitive study of the Mars samples are both very large. It is not practical to construct a quarantine facility large enough to house them. It is essential that a means be found to safely remove aliquots of the samples from the quarantine facility and distribute them to qualified members of the international scientific community, so that the full complement of modern scientific instrumentation and individual talents can be brought to bear on the analysis and interpretation of the samples. The prospect of using the large and very sophisticated laboratory instruments available throughout the world for this purpose was the main justification for returning samples to Earth rather than trying to study Mars materials in situ on that planet. Even if life detection were the only scientific issue that needed to be addressed, not all of the required life-detection work could be carried out in the quarantine facility. Moreover, research beyond life detection cannot be ignored: Geochemical studies of the samples will be very important, to provide a sample context for the scientists searching for life, and also to expand current knowledge of the geologic history of Mars and allow a more informed choice of landing sites for subsequent sample-return missions.

COMPLEX recommends sterilization[1] of a subset of the samples as a means of safely transferring them into the laboratories of the international scientific community. Commonly employed techniques of sterilization are reviewed in Table 5.1. The techniques that appear to be best suited for this application are gamma irradiation and dry-heat sterilization. Sterilization techniques that involve gases and liquids were not chosen because they lack the ability to penetrate to the centers of the samples. Electron-beam irradiation was excluded both because of its limited penetration and the size and expense of the equipment necessary. Both gamma-ray sterilization and dry heat have the advantages that they penetrate to the centers of samples, and they can be implemented on a relatively modest scale.

DAMAGE TO SCIENTIFIC INFORMATION IN SAMPLES AS A RESULT OF THEIR STERILIZATION

The sterilization techniques listed in Table 5.1 degrade samples to varying degrees, causing a loss of the scientific information in them. Some scientific studies are little affected by heat (less than 150 °C) or gamma-ray

[1]Throughout this report, COMPLEX uses the words "sterilized" and "sterilization" as being synonymous with treatment by heat and/or gamma radiation to such a level as to kill any known terrestrial organism.

TABLE 5.1 Potential Sterilization Techniques

	Conditions	Applicability		Problems
		Surface	Interior	
Heat				
Dry[a]	135 °C, 24 h	Yes	Yes	Alters organics, volatilizes
Wet/steam	125 °C, 24 h	Yes	Yes	Alters organics, volatilizes
Radiation				
Gamma (^{60}Co)	>1 Mrad	Yes	Yes	Alters organics
Electron beam	—	Yes	Limited	Large facility; untested[b]
Alkylating chemicals				
Formaldehyde	Liquid, 80 °C	Yes	No	Residual organics
Ethylene oxide	Vapor, 60 °C	Yes	No	Residual organics
Oxidizing chemicals				
Hydrogen peroxide	Vapor, 50 °C	Yes	No	Some residuals? Untested[b]
Chlorine dioxide	Gas, 50 °C	Yes	No	Some residuals? Untested[b]
Ozone	Gas, 50 °C	Yes	No	Some residuals? Untested[b]
Peracetic acid	Liquid, 50 °C	Yes	No	Some residuals? Untested[b]
Hydrogen peroxide/plasma	50 °C	Yes	No	Some residuals? Untested[b]
Mixed chemical/plasma	50 °C	Yes	No	Some residuals? Untested[b]

[a]Method used to reduce bioload on the Viking spacecraft, 1976.
[b]Untested on rock-soil-microbial mixes.

irradiation. These include most of the inorganic (geochemical, isotopic, and mineralogic) analyses. An exception to this is studies of volatile components such as adsorbed water. Water and other volatile components will certainly be lost by heat sterilization. Probably the most serious compromises occur for the organic compounds in the samples, which is unfortunate because these include the biomarkers that researchers hope will yield information about life processes on Mars. Many of the organic compounds are very labile and are destroyed by heat or radiation.

Table 5.2 lists the compounds that are of interest and indicates current understanding of the effect of different types of sterilization on them. As can be seen, many of the compounds will either be destroyed by the process or at least compromised to the point that the analyses may be hard to interpret. It is also apparent that there is much uncertainty about the extent of damage caused to organic compounds by sterilizing procedures. One mitigating circumstance is that many of the biomarker compounds can be extracted by thermal desorption and supercritical fluids within the quarantine facility and, pending verification that the procedure effectively sterilizes the extracts produced, the latter can be distributed to laboratories for studies without further treatment (Figure 4.1; see also "The Study of Extracts" in Chapter 4).

Recommendation. A program of research should be initiated to determine the effects on organic compounds in rocky matrices, and also on microscopic morphological evidence of life, of varying degrees of application of heat and gamma irradiation. This research should be started well in advance of the return of the Mars samples, so that treatment protocols can be designed intelligently and data obtained from analyses of treated samples can be interpreted with minimal ambiguity.

INTENSITY OF STERILIZATION

The question of intensity of sterilization is crucial, but the choice of actual parameters is an issue of implementation and is beyond the scope of this report. Qualitatively, very vigorous sterilization measures should be used, as

TABLE 5.2 Effect of Sterilization Treatments on Biomarker Compounds (and Kerogens)

	Heating in Dry Inert Atmosphere			^{60}Co γ-irradiation			
Compound Class	12 h at 125 °C	3 d at 125 °C	7 d at 150 °C	0.3 Mrad	300 Mrad	Extractability[a]	Thermal Desorption
Amino acids						√	
Decomposition[b]	√	√	√	√	X		
Racemization[c]	√	√	√	√	X		
Sugars	X[d]	X[d]	X[d]	X?	X?	√	e
Nucleotides	√?	√?	X?	X	X	√	e
Unsaturated lipids	√?	√?	√?	?	?		√
Aliphatic hydrocarbons	√	√	√	X?	X?		√
Tetrapyrroles	X?	X	X	?	?		e
Carotenoids	X?	X	X	?	?		e
Geobiopolymers (kerogen)	√	√	√	√?	√?		f

NOTE: √, survives; X, destroyed.

[a]Capacity for being extracted, at least partially, by washing the sample with polar solvents.
[b]Stable (?) at 150 °C under wet conditions.
[c]*Racemization* is the destruction of chirality: numbers of right- and left-handed molecules are randomized. Under wet conditions, racemization occurs rapidly at 125 °C.
[d]Dehydrated to form secondary products.
[e]Characteristic secondary products released.
[f]Presence detectable by release of simple products at high temperatures.

it will be difficult to assess the effectiveness of any more modest sterilization conditions. For the case of dry heat sterilization, one plausible standard is that commonly used in the surgical suites of hospitals. This is a stringent standard because the tools sterilized have come from the hospital environment, a very dirty environment with respect to microbial contamination, and after sterilization they will be inserted into patients with weakened immune systems. The typical conditions used in a hospital steam autoclave are 121 °C for 15 minutes or 134 °C for 4 to 5 minutes. The contact time may be lengthened for substantial loads. For dry heat sterilization the temperature and contact times vary from 170 °C for 1 hour to 140 °C for 3 hours. A more conservative treatment may be considered necessary for the martian samples.

Gamma irradiation devices small enough to be incorporated in a quarantine facility are obtainable (Figure 5.1). Gamma irradiation is sometimes used to eliminate specific classes of organisms from foodstuffs, although total sterilization is not attempted because the doses needed can make food rancid and unpalatable. Table 5.3 lists some useful and/or critical gamma irradiation doses and their effects.

However, the target organisms in food sterilization are relatively vulnerable to the effects of ionizing radiation, and the possibility must be entertained that organisms with much greater resistance may have survived on Mars. Attention has been drawn to the terrestrial bacterium *Deinococcus radiodurans* (Figure 5.2), which is in this category. Comparison of Figure 5.3 with Table 5.3 shows that *D. radiodurans* is vastly more resistant to radiation than are other familiar organisms. The case has been made that if life has formed and survived on Mars, it is probably in the form of species that have the same exceptional ability to repair genetic damage that *D. radiodurans* has (see Appendix A). Note that the radiation dose required to kill *D. radiodurans* is sufficient to kill viruses (Table 5.3).

One factor that should temper speculation about organisms able to survive radiation sterilization is that if they are present on or near the surface of Mars (a necessary condition for them to be included in a returned sample), then similar material has already been transported to Earth in the form of martian meteorites (see Box 5.1). Mars

FIGURE 5.1 Example of a small gamma-irradiation sterilizer: the Gammacell 220 Excel research irradiator, marketed by MDS™ Nordion™, Kanata, Ont., Canada. The unit is 2.1 m (7 ft) tall and weighs 4,000 kg (8,818 lb). SOURCE: Figure courtesy of MDS™ Nordion™.

TABLE 5.3 Some Gamma-ray Irradiation Doses and Their Effects or Applications

Dose (Mrad)	Effect or Application
0.0003-0.0004	50% killing of human beings exposed and not given medical treatment[a]
0.005	Will promptly kill human beings by central nervous system damage[a]
0.005-0.015	Used to inhibit sprouting of white potatoes[b]
0.02-0.05	Used for control of mold in wheat flour[b]
0.02	90% killing[c] of *Salmonella typhimurium* (potentially pathogenic bacterium)[d]
0.024-0.031	90% killing of *Escherichia coli* O157:H7 (a pathogenic bacterium)[e]
0.04-0.08	90% killing of *Salmonella typhimurium*[f]
0.03-0.1	Used to control *Trichinella spiralis* (parasites) in pork[b]
0.05	90% killing of a typical yeast or fungus[d]
0.07	90% killing of *Salmonella typhimurium*[g]
0.08	90% killing of a typical growing bacterial culture[h]
0.1	Used to control insects and increase shelf life of fruits and vegetables[b]
0.1-0.3	Equivalent to 10,000 years in space

continued

TABLE 5.3 Continued

Dose (Mrad)	Effect or Application
0.1-0.5	90% killing of the insects in a population[d]
0.15	90% inactivation of Ebola virus[i]
0.15-0.3	Used to reduce bacterial pathogens (especially *Salmonella*) in poultry[b]
0.2-2	Used for control of *Salmonella* in animal feed and pet food[j]
0.24-0.33	90% killing of Gram-positive anaerobic spore-forming bacteria[d]
0.33	90% inactivation of Polio virus[k]
0.37	90% killing of the cells in a deep-frozen bacterial culture
0.45	Used to reduce bacterial pathogens in red meat[b]
0.53	90% inactivation of hoof-and-mouth disease virus[l]
1.04	90% killing of *Deinococcus radiodurans* cells (one of the most radiation-tolerant organism known)[h]
1.07	90% inactivation of minute virus of mice[l]
2–5	90% inactivation of the molecules of a typical enzyme[d]
2.5	Inactivation of HIV-I by a factor of 10^{-5} to 10^{-6} [m,n]
2.5	Dose recommended by International Atomic Energy Agency for the sterilization of medical products
3.0	Sufficient to destroy HIV-I DNA as assayed by PCR[o]
3.0	Used to kill insects and decontaminate dry herbs and spices[b]
4.4	Used to sterilize meat frozen and packaged for NASA[p]

NOTE: Some of the values given can serve only as guidelines, since the values for the radiation sensitivity of specific organisms vary significantly in the literature (see van Gerwen, S.J., Rombouts, F.M., van't Riet, K., and Zwietering, M.H. 1999. A data analysis of the irradiation parameter D10 for bacteria and spores under various conditions. J. Food Prot. 62: 1024-1032). For example, it is very commonly stated and published that *Deinococcus radiodurans* has "100% survival at 1.5 Mrad," yet published values for 90% killing range from 0.5 to 1 Mrad.

[a]See <http://www.jlab.org/div_dept/train/rad_guide/effects.html>.
[b]See <http://www.cdc.gov/ncidod/dbmd/diseaseinfo/foodirradiation.htm>.
[c]The dose at which 90% of the individuals in a population are killed is called D10 (dose of 10% survival).
[d]See <http://www.agen.ufl.edu/~chyn/age4660/lect/lect_27/radiatio.htm>. But see also *f* and *g*.
[e]Clavero, M.R., Monk, J.D., Beuchat, L.R., Doyle, M.P., and Brackett, R.E. 1994. Inactivation of *Escherichia coli* O157:H7, salmonellae, and *Campylobacter jejuni* in raw ground beef by gamma irradiation. Appl. Environ. Microbiol. 60: 2069-2075.
[f]U.S. Food and Drug Administration. 1997. Irradiation in the production, processing and handling of food. Federal Register 62(232): 64107-64121. But see also *d* and *g*.
[g]See <http://ans.neep.wisc.edu/~ans/meetings/98-99/mathews.html>. But see also *d* and *f*.
[h]van Gerwen, S.J., Rombouts, F.M., van't Riet, K., and Zwietering, M.H. 1999. A data analysis of the irradiation parameter D10 for bacteria and spores under various conditions. J. Food Prot. 62: 1024-1032.
[i]Elliott, L.H., McCormick, J.B., and Johnson, K.M. 1982. Inactivation of Lassa, Marburg, and Ebola viruses by gamma irradiation. J. Clin. Microbiol. 16: 704-708.
[j]See <http://www.foodsafety.org/ga/ga022.htm>.
[k]Kaupert, N., Burgi, E., and Scolaro, L. 1999. Inactivation of poliovirus by gamma irradiation of wastewater sludges. Rev. Argent. Microbiol. 31: 49-52.
[l]House, C., House, J.A., and Yedloutschnig, R.J. 1990. Inactivation of viral agents in bovine serum by gamma irradiation. Can. J. Microbiol. 36: 737-740.
[m]Salai, M., Vonsover, A., Pritch, M., von Versen, R., and Horoszowski, H. 1997. Human immunodeficiency virus (HIV) inactivation of banked bone by gamma irradiation. Ann. Transplant. 2: 55-56.
[n]Hiemstra, H., Tersmette, M., Vos, A.H., Over, J., van Berkel, M.P., and de Bree, H. 1991. Inactivation of human immunodeficiency virus by gamma radiation and its effect on plasma and coagulation factors. Transfusion 31: 32-39.
[o]Fideler, B.M., Vangsness, C.T., Jr., Moore, T., Li, Z., and Rasheed, S. 1994. Effects of gamma irradiation on the human immunodeficiency virus. A study in frozen human bone-patellar ligament-bone grafts obtained from infected cadavera. J. Bone Jt. Surg., Am. 76: 1032-1035.
[p]See <http://www.wisc.edu/fri/foodirrd.htm>.

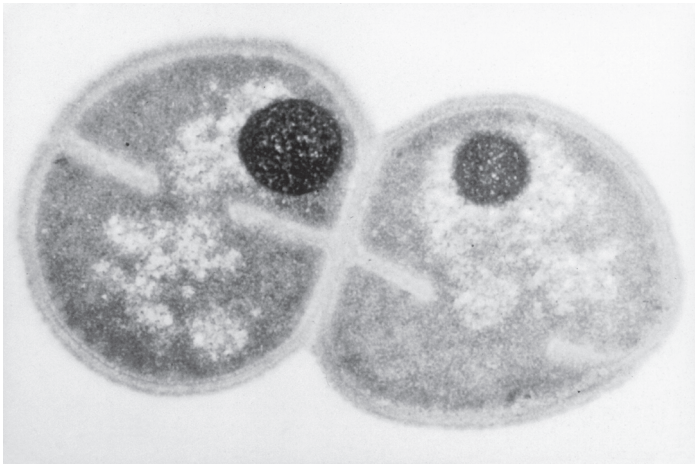

FIGURE 5.2 A pair of *Deinococcus radiodurans* cells, in the act of dividing into a tetrad. Transmission electron microscope image of a cross section of the cells, approximately 3 micrometers wide. SOURCE: Image courtesy of M.C. Henk and J.R. Battista.

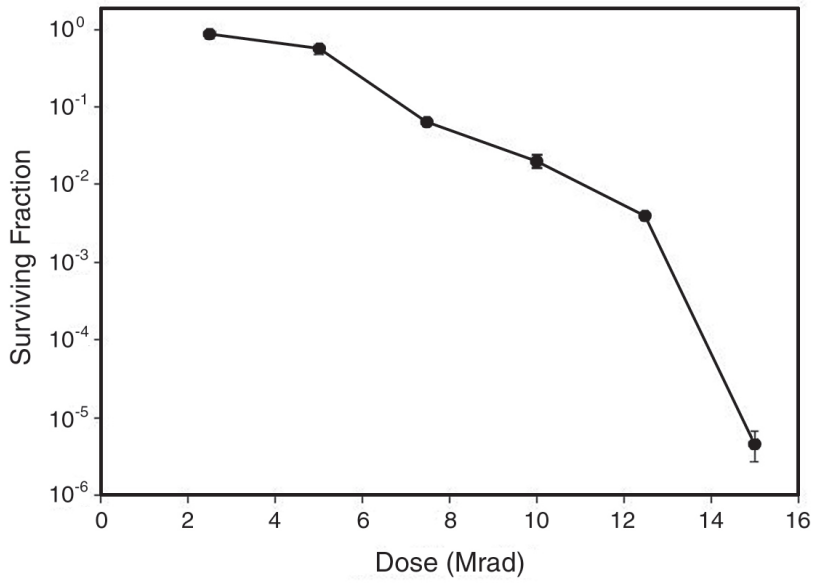

FIGURE 5.3 "Killing curve" for *Deinococcus radiodurans* in a state of desiccation, when it is most resistant to gamma radiation. Colonies of *D. radiodurans* were dried on glass plates at 5% relative humidity for a week, irradiated in the dry state, and then rehydrated and cultured. Exposure to 17.5 Mrad of radiation left no detectable viable organisms. SOURCE: Unpublished data of J. Battista, Louisiana State University. See also Auda, H., and Emborg, C. 1973. Studies on post-irradiation degradation in *Micrococcus radiodurans*, Strain RII5. Rad. Res. 5: 273-280.

Box 5.1
Meteorites from Mars

Most meteorites that fall to Earth are fragments of asteroids. However, about 0.5% of the meteorites that fall, the SNC category of meteorites, are pieces of Mars. The young radiometric ages of SNC meteorites require that they come from a planet large enough to retain some geologic activity, not from asteroids, which cooled and became inactive soon after they were formed. Mars as a source of the SNCs was confirmed when one of them was found to contain noble gases in the same proportions and with the same isotopic compositions as noble gases in the martian atmosphere (which had been analyzed by a Viking lander in 1976).[1]

Some of the debris from cratering impacts on the surface of Mars, derived from a range of depths in the planet, is launched into space. The heliocentric orbits of the debris fragments evolve with time because of gravitational perturbations and random collisions they experience. Some eventually become Earth-crossing orbits, and the fragments in these orbits may fall to Earth as SNC meteorites.[2] The abundances of cosmic-ray-produced isotopes in known SNC meteorites show that they wandered in space, absorbing cosmic rays, for 0.5 to 15 million years between the time they were broken out of Mars and the time when they fell to Earth.[3]

[1]Bogard, D.D., and Johnson, P. 1983. Martian gases in an Antarctic meteorite? Science 221: 651-654.
[2]Gladman, B.J., Burns, J.A., Duncan, M., Lee, P., and Levinson, H.F. 1996. The exchange of impact ejecta between Terrestrial Planets. Science 271: 1387-1392.
[3]Eugster, O., Weigel, A., and Polnau, E. 1997. Ejection times of Martian meteorites. Geochim. Cosmochim. Acta 61: 2749-2757.

meteorites that were 0.5 to 15 million years in transit to Earth (Box 5.1) have absorbed 10 to 300 Mrad of cosmic radiation.[2] The high end of this range of doses almost certainly would be adequate to kill any organisms they might contain. However, the low end of the range, 10 Mrad, is not enough to kill some known strains of terrestrial organisms (see Figure 5.3). And the SNC meteorites that have been collected and studied represent only a tiny fraction of the Mars material that has been transported to Earth; some of this material is certain to have come to Earth within less than 0.5 million years, and it received a smaller radiation dose than 10 Mrad. (Indeed, orbital simulations show that some martian ejecta immediately enter Earth-crossing orbits, and a portion of that almost certainly is delivered to Earth in less than a few years.[3]) If this material contained organisms sufficiently resistant to radiation to survive the relatively small dose they received, then Earth already has been infected by those organisms.[4]

In practice the sterilization dose used should be the minimum dose that will kill everything known, with some extrapolation factor for the possibility of unknown, more resistant, life forms. The key question is the size of the extrapolation factor. Before *D. radiodurans* was discovered, the most radiation-resistant organisms known were about a factor of 10 less resistant than it is. Thus extrapolation of a sterilizing irradiation dose by a factor of 2, or even 5, from that adequate to kill the previous record holder might easily have led to a dose that would not effectively sterilize a sample containing *D. radiodurans*. (Radiation resistance of *D. radiodurans* appears to have been a consequence of adaptation to conditions of severe dessication.[5]) However, in spite of previous bad

[2]Clark, B.C. 2001. Planetary interchange of bioactive material: Probability factors and implications. Origins of Life and Evolution of the Biosphere 31: 185-197.
[3]Gladman, B. 1997. Destination: Earth. Martian meteorite delivery. Icarus 130: 228-246.
[4]Gladman, B., and Burns, J.A. 1996. Mars meteorite transfer: Simulation. Science 274: 161-162.
[5]Mattimore, V., and Battista, J.R. 1996. Radioresistance of *Deinococcus radiodurans*: Functions necessary to survive ionizing radiation are also necessary to survive prolonged desiccation. J. Bacteriol. 177: 5232.

extrapolations, the strategy is sound. There must be a dose at which sufficient chemical damage is done to a cell for recovery to be impossible.

VERIFICATION OF STERILIZATION

The verification and *certification* that a sample does not constitute a biohazard is a difficult issue. Just as there are substantial limitations on researchers' ability to detect life (Chapter 2), there are corresponding limitations on their ability to verify that sterilization has been successful. In essence, it is not logical to expect to verify that an organism has been killed if researchers are incapable of growing it. However, it is possible to certify that samples have been effectively sterilized even without the detection of life and subsequent killing.

Three cases can be considered: (1) abundant life is detected before sterilization, (2) little life is detected before sterilization, and (3) no life is detected before sterilization. However, it will be seen that in many respects, all samples will need to be treated as in the third case.

If abundant viable life is detected ("viable" meaning than it can be cultivated, or at least it can perform reactions that require the input of metabolic energy), then preliminary experiments can be performed to produce a "killing curve," quantifying the efficiency of killing as a function of the sterilization dose (see, for example, Figure 5.3). Extrapolation of the killing curve to doses for which substantially less than one survivor would be expected in the total sample provides a lower bound on the sterilizing dose. (In practice, it is more conservative to assume that the sample might contain an undetected more resistant organism, and that the actual dose needed will be greater than this. However, it is important to demonstrate that the treatment really is killing all the types of organisms known to exist in the sample.) As part of verifying the sterilization of a given subsample, a small portion is tested after sterilization to verify that the organisms initially present are no longer viable. The test aliquot can be removed after sterilization, or it can be removed before sterilization, packaged in the same manner as the balance of the sample, and sterilized simultaneously in the same device. This test is only meaningful if the organisms in the presterilized samples are sufficiently abundant that the test sample is expected to have a large number of viable organisms prior to sterilization, so killing them makes an observable difference.

However, if life is not very abundant in the sample, a small subsample might lack life simply because of sampling statistics, not as a result of the sterilization treatment. In this case, measuring loss of life is impractical. The same would be true if no life at all were detected to begin with. In these cases, and in the first case as well, it is appropriate to assume that the sample contains some small amount of an undetected, sterilization-resistant organism at the outset. Then the main issue is devising a model for the organism that is "realistic." For example, scientists know the thermal tolerance of many organisms and do not know of any that comes close to surviving sterilization at 150 °C for several hours. Similarly, *Deinococcus radiodurans* is the most radiation-resistant organism known, and researchers know what dose is required to kill a particular number of cells with a given confidence. Whatever sterilization regime is chosen for the samples, it is necessary to physically demonstrate that it does kill the most resistant organisms known. So if we want 99.99% confidence that we can kill all of the 10^8 *D. radiodurans* cells in a Mars sample (the expected number of surviving cells is less than 10^{-4} per 10^8 input cells, which is equivalent to $<10^{-12}$ per input cell), we should demonstrate that there are no surviving cells out of the $\sim 10^{12}$ cells initially present in an irradiated test sample.

The preceding section in this chapter argued that if there are Mars organisms sufficiently robust to survive a realistic sterilization treatment in the quarantine facility, then some of these resistant organisms also would have survived transit to Earth in meteorites, and our planet already has been infected by them. Thus sample certification as "effectively sterilized" is appropriately based on verifying that the treatment used kills the most resistant known terrestrial organisms, and that the treatment is at least as harsh as that experienced by recent meteorites in Mars to Earth transit. Being substantially harsher than this will not be necessary.

6

The Quarantine Facility

The quarantine facility for Mars samples called for in previous chapters has several purposes. First, it will prevent back contamination of Earth's environment by martian organisms, if the samples contain them, by sealing the samples in a biologically contained space. Second, it will prevent contamination of the samples by terrestrial organisms and chemical contaminants. Third, it will contain a laboratory and the necessary equipment for initial processing of the samples: unpacking, preliminary examination, baseline characterization, weighing, photography, splitting, repackaging, and storage. Another important operation will be the preparation of heat- and/or radiation-treated samples for distribution to the scientific community. In addition, certain life-detection studies that cannot be made on sterilized[1] samples—such as testing for biohazards—will have to be carried out in the quarantine facility. There is widespread agreement that the quarantine facility should be a biological containment facility of the most rigorous possible design, technically equivalent to the laboratories in which the most dangerous pathogens known are studied at the Centers for Disease Control and Prevention (CDC, Atlanta, Georgia) and at the U.S. Army Medical Research Institute of Infectious Diseases (USAMRIID, Ft. Detrick, Maryland).

BIOLOGICAL CONTAINMENT FACILITIES

Four categories of biological containment facilities are recognized, ranging from biosafety laboratories in which minimal common-sense practices are followed (BSL-1; a high school biology laboratory would qualify) to highly engineered, tightly sealed facilities where diseases that cause high mortality, and for which no immunization or treatment are known, are studied (BSL-4). Table 6.1 summarizes the major distinctions among the four biosafety levels. The higher levels of BSL facilities employ ventilated Biological Safety cabinets (BSCs) as work stations. These, too, are numbered, but there is no correspondence between the BSL number of a facility and the BSC number of the cabinets in it. The properties of three classes of BCSs are shown in Table 6.2.

The maximum level of biological security is furnished by a BSL-4 facility, and the Mars Quarantine Facility should be designed to that level. There are two principal forms a BSL-4 laboratory can take (Figure 6.1). (1) Inside an augmented BSL-3 facility, the samples can be enclosed in Class III Biological Safety cabinets with

[1]Throughout this report, COMPLEX uses the words "sterilized" and "sterilization" as being synonymous with treatment by heat and/or gamma radiation to such a level as to kill any known terrestrial organism.

TABLE 6.1 A Summary of the Four Formally Defined Levels of Biological Containment

	Biosafety Laboratory Level			
	BSL-1	BSL-2	BSL-3	BSL-4
Biological agents present	Well-characterized agents not known to cause disease in healthy adult humans (high school, college biology labs are BSL-1)	Agents of moderate potential hazard to personnel and environment, for example, measles virus, salmonellae, hepatitis B virus	Agents capable of causing serious or lethal disease by inhalation, for example, tuberculosis, St. Louis encephalitis virus	Dangerous and exotic agents that pose a high risk of aerosol-transmitted infections and life-threatening disease, for example, hanta, ebola, arena viruses
Building	(No restriction)	(No restriction)	Separate building or isolated zone; double-door entry; negative air pressure (inward air flow); room penetrations sealed	As in BSL-3, plus independent air supply and HEPA-filtered exhaust; rooms sealed to permit gaseous decontamination
Laboratory facilities	Designed for ease of cleaning and decontamination	As in BSL-1, plus Class II biosafety cabinets, autoclave, eyewash station available	As in BSL-1, BSL-2	Class III biosafety cabinets or Class II cabinets with spacesuits
Safety equipment	Lab coats, gloves, face and eye protection if needed	As in BSL-1, plus Class II cabinets for work with infectious agents involving aerosols and splashes, large volumes, high concentrations	As in BSL-2, plus Class II or Class III biosafety cabinets to manipulate infectious material; respiratory protection if needed	BSL-3 where applicable; interlocking double-door pass-through autoclave
Laboratory operations	Mechanical pipetting, hand washing, restricted access; no eating, drinking, smoking; splashes and aerosols minimized; daily decontamination of work surfaces; decontamination of waste, control of insects and rodents	As in BSL-1, plus emphasis on gloves, mechanical pipetting; extreme precaution with contaminated needles, sharp instruments, broken glass; use of plasticware where possible; restricted entry; biosafety training and biosafety manual specific to lab; personnel immunized, baseline serum samples taken; spills and accidents reported; supervisor is a competent scientist	As in BSL-2, plus all work in biosafety cabinets; use of bioaerosol-containing equipment; prompt decontamination of spills; personnel strictly follow guidelines, demonstrate proficiency, participate in medical surveillance	BSL-3 where applicable, plus liquid, solid waste sterilized; exhaust air HEPA filtered; all work in Class III biosafety cabinets or Class II cabinets with spacesuits; all surfaces decontaminated before removal; no infectious organisms can leave the laboratory except in sealed containers

TABLE 6.2 Summary of Properties of Three Classes of Biological Safety Cabinet

	Class of Biological Safety Cabinet		
	Class I	Class II	Class III
Interior pressure	Ambient	Ambient	Negative pressure (closed cabinet)
Air supply	Through opening for arms	HEPA-filtered supply, also through arm opening	HEPA-filtered supply only
Air exhaust	HEPA-filtered	HEPA-filtered	Doubly HEPA-filtered
Glove ports	Optional	Optional	Required

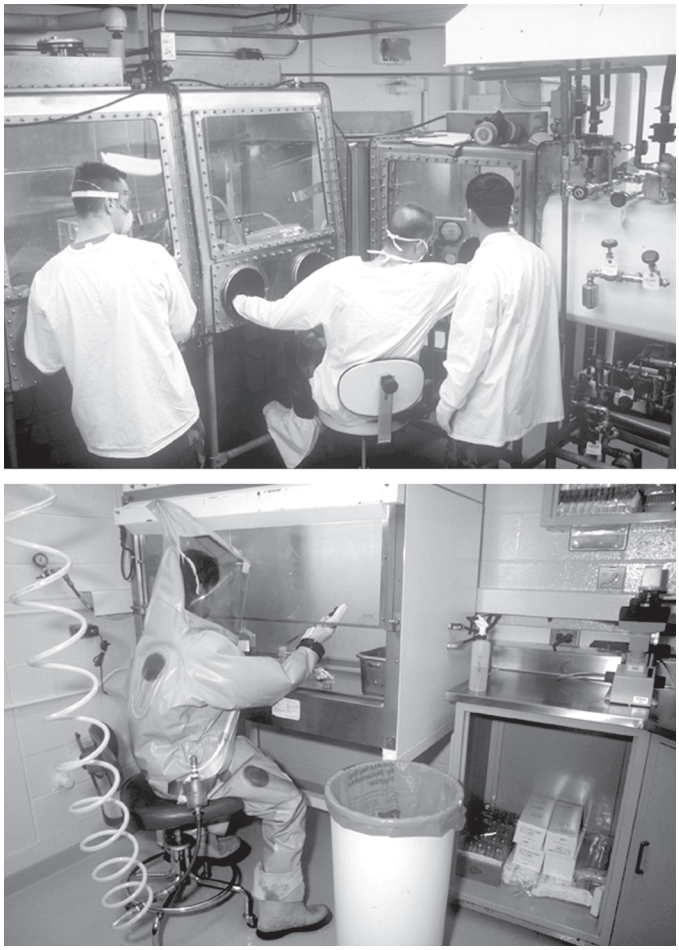

FIGURE 6.1 Different work modes in a BSL-4 laboratory at USAMRIID. At the top, hazardous samples are enclosed in a Class III Biological Safety cabinet. Below, the samples are more exposed in a Class II cabinet, but the worker is protected from them by a spacesuit. Using a glove box (Class III cabinet) is preferable for repetitive tasks that require limited lateral mobility. The spacesuit offers more flexibility, but at the cost of increased fatigue. SOURCE: Photographs courtesy of Steve Ferendo, USAMRIID.

glove ports, which are used by operators with only minimal protective garments (top of Figure 6.1). The box is typically made of stainless steel, glass, or Plexiglas, and gloves are sealed to the arm holes. (2) The samples can be manipulated in Class II cabinets that are ventilated but not tightly sealed, by operators wearing ventilated protective garments that completely isolate them from the environment they are working in (bottom of Figure 6.1). These garments are formally *positive pressure personnel protection suits,* but in practice they are called "spacesuits."

In the first case, the Class III Biological Safety cabinets have internal gas pressures that are less than atmospheric, so if there is slight leakage, the gas flow is from the surrounding room into the cabinet and pathogens in the cabinet have no path out of it. The surrounding room is effectively a large Class III cabinet itself. In the second case, the spacesuits have internal gas pressures higher than that of the room being worked in, so leaking gas flows out of the suits and pathogens in the laboratory cannot enter the suits. Ideally, a BSL-4 laboratory offers both work environments.

All waste products from BSL-4 laboratories must be specially treated. Exhaust air is passed through two high-efficiency particulate air (HEPA) filters. Liquid effluents are routinely decontaminated by a heating process before discharge to a sanitary sewer. In small operations, this decontamination can be accomplished with chemical treatment. Other waste materials are decontaminated by steam sterilization in an autoclave. Large objects (e.g., equipment) may be sterilized by exposure to gaseous formaldehyde, hydrogen peroxide, ethylene oxide, or some other sterilant. Small quantities of material are sometimes gamma-irradiated.

Some Operational Details[2]

Flexibility and adaptability are essential in the design of a BSL-4 facility. Experience has shown that the ideal BSL-4 environment is one in which a Class III cabinet line is joined to a spacesuit laboratory. After training and acclimatization, most workers prefer the flexibility of the spacesuit environment but report that they cannot perform satisfactorily for more than about 4 hours (continuously) in that environment. To the extent that segments of the work routine can be compartmentalized, those elements that require little lateral mobility can be performed with more comfort in a cabinet. The cabinet can be held at modest negative pressure; 0.98 to 0.99 atmospheres is sufficient. The gloves need be no thicker than those on spacesuits, provided that they are checked regularly for perforations.

The responsibilities surrounding a BSL-4 facility are not to be taken lightly. Engineering controls for such a system must be monitored around the clock, and support staff must be on continuous alert to correct any problems that arise. Access to BSL-4 laboratories must be controlled; passage through control points is monitored by guards who can track movement via key cards and visually, through windows to the corridors and with television monitors. Firefighters and emergency medical care providers also must be trained for the contingency that they might be required to enter an active BSL-4 suite. Backup generators to supply power to all critical systems must be available and routinely tested. The necessary systems must incorporate redundancy so that no critical system is ever compromised, even momentarily. Battery backups for such systems are also advisable, and they also should be routinely exercised and performance tested. It is also essential to have a backup supply of breathing air.

Extensive documentation regarding design and maintenance specifications, environmental impact statements, internal and external security systems, emergency response plans, and standard operating procedures (especially with regard to decontamination) must be published.

Staffing and Training

A fully functional BSL-4 facility requires more than the elaborately engineered laboratory itself. The physical plant is useless without a highly trained and dedicated cadre of scientists and support personnel. At most BSL-4

[2]The technical discussions in this subsection and in the next ("Staffing and Training") are from the experience of COMPLEX member P. Jahrling, USAMRIID. Some of the guidelines differ at other containment facilities, such as the National Center for Infectious Diseases at the CDC.

laboratories, technical staff and scientists who wish to work in that environment must first demonstrate that they can perform whatever manipulations are contemplated in a less hazardous (BSL-2) environment. Typically, these personnel begin their special immunization series (required to work at BSL-3) while working at BSL-2. After a period of several months they graduate to BSL-3, where they will work for at least several additional months. Experience has shown that a number of potential BSL-4 workers may drop out at this stage, for a variety of reasons, but others will elect to undergo the specialized training for BSL-4. This training involves familiarization with wearing and adjusting the spacesuit, and understanding the critical life support systems (breathing air supply and backups), communications, redundancy of all systems, and emergency procedures. The neophyte never works alone in the early stages of training; he is assigned a mentor who oversees his every move. Although most special situations are detailed in written procedures, it is only through personal supervision and daily reinforcement that these rules become ingrained and routine. Working with authentic pathogens removes the abstraction from such rules. It is unlikely that any worker who has not handled pathogenic agents will fully appreciate and respect the importance of strict adherence. A worker without this experience is more likely to cut corners, especially if he believes the possibility of encountering a true pathogen to be remote. At least 1 year of experience is required before BSL-4 workers should be permitted to work on their own, or to train others in the principles of bio-containment.

At the Mars Quarantine Facility, the goal of rigorous adherence to biosafety principles will be facilitated by providing a core staff of veterans to run the laboratory and serve as mentors for new recruits and visiting scientists. A policy of zero tolerance for shortcuts or lapses should be enforced. The core staff should include scientists with training similar to that of the visiting specialists, in addition to experts in biosafety, decontamination, tissue culture, media preparation, communication, and conceivably animal care.

In facilities used for the study of disease, the first indication that there has been a break in containment may come when a worker develops a febrile illness. In this case, circumstances may suggest that the prudent course is to place the worker in BSL-4 quarantine. This requires that there be a dedicated medical care facility at hand, and trained medical care providers. Again, experience with authentic agents is desirable, since highly trained workers who lack this experience sometimes panic when faced with the reality of a truly sick patient. This experience can come only from working at a facility where such patients are cared for on a routine basis.

These measures sound drastic, in the context of handling samples that are unlikely to contain life and still less likely to contain pathogenic life; but if maximum precautions are to be taken, they must be adhered to. COMPLEX concurs with the conclusion reached by earlier studies that initial BSL-4 containment is dictated for the Mars samples.[3-7]

THE QUARANTINE FACILITY FOR MARS SAMPLES

The initial processing of returned martian samples should be restricted to a BSL-4 laboratory in the quarantine facility (Chapter 6). A very modest gas-tight glove box (Class III cabinet) in a “clean room” (class 10; however, see following section) will be sufficient for this purpose. Within this cabinet, the samples should be inventoried and subdivided for various analyses. One portion of each sample should be sterilized and sent to an outside laboratory to be analyzed for total reduced carbon (organic carbon; see Chapter 2). If this analysis finds all samples to be devoid of reduced carbon, it will be reasonable to conduct almost all additional analyses under less

[3]Space Studies Board, National Research Council. 1997. Mars Sample Return: Issues and Recommendations. National Academy Press, Washington, D.C.

[4]NASA and the Jet Propulsion Laboratory. 1999. Mars Sample Handling and Requirements Panel (MSHARP) Final Report. NASA/TM-1999-209145.

[5]Mars Sample Handling, Distribution and Analysis Workshop Report. Report of a workshop organized at the request of the Mars Architecture Definition Team and held February 16-17, 1999, California Institute of Technology, Pasadena, Calif.

[6]NASA. 1990. Scientific Guidelines for Preservation of Samples Collected from Mars. NASA Technical Memorandum 4184.

[7]NASA. 1999. Mars Sample Quarantine Protocol Workshop Report, D.L. DeVincenzi, J. Bagby, M. Race, and J. Rummel, eds. NASA/CP-1999-208772.

stringent conditions of biocontainment (BSL-3). However, if additional effort is invested in isolating replicating agents by encouraging them to grow in artificial media, or to amplify their genomes by polymerase chain reaction or related techniques, this work should be restricted to BSL-4. The small original sample sizes will preclude testing in a variety of animals; animal infectivity studies will be practical only in the unlikely event that a seed stock of a microbe is successfully replicated from the minute sample of original material. The need for extensive BSL-4 facilities, especially animal facilities, is remote.

Special Requirements

Earlier studies have recognized a special problem associated with the Mars Quarantine Facility,[8-9] in that it must be not only a biological containment facility (to protect the environment), but also a clean room (to protect the samples). No existing facility combines these qualities. BSL-4 facilities are usually rather "dirty," both chemically and biologically. Clean conditions are needed to avert false biological positives, and to make precise isotopic dating and other geochemical measurements possible.

The need for cleanliness to protect the samples from terrestrial contamination is even more important now than it was at the time of the Apollo program (Appendix B). Analytical techniques have improved to the point that low levels of contamination that would have escaped detection 30 years ago are now significant signals. For example, analyses of amino acids are commonly made on samples with a sensitivity of 10^{-11} g, and 10^{-14} g can be achieved with state-of-the-art apparatus. By the time the samples are returned, it is reasonable to expect sensitivities substantially better than these. In the area of geochemical analyses of inorganic minerals, the sensitivity for measurements of isotopic ratios has improved to the point that, for example, researchers can typically measure the ratio of $^{143}Nd/^{144}Nd$ to a precision of 1 part in 10^5 on a mass of 10^{-9} g of the element. It is only because of such high sensitivity and precision that many of the new isotopic dating schemes in use have become possible. The tiniest amount of terrestrial contamination can alter the isotopic ratio of a sample to the point that application of a dating technique gives an erroneous result.

Contamination can be less of a problem when isotopic ratios are measured by secondary ion mass spectrometry (SIMS) on polished sections of rocks, which can be freshly cut and kept relatively clean. Technological improvements in the capability of SIMS have made possible some forms of isotopic dating, but these SIMS techniques are only applicable to samples where there are large chemical fractionations and thus large isotope-ratio differences. Such large fractionations have been found in samples thought to contain interstellar grains, but they are not expected in the Mars samples. Our ability to understand the Mars samples would be severely limited if quarantine or curation activities allowed contamination of the samples to a degree that precluded all dating techniques except SIMS.

The problem of combining quarantine with cleanliness is technically challenging, because contradictory measures (i.e., pressure differentials) are normally employed to produce biological containment and a clean room environment. Biological Safety cabinets are held at negative gas pressures, so leakage (which must be considered inevitable) will produce gas flow into the cabinets, away from personnel in the room with the cabinets. Clean rooms are held at positive gas pressures, relative to ambient, so gas will flow out of them and contaminants cannot flow in. Nesting the two types of enclosures (Figure 6.2) would seem to offer a first-order solution to the problem, but that stratagem raises new difficulties. If a BSL-4 cabinet is housed in a clean room, the clean room protection is partly negated because contamination from workers in the room and the ambient atmosphere is drawn by leakage into the cabinet. A clean cabinet in a BSL-4 enclosure would protect both samples and environment, but workers would have to wear spacesuits *and* use glove ports in the clean cabinet, which would limit their dexterity

[8]NASA and the Jet Propulsion Laboratory. 1999. Mars Sample Handling and Requirements Panel (MSHARP) Final Report. NASA/TM-1999-209145.

[9]Mars Sample Handling, Distribution and Analysis Workshop Report. Report of a workshop organized at the request of the Mars Architecture Definition Team and held February 16-17, 1999, California Institute of Technology, Pasadena, Calif.

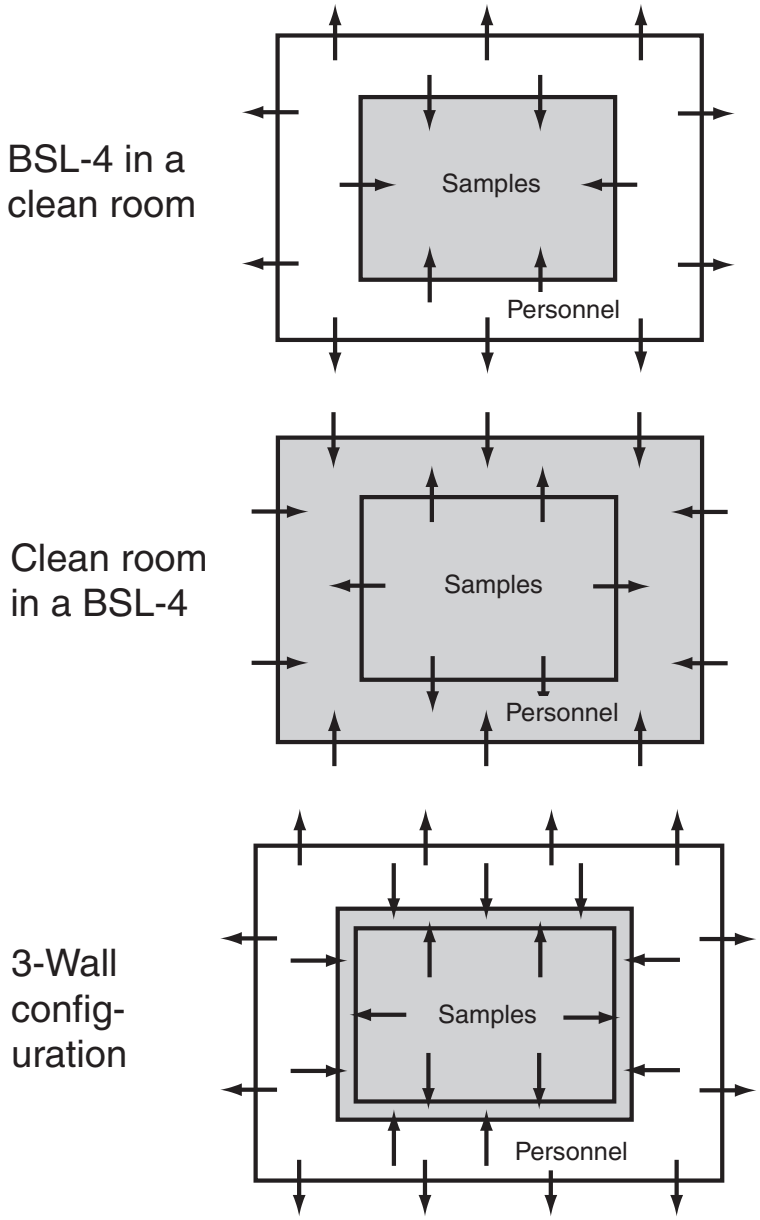

FIGURE 6.2 *Top and center:* simple options for the combination of a biological containment facility with a clean room. Arrows show gas flow (via leakage) caused by pressure differentials in the spaces shown. Gray areas are potentially contaminated by any organisms the Mars samples might contain. *Bottom:* a more complex arrangement with double walls separating workers from samples in which the sequence of gas pressures is personnel space > outside atmosphere > sample cabinet > exhaust space between double walls.

to a degree that might prevent useful work from being done. The bottom panel of Figure 6.2 suggests an approach in which double walls separate workers from the samples, with the lowest gas pressure in the space between the double walls. Leakage from both the sample cabinet and the workers' space would be collected between the walls, sterilized, and exhausted. Glove ports would have to penetrate both walls, however, and leakage of air through pinholes in the gloves would still have the potential of contaminating the samples, but gas in the sample cabinet could be monitored for traces of contaminant gases as was done in the Lunar Receiving Laboratory (Figure B.2, Appendix B), and glove ports sealed if it is detected.

A possibility that should be explored is the use of remote or robotic manipulation of samples within the containment facility, in lieu of human hands.

The solution of this problem is in the area of implementation, and beyond the scope of a COMPLEX study. However this committee recognizes the importance of the problem, and the fact that design work on a quarantine facility cannot proceed until it is solved.

Recommendation. A major obstacle to the design of the Mars Quarantine Facility is the problem of combining biological containment with clean-room conditions. It is essential that work on the solution of this problem be started immediately, to include mockups of containment/clean-room combinations whose efficacy can be tested, so that the design of a quarantine facility can proceed.

Scale and Use of the Facility

It must be understood that imposing biological containment and clean-room conditions on any kind of scientific operation complicates it by a huge factor. Working through glove ports or spacesuit gloves is inherently clumsy: Fairly coarse, simple operations are possible but impeded; delicate operations on a fine scale may be prohibited. The use of bulky, complex, failure-prone scientific instrumentation within such an enclosure, with the potential for system contamination by expendable supplies the instrument uses, and the need to accommodate the technicians and repair personnel who keep the instrument working, would be a nightmare, and an expensive one. When thought is given to the many kinds of studies (and attendant instrumentation) the international scientific community will want to subject the Mars samples to, it is clear that the required instrumentation and personnel cannot possibly be housed within the quarantine facility. The quarantine facility should contain a minimum amount of scientific equipment, only the devices needed to carry out research that cannot be done elsewhere. Other research should be conducted on sterilized samples, in the home laboratories of investigators approved for the Mars sample research program. Chapter 4 identifies the types of studies that should be done in the quarantine facility: first-order characterization of the samples, and studies that cannot be carried out on sterilized samples because of damage that would be done to them by the sterilization procedure, especially efforts to demonstrate the presence of microbial life by methods of microbial replication. (It is possible, however, that technological advances during the time before sample return may make available compact analytical devices that could feasibly be included within the quarantine facility, which would allow searches for biological structures and/or reduced carbon within the facility.)

Recommendation

- The Mars Quarantine Facility should be designed to the smallest and simplest possible scale consistent with its role as a biological containment and clean room facility. No scientific investigations should be carried out in the quarantine facility that can be executed on sterilized samples outside the facility.
- Protocols should be developed that specify in detail the steps and procedures to be followed for handling Mars samples in the quarantine facility. Necessary protocols include those for inventorying and preliminary analyses of the samples, searching for evidence of biological activity, testing for biohazards, and preparing sterilized aliquots of the samples for distribution to the scientific community.

• Because it cannot be carried out on sterilized samples, biohazard assessment should be performed in the quarantine facility prior to any release of samples from the facility. Elements of these studies might include culturing experiments; attempts to infect animals, plants, and cell cultures; and genome detection via the polymerase chain reaction or similar techniques.

Siting of the Facility

The quarantine facility could be built as an independent, free-standing laboratory, or it could be physically part of and affiliated with an institution that already contains BSL-4 laboratories (USAMRIID, in Ft. Detrick, Maryland; CDC, in Atlanta, Georgia; or the Medical Branch of the University of Texas at Galveston, where a BSL-4 facility is being constructed). COMPLEX considers that the second of these options, affiliation with an ongoing containment facility, is preferable for several reasons.

1. *Institutional support*. A collaborative agreement with a host institution would mean that the Mars Quarantine Facility could draw on that institution for personnel, training, experience, security, and specialized utilities (in return for appropriate augmentation of the institution's support).
2. *Economy*. Sharing the resources named under 1 above should effect a large economy in operation of the Mars Quarantine Facility.
3. *Environmental impact*. Clearing an environmental impact statement for a BSL-4 facility can take years. Ideally, the Mars facility would operate under the environmental impact statement (EIS) of its host institution. Failing this, existence of the original EIS should pave the way for approval of a new statement applicable to the quarantine facility.

The Mars Quarantine Facility should be either a free-standing building located at an existing institution, or a segregated portion of an extant building in that institution's complex. The BSL-4 laboratory that handles the martian samples must not be shared physically with other activities that involve BSL-4 agents, but this restriction would not prevent the Mars Quarantine Facility from sharing the manpower pool of the host institution.

The Mars Quarantine Facility should remain effectively under the control of NASA, which is able to supply the science perspective that will guide study of the samples without compromising the required standard of biological containment and planetary protection. It is important that the laboratory be located in or near an active biological science community, where those workers using the facility would be able to draw from a critical mass of supporting scientific disciplines and infrastructure, including other BSL-4 facilities. Without this support, operation of the Mars Quarantine Facility would be greatly handicapped.

Recommendation. The Mars Quarantine Facility should be affiliated with an ongoing containment facility that has BSL-4 capability and should be physically part of it or proximate to it, but control of the Mars Quarantine Facility should be under the jurisdiction of NASA.

TIME FOR CONSTRUCTION

The design of a BSL-4 facility dedicated to the processing of martian samples will be more complex than any BSL-4 laboratory constructed to date, because of the need to prevent contamination of the samples with terrestrial organisms. The design will probably entail enveloping a conventional BSL-4 facility within a class 10 clean room, and operational controls will have to be implemented to reduce the likelihood of terrestrial organisms being carried on clothing, instruments, or reagents into the BSL-4 zone (either cabinet or hood line).

Conventional BSL-4 laboratories (without the clean room envelope) are being constructed now with increasing frequency, both in the United States and abroad. The record for speed of construction, from inception to commissioning, belongs to a private laboratory built by Pasteur Merrieux Connaught in Lyon, France. This

laboratory progressed from first design to completion in exactly 2 years, although the commissioning process and shakedown have consumed an additional 18 months. This is a conventional spacesuit laboratory, with tissue culture laboratories, animal (primate) laboratories, and a surgery/necropsy suite; however, no class III cabinets are installed. A smaller spacesuit-only lab was created at the National Institutes of Health in Bethesda, Maryland, using existing BSL-3 space. Construction and commissioning took 4 years, although some of this time was expended on a divisive public-relations problem with concerned residents in the community. A more extensive BSL-4 facility has been constructed by the Canadian Departments of Health and Agriculture in Winnipeg; more than 5 years have elapsed, and it has still not been commissioned.

With the exception of the clean room envelope, a BSL-4 laboratory dedicated to processing the martian samples should be designed with simplicity and flexibility in mind. Experience has shown that the ideal BSL-4 environment is one in which a Class III cabinet line (see Table 6.2) is joined to a spacesuit laboratory. Class III cabinetry should be selected "off the shelf." Custom-designed Class III BSC hoods that accommodate specialized equipment items are very expensive and inherently inflexible; such equipment is usually better accommodated in a spacesuit laboratory. An optimistic estimate for design and construction of this dedicated laboratory is 4 years, plus shakedown and commissioning. Cross-training of personnel in adjacent or affiliated BSL-4 facilities would reduce the start-up time in activating a trained BSL-4 cadre.[10]

A schedule for the planning, building, and certification of the Mars Quarantine Facility is given in Table 6.3. This schedule assumes that the facility is affiliated with an ongoing containment facility with BSL-4 capability; for a free-standing facility, more time would be required to clear an environmental impact statement, establish needed support services, and train personnel (since these activities cannot proceed in parallel with facility construction). An estimated 7 years is required from beginning to end. This estimate is not padded; it is based on experience with the Apollo Lunar Receiving Laboratory (Chapter 7) and contemporary biological containment facilities. The schedule cannot be shortened without compromising the quality and effectiveness of the facility. Thus it is imperative that planning and construction of the facility be put in motion 7 years prior to projected sample return, which is the most important recommendation that COMPLEX makes in this report.

Recommendation. It is imperative that planning and construction of the Mars Quarantine Facility be begun at least 7 years in advance of the anticipated return of Mars samples. This responsibility cannot be deferred without compromising the quarantine and study of the Mars samples.

INTERNATIONAL ACCESS TO THE MARS SAMPLES

It is possible if not probable that the Mars sample return program will be an international venture, with other nations playing an important role in flight operations. Plans for the original Mars Surveyor 2005 mission called for a substantial part of the program to be carried out by France. The role of international partners in a sample-return program should be carefully defined. The question of international access to the samples is not an issue; traditionally the United States has freely shared the fruits of its space program with other nations (e.g., the Apollo lunar samples). However, the potentially sizeable contribution of another nation to the Mars program raises questions of how the earliest access and ultimate curation of the samples will be shared. The simplest option would be a single path through quarantine and curation, with management and operation of the facilities, and continuing studies within a quarantine facility in the United States, shared by the international partners. Separate curation facilities in the United States and abroad could also be pictured.

It is beyond the scope of COMPLEX's charge to comment on the ultimate curation of the samples, but the committee believes strongly that their preliminary examination, baseline description, cataloguing, and packaging should be carried out at a single quarantine facility in the United States. The sample canister containing Mars samples probably will be landed in the continental United States, recovered by a mobile retrieval unit, and then

[10]This technical discussion is from the experience of COMPLEX member P. Jahrling, USAMRIID. Some of the guidelines differ at other containment facilities, such as the National Center for Infectious Diseases at the CDC.

TABLE 6.3 An Estimate of the Time Required to Construct, Prepare, and Certify the Mars Quarantine Facility

Time	Action: Facility	Action: Personnel
0 years	Funding of facility is authorized. Negotiate site for laboratory and collaborative relationship with in-being containment facility. Determine requirements for sample sterilization, certification of absence of biohazardous material, and sample handling and storage. (12 months)	
1 year	Write draft protocols designed to meet requirements named above and for other critical procedures, such as those for: • Sample entry, • Sample inventory, • Preliminary analyses, • Search for evidence of biological activity, • Assessment of whether sample contains biohazardous material, • Sterilization of aliquots of sample in preparation for removal from the facility, • Removal of samples from the facility, • Storage of samples within the facility, and • Sterilization/cleaning of laboratory facility in preparation for sample entry. (6 months)	
1.5 years	Design a facility around the requirements for Mars sample quarantine and the particular protocols that have been written. (24 months[a])	
3.5 years	Begin construction. (12 months[a])	Begin recruiting, training, and inoculation regime of staff for the facility. (18 months[b])
4.5 years	Begin troubleshooting and certification of mechanics of facility (air, water, pumps, backup systems, etc.). (6 months)	
5 years	Approve and officially certify building and laboratory mechanics (air, water, pumps, backup systems, etc.). Perfect all protocols that will be used in the facility (listed above). Test them with a full dress rehearsal. (12 months)	Training of initial staff completed[b]
6 years	Review and, as necessary, revise specified requirements for critical procedures in light of demonstrated facility capabilities. (6 months)	
6.5 years	Clean the facility, which is now biologically contaminated and organically dirty. (6 months)	
7 years	Facility is certified as ready to receive Mars samples.	

[a]From Lunar Receiving Laboratory experience (Chapter 7).
[b]From the section "Staffing and Training" in this chapter.

transported to a fixed quarantine facility for opening and initial processing. It would be a mistake to attempt to open and divide the sample before it entered the quarantine facility, inviting contamination of the sample and environment. Once in the facility, it would be a mistake to divide the sample before baseline characterization was completed, as this would compromise optimal allocation of the samples to outside investigators for study.

Recommendation. All samples in the initial collection returned from Mars should be placed in a quarantine facility in the United States, at least until the preliminary examination of the samples has been completed. Management and operation of the Mars Quarantine Facility should be shared between the United States and major international partners that participated in the collection of martian samples.

After sample inventory, an international partner may wish to establish an independent BSL-4 facility in which to study unsterilized samples of martian material. Division of the samples in this manner is not excluded by COMPLEX's recommendation, providing that containers and transfer procedures conform to protocols established by a panel of experts (e.g., from the CDC) in containment (Chapter 4).

It is important that formal agreements (memorandums of understanding) be completed with major partners, in the planning stages of an international program of Mars sample return, to define each partner's degree of participation in the preliminary examination of the samples and its entitlement to a share of samples for an independent quarantine facility (and/or its degree of participation in a continuing U.S. quarantine facility).

OVERSIGHT COMMITTEE

COMPLEX endorses the 1997 National Research Council report's recommendation that:

> A panel of experts, including representatives of relevant governmental and scientific bodies, should be established as soon as possible once serious planning for a Mars sample-return mission has begun, to coordinate regulatory responsibilities and to advise NASA on the implementation of planetary protection measures for sample-return missions. The panel should be in place at least one year prior to the establishment of the sample-receiving facility (at least three years prior to launch).[11]

The panel described would be equivalent to the Interagency Committee on Back Contamination (ICBC) that provided oversight during the Apollo program (Appendix B). However, Appendix B also describes another committee, the Lunar Sample Analysis and Planning Team (LSAPT), which played a different and equally important role in the lunar program. The formal charge of LSAPT was to advise NASA on the following:

1. Equipment and procedures for use in the Lunar Receiving Laboratory for conduct of time critical experiments, gross preliminary examinations of samples and curatorial functions.
2. Proficiency, capability and adequacy of Lunar Receiving Laboratory and its staff to carry out functional roles of Lunar Receiving Laboratory.
3. Sequence of sample analysis and the allocation and distribution of samples after quarantine release.
4. Review findings of preliminary examination team during quarantine and advise on operational procedures and techniques.[12]

The responsibilities of ICBC lay mostly in the quarantine period (limited to three weeks after return of the lunar samples), and LSAPT's responsibilities were mostly in the curation and allocation period after that, but there was overlap of the committees' roles, and, inevitably, conflict. Put in simplest terms, the job of ICBC was to tighten the screws, while LSAPT tried to loosen them and move the samples. In this LSAPT was very effective,

[11]Space Studies Board, National Research Council. 1997. Mars Sample Return: Issues and Recommendations. National Academy Press, Washington, D.C., pp. 5-6.

[12]Memorandum from W.N. Hess, MSC Director of Science and Applications, to members of LSAPT and LSPET, Dec. 6, 1968.

and much of the timeliness of the Apollo scientific program is attributable to LSAPT's exertions. Since the lunar samples proved to be devoid of life or its products, the efforts of ICBC are less celebrated.

The Mars Quarantine Facility would profit from the efforts of a committee modeled after LSAPT. It should differ from the planetary protection panel described above in being more of an advocacy group, concerned with getting the most science possible from the samples, and making political representations where necessary. It should differ from LSAPT in having a larger component of biological scientists, and a more deeply felt appreciation for planetary protection and the potential of the Mars samples to be repositories of life. But like LSAPT it should be a continuing committee, with the capacity and motivation to follow through on the reception of its recommendations, something that advisory groups like COMPLEX, the Space Studies Board Task Group on Issues in Sample Return,[13] and MSHARP (the NASA Mars Sample Handling and Requirements Panel[14] cannot do.

The Mars sample advocacy committee should consist mostly of experts from outside NASA. Qualified persons from outside NASA (e.g., from the National Academy of Sciences) should be substantially involved in nominating the committee. The committee should report to a high level in NASA, either the Office of the Associate Administrator for Space Sciences or the Office of the Director of the NASA center principally concerned with Mars sample quarantine and curation.

Recommendation. A continuing committee of senior biologists and geochemists that includes appropriate international representation should be formed and charged with reviewing every step of the planning, construction, and employment of the Mars Quarantine Facility. The committee should be formed during the earliest stages of planning of a Mars sample-return mission. Members of the committee should also participate in the design of the spacecraft and those portions of the mission profile where biological contamination is a threat.

Early formation of the committee is advocated because there is work for it to do at every stage of planning, including response to the recommendations of this report and earlier reports.[15-17]

[13]Space Studies Board, National Research Council. 1997. Mars Sample Return: Issues and Recommendations. National Academy Press, Washington, D.C.

[14]NASA and the Jet Propulsion Laboratory. 1999. Mars Sample Handling and Requirements Panel (MSHARP) Final Report. NASA/TM-1999-209145.

[15]NASA and the Jet Propulsion Laboratory, 1999; see footnote 14 above.

[16]Mars Sample Handling, Distribution and Analysis Workshop Report. Report of a workshop organized at the request of the Mars Architecture Definition Team and held February 16-17, 1999, California Institute of Technology, Pasadena, Calif.

[17]NASA. 1990. Scientific Guidelines for Preservation of Samples Collected from Mars. NASA Technical Memorandum 4184.

7

Lessons Learned from the Quarantine of Apollo Lunar Samples

The martian samples will not be the first extraterrestrial materials that NASA has quarantined. A need was seen to quarantine the lunar samples collected by the Apollo astronauts beginning in 1969, as well as the astronauts themselves, and a quarantine program and quarantine facility (the Lunar Receiving Laboratory; LRL) were set up for that purpose. Much valuable experience was gained during the lunar quarantine, and it is important for the Mars quarantine program to profit from this history wherever it can.

A brief chronology of events relating to quarantine and handling (which are inextricably mingled) of the lunar samples is given in Appendix B. COMPLEX draws the following conclusions from this history.

1. Many factors that strongly shaped the Apollo quarantine experience will be absent from Mars sample return missions and quarantine. It will not be necessary to worry about ensuring the safety of astronauts; astronauts as vehicles for organisms; the need to introduce the concept of planetary protection to NASA; lack of experience with extraterrestrial samples; the constraint of President Kennedy's "end of this decade" time scale; or handling the very large amounts of sample material that were collected on the moon. Also missing will be the "crash program" resources and mentality that Apollo enjoyed.

2. The preliminary examination, curation, and distribution for study of lunar samples from the LRL were generally successful. A community of outside investigators had been selected and funded by the time the Apollo 11 crew returned to Earth, and they had had some time to equip their laboratories and simulate analyses of lunar samples. Real lunar samples were distributed to them a few weeks after Apollo 11 (and subsequent missions) returned to Earth. The samples were allocated in a reasonably rational way, and with some exceptions they were protected from serious contamination.

On the other hand, the quarantine program would have to be judged a failure. It greatly complicated sample processing, yet if lunar material had contained lethal microorganisms Earth would have been infected in two places: the Pacific Ocean, and Houston, Texas.

3. NASA, for the most part, made the right decisions and acted in a timely way in preparing for the receipt of lunar samples in Houston. The agency made very extensive use of external panels of experts for scientific advice. The Manned Spacecraft Center (MSC) was unstinting in its support of the planned scientific studies. NASA accepted the need for planetary protection measures as soon as external advice drew attention to it, and factored it

into the program. In the end NASA gave a higher priority to successful execution of the missions, and the welfare of astronaut crews, than to planetary protection; but that was the imperative that had been handed to NASA.

The difficulties that were encountered can be ascribed mostly to some poor managerial choices made by NASA, the small amount of time available to set up and test a new and unprecedented enterprise, and a certain amount of internecine warfare that developed (Headquarters versus the Manned Spacecraft Center, Office of Space Science and Applications versus Office of Manned Space Flight, physical science versus biology).

4. Reduced to the simplest terms, 2 years were spent planning LRL (only the last year of which included planning for a quarantine), 1 year was spent building the facility, and after that 2 more years were available for staffing and training of personnel before the first samples arrived. There is widespread agreement that this was not enough time to do the job right. This experience with scheduling translates fairly straightforwardly to the time line for the Mars Quarantine Facility (Chapter 6).

5. The momentum of Apollo, and the goal set by President Kennedy, forced the Interagency Committee on Back Contamination to accept halfway measures in enforcing planetary protection. The reentry and splashdown procedures implemented had the potential for infecting the atmosphere and ocean. These compromises did not escape the notice of personnel in the LRL quarantine facility (6, below). In the absence of a Cold War imperative and concern for the welfare of astronauts, and with the benefit of Apollo experience, it should be possible to design and enforce a truly rigorous quarantine program. The management structure of the Mars sample program should not permit planetary protection to be overruled for reasons of operational expediency.

6. Many of the scientists (especially physical scientists) and technical staff of LRL were poorly motivated to endure the many inconveniences of quarantine. It was obvious to them from first principles that the Moon is a hostile, sterile place, and the risk of lunar pathogens there is negligible. In addition, the obvious gaps in planetary protection associated with return of the Apollo spacecraft to the Pacific Ocean made security in Houston seem less than compelling. Consequently, there were many breaches of quarantine security in LRL that went unreported.

It is essential that quarantine personnel be motivated to observe quarantine. It is not enough to simply order them to do so. This is especially true in a situation where (unlike the case at the Centers for Disease Control and Prevention or the U.S. Army Medical Research Institute of Infectious Diseases) the probability that infectious organisms are present is small. Essential ingredients of motivation are an airtight, uncompromised quarantine plan, and effective and complete communication of the quarantine concept to all parties.

7. Many of the worst problems encountered in LRL, affecting both sample processing and quarantine, stemmed from the F-201 vacuum chamber and its glove ports. The concept of a vacuum glove box was a very novel and ambitious one that, in the end, came to grief. The lesson for the Mars Quarantine Facility is to strive for simplicity. The requirements of quarantine and chemical cleanliness already dictate a fairly complex system; the Apollo lesson is, don't complicate the system beyond that point unless the reasons for doing so are very compelling. There were fairly good reasons for wanting to maintain a vacuum environment for the lunar samples—the intellectual climate of the time, which is largely forgotten now, said *we have no idea what kind of material the lunar samples will turn out to be*—but in the end maintaining the lunar samples in a vacuum did no particular good.

The analogous potential complication for the Mars Quarantine Facility is storing and handling the samples at Mars (subfreezing) temperatures. While storing samples at low temperatures in the quarantine facility would probably be straightforward, attempting to handle and process them at low temperatures would greatly complicate the facility's design and operating procedures. Although reasons can be found for keeping the Mars samples at low temperatures, none are compelling enough to justify the added difficulties and risks to the quarantine facility. An effort to reproduce the martian environment (gas composition, pressure) in the facility is probably also not justified, though to the maximum extent feasible the samples should be stored and handled in an inert atmosphere such as purified dry nitrogen gas (or helium or argon).

Recommendation. It is essential that the design for the Mars Quarantine Facility be kept as simple as possible, consistent with the facility's mission of protecting Earth's environment and the samples. Although it may be feasible to store the samples at low temperatures, an effort to try to maintain a Mars environment (temperature, pressure) during sample handling would complicate the design and operation of the facility to a very large degree, probably unnecessarily, and it should not be attempted for the first Mars sample return.

8. LRL profited greatly from the advice and advocacy of panels of outside experts. The Lunar Sample Analysis Planning Team (LSAPT) won the confidence of the NASA Manned Spacecraft Center's director to such an extent that its recommendations were almost always implemented.

Panels of this sort are extremely important (Chapter 6), but the question of how much power to invest them with is a complicated one. Their effectiveness depends on particular personalities that might be on the panel. LSAPT was only one example, and its membership included some exceptional people; for this reason it is probably not safe to generalize about powerful advisory panels from the Apollo experience.

8

Conclusions and Recommendations

Briefly stated, the conclusions of the COMPLEX study on the quarantine and certification of martian samples are as follows.

1. COMPLEX agrees with the findings of earlier panels and committees, that Mars samples should be treated on the assumption that they contain dangerous microorganisms, and they should be subjected to an effective quarantine upon arrival on Earth.

2. It will take a substantial amount of time (the estimate of COMPLEX is 7 years) to design, construct, and implement an effective quarantine facility, even if (as COMPLEX recommends) the design and quarantine plan are kept as simple as possible. The facility is complicated unavoidably by the need to combine biological containment with clean-room conditions. **It is imperative to begin planning, construction, and staffing of the facility at least 7 years prior to projected sample return.** This schedule cannot be shortened without compromising the effectiveness of the quarantine facility. The time of launch of a Mars sample-return mission should be constrained by the schedule of construction of such a facility; an element of the Launch Readiness Review should be a Mars Quarantine Facility Readiness Review.

The possible need to clear an environmental impact statement for the quarantine facility should be borne in mind. Extra time needed for this task must be added to the projected 7-year schedule for construction and implementation.

3. Several initiatives should be begun *prior to* design of the quarantine facility and planning of quarantine protocols, i.e., immediately:

- A program of research should be begun to determine the efficacy of supercritical fluids and commonly used organic solvents in killing organisms.
- A program of research should be begun to determine the effects on organic compounds in rocky matrices of varying degrees of application of heat and gamma irradiation.
- Work should begin on the problem of combining biological containment with clean-room conditions, to include mocking up containment/clean-room combinations whose efficacy can be tested.
- A continuing committee of senior biologists and geochemists should be formed and charged with reviewing these initiatives and every step of the planning, construction, and ultimately the employment of the Mars Quarantine Facility.

4. The probability is large that the Mars samples will display properties that at least some people consider suggestive of life forms or life processes, although other observers disagree. In the event of such equivocal evidence, plans should be in place to begin distributing sterilized samples immediately to approved investigators (biological and geochemical) for study in their home institutions. This distribution should not wait upon resolution of the significance of the equivocal evidence, which is likely to take a very long time.

5. Rigorous programs of heat and/or gamma-ray sterilization should be planned for samples that are to be removed from the quarantine facility, more than sufficient to kill any known terrestrial organism (e.g., twice the necessary dose of gamma irradiation).

The specific recommendations of COMPLEX are presented in preceding chapters and are assembled in the Executive Summary of this report.

Appendixes

Appendix A

Deinococcus radiodurans as an Analogue to Extremophile Organisms That May Have Survived on Mars

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The only genetic systems within which the equivalent of millions of years' worth of background radiation accumulated as genetic damage have been studied in a living organism are those developed by the terrestrial bacterium *Deinococcus radiodurans*.[1-7] This bacterium is capable of repairing massive genetic damage without lethality or increasing mutation frequency. As such, *D. radiodurans* is an excellent organism in which to consider the potential for survival and biological evolution beyond its planet of origin, as well as the ability of life to survive extremely long periods of metabolic dormancy in high-radiation environments. Ultimately, the survival of any organism in such environments will be determined by its ability to repair, and recover from, accumulated genetic damage. *D. radiodurans* could likely survive extended periods of metabolic dormancy near the surface of Mars, periods of time in which the equivalent of Mrads of radiation damage would be accumulated in any genetic material.

It is likely that during the first 500 million years (0.5 Ga) of its existence, Mars had a warmer and wetter climate than it has now, and an active hydrologic cycle. This may slightly predate the time at which Earth first began to support water and life; evidence of chemically evolved life on Earth dates to ~0.6 Ga after its formation.

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[1]Battista, J.R., Earl, A.M., and Park, M.-J. 1999. Why is *Deinococcus radiodurans* so resistant to ionizing radiation? Trends Microbiol. 7: 362.

[2]Minton, K.W. 1996. Repair of ionizing-radiation damage in the radiation resistant bacterium *Deinococcus radiodurans*. Mutat. Res. DNA Repair 362: 1.

[3]Mattimore, V., and Battista, J.R. 1996. Radioresistance of *Deinococcus radiodurans:* Functions necessary to survive ionizing radiation are also necessary to survive prolonged desiccation. J. Bacteriol. 177: 5232.

[4]Lange, C.C., Wackett, L.P., Minton, K.W., and Daly, M.J. 1998. Engineering a recombinant *Deinococcus radiodurans* for organopollutant degradation in radioactive mixed waste environments. Nat. Biotechnol. 16: 929.

[5]Brim, H., McFarlan, S.C., Fredrickson, J.K., Minton, K.W., Zhai, M., Wackett, L.P., and Daly, M.J. 2000. Engineering *Deinococcus radiodurans* for metal remediation in radioactive mixed waste environments. Nat. Biotechnol. 18: 85-90.

[6]Daly, M.J., Ouyang, L., Fuchs, P., and Minton, K.W. 1994. In vivo damage and recA-dependent repair of plasmid and chromosomal DNA in the radioresistant bacterium *Deinococcus radiodurans*. J. Bacteriol. 176: 3508.

[7]White, O., Eisen, J.A., Heidelberg, J.F., Hickey, E.K., Peterson, J.D., Dodson, R.J., Haft, D.H., Gwinn, M.L., Nelson, W.C., Richardson, D.L., Moffat, K.S., Qin, H., Jiang, L., Pamphile, W., Crosby, M., Shen, M., Vamathevan, J.J., Lam, P., McDonald, L., Utterback, T., Zalewski, C., Makarova, K.S., Aravind, L., Daly, M.J., Minton, K.W., Fleischmann, R.D., Ketchum, K.A., Nelson, K.E., Salzberg, S., Smith, H.O., Venter, J.C., and Fraser, Claire M. 1999. Genome sequence of the radioresistant bacterium *Deinococcus radiodurans* R1. Science 286: 1571-1577.

Subsequent to this watery period martian life, if it existed, may have become subterranean and/or dormant, or it may have become extinct during the transition to a hostile environment caused by Mars's loss of atmosphere and water. The time when conditions effectively became hostile to life are not known; the loss of atmosphere and water from Mars was gradual and is still occurring.

It is not justified to assume a priori that all martian life, if it existed, has become extinct. If life existed on Mars, it likely evolved to survive the increasingly harsh conditions, and/or it found environments that are shielded from the planet's present extreme climate. As our knowledge of Earth's biosphere increases, extreme environments originally believed to be far too harsh to support life are regularly being found to support flourishing microbial communities. Organisms discovered in these extreme environments are called *extremophiles,* and collectively they demonstrate the remarkable ability of life to thrive over a wide range of extremely hostile environments, e.g., high temperature (these organisms are called *thermophiles*) or high pressure (*barophiles*). Similarly successful adaptations may have been made by microbes on Mars in environments at least equally hostile.

D. radiodurans is an excellent example of an extremophile. It can grow continuously, without induced mutation or any effect on its growth rate, in the presence of 6,000 rad of radiation per hour.[8,9] Further, it demonstrates astounding survival: it can withstand an acute dose of radiation of 1.5 Mrad (at 0 °C) or 4.0 Mrad (at –70 °C) without lethality. Even more extraordinary is the fact that following such massive irradiation, these cells can repair their genomes within 12 to 24 hours.[10] Unpublished work by J. Battista has shown that desiccated *D. radiodurans* can survive exposure at higher doses, as much as 15 Mrad (Figure 5.3 in Chapter 5). These qualities are key to answering the question of whether life is extinct or extant on Mars, where the commonly accepted "post-transition period" from a life-supporting to a life-challenging biosphere might subject a dormant microbe to up to 2 billion years' worth of accumulating background radiation, along with freezing and/or desiccation. The background cosmic radiation on Mars is similar to that on Earth, ~0.5 rad/year. Thus on the order of 1,000 Mrad of accumulated radiation dose could have been delivered to a microbe locked in a cryptobiotic state at the end of the transition period. As a single acute dose, this amount of radiation damage would overwhelm *D. radiodurans* and any other known organism. However, fluctuations in the Mars surface environment, and other events (such as burial by crater ejecta), could present the microbial population with periodic opportunities to become active, repair damage, and replenish their numbers while the radiation occurred.

In the context of current technological and financial constraints, the question of where life on Mars is most likely to be metabolically active is distinctly separate from the question of where preserved life is most likely to be detected on Mars by missions to the planet in the near future. By analogy to the extremophiles on Earth, it is likely that metabolically active life on Mars would occur in geothermally heated subsurface water tables or spaces where it would be shielded from the harsh surface conditions. Such Mars subterranean environments will, however, be beyond human exploration for many years.

In the foreseeable future, exploration of Mars will be limited to surface and near-surface environments. Although these accessible environments are exposed to harsh atmospheric and solar stress, such conditions do not preclude dormant life. It is possible that periodic melts of subsurface water caused by geothermal events and/or orbital variations bring subsurface microorganisms into surface and near-surface environments where they become frozen and cryopreserved in ground ice. If a martian microorganism had a proficient genetic repair system, it could survive frozen in such a site for millions or even hundreds of millions of years. If intermittently, though rarely, the local surface environments also underwent thawing for the same reasons, it would allow revival of organisms, repair of accumulated genetic damage, and repopulation of their environments. *D. radiodurans* provides a good

[8]Lange, C.C., Wackett, L.P., Minton, K.W., and Daly, M.J. 1998. Engineering a recombinant *Deinococcus radiodurans* for organopollutant degradation in radioactive mixed waste environments. Nat. Biotechnol. 16: 929.

[9]Brim, H., McFarlan, S.C., Fredrickson, J.K., Minton, K.W., Zhai, M., Wackett, L.P., and Daly, M.J. 2000. Engineering *Deinococcus radiodurans* for metal remediation in radioactive mixed waste environments. Nat. Biotechnol. 18: 85-90.

[10]Daly, M.J., Ouyang, L., Fuchs, P., and Minton, K.W. 1994. In vivo damage and recA-dependent repair of plasmid and chromosomal DNA in the radioresistant bacterium *Deinococcus radiodurans*. J. Bacteriol. 176: 3508.

model system in which to consider preservation and irradiation on the accessible surface and in near-surface environments of Mars.

The following factors are known to dramatically affect an organism's radiation resistance:

- *Genome size*. The smaller the genome, the more resistant it is.
- *Ambient nutrient conditions*. The presence of nutrients supports DNA repair.
- *Temperature*. Typically, the colder an organism is, the more resistant to radiation it is. Freezing increases radiation resistance.
- *Hydration*. Organisms are much more resistant to radiation when desiccated.

Appendix B

A History of the Lunar Receiving Laboratory

May 25, 1961

President John F. Kennedy, in a special message to Congress on "urgent national needs," sets a goal for the United States: ". . . I believe this nation should commit itself to achieving the goal, before this decade is out, of landing a man on the moon and returning him safely to the earth. No single space project in this period will be more impressive to mankind, or more important for the long-range exploration of space; and none will be so difficult or expensive to accomplish."

October 1961

The Space Task Group at NASA Langley Field, which has been executing the Mercury Program, begins to move to temporary quarters in Houston in preparation for setting up the new Manned Spacecraft Center (MSC) there.

February 1964

Elements of MSC begin occupying their newly constructed center at Clear Lake City, Texas.[1]

February 25, 1964

An internal MSC memorandum is sent from the Assistant Chief for Space Environment to the Director of Engineering and Development in which the need for a central facility to accept and handle lunar samples is first identified. The original concept is for a $10' \times 10' \times 7'$ vacuum chamber, 10^{-7} bar pressure, in which lunar sample containers will be opened and the samples characterized, subdivided, and packaged for distribution to external investigators. Remote manipulators are to accomplish these operations in the vacuum chamber.[2,3]

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[1]King, B. 1989. Moon Trip. University of Houston, Houston, Texas.

[2]Annexstad, J.O. 1978. The Lunar Receiving Laboratory: A Management Study. Unpublished manuscript submitted to the Graduate Program in Public Administration, University of Oklahoma, Norman, Oklahoma, in partial fulfillment of requirements for the Master of Arts in public administration.

[3]Compton, W.D. 1989. Where No Man Has Gone Before: A History of Apollo Lunar Exploration Missions. NASA SP-4214. Superintendent of Documents, Washington, D.C., 415 pp (and original documents).

The rationale for vacuum processing is to preserve loosely bonded lunar gases in the samples for analysis, and also to guard against the possibility that the samples might be reactive with the terrestrial atmosphere.

March 1964

The Engineering Division of MSC completes planning of a preliminary engineering concept for a lunar facility and presents it to MSC management. The projected cost is $11 million, and operational status will be reached in 1968.[4]

June 15, 1964

The Apollo Science Teams of the Planetology Subcommittee of NASA's Office of Space Sciences and Applications (OSSA) is asked by NASA management to consider the necessity and requirements for a central lunar facility. The Apollo Science Teams meet in Houston. Their report is issued in December 1964.[5,6]

July 7, 1964

The projected design of the lunar sample facility at MSC is upgraded to something that more nearly resembles the Lunar Receiving Laboratory (LRL) that is later built. The facility is to include a vacuum chamber and also a cabinet filled with purified N_2 gas for processing samples, ancillary laboratories for science, and offices, occupying in all ~8,000 square feet. At this time there is no plan for quarantine against back contamination. Manual versus mechanical manipulation is not specified for the vacuum chamber.[7]

July 29, 1964

The Space Science Board (SSB) of the National Research Council convenes a conference of representatives from the Public Health Service, the Department of Agriculture, the Fish and Wildlife Service, the National Academy of Sciences, and NASA to assess the back-contamination problem. The conference concludes that the existence of life on the Moon cannot be precluded, and it recommends that astronauts, spacecraft, and lunar materials should be received into an isolation unit; the astronauts should be held in rigid quarantine for at least three weeks; and preliminary examination of the samples should be conducted behind "absolute biological barriers, under rigid bacterial and chemical isolation."[8]

October 1964

NASA Headquarters challenges the scale of the sample-handling facility MSC has proposed. OSSA establishes an ad hoc committee to consider the requirements and concepts for a lunar laboratory, which meets in November and December in Houston.[9,10]

January 1965

NASA requests that the National Research Council's SSB review a draft of the ad hoc committee's report. The SSB concludes that there is a need to quarantine the astronauts and lunar samples until they are proven biologically innocuous. Quarantine can be at some existing Public Health Service or U.S. Army installation. A

[4]Annexstad, 1978; see footnote 2.
[5]Annexstad, 1978; see footnote 2.
[6]Compton, 1989; see footnote 3.
[7]Compton, 1989; see footnote 3.
[8]Compton, 1989; see footnote 3.
[9]Annexstad, 1978; see footnote 2.
[10]Compton, 1989; see footnote 3.

minimal quarantine facility, including a radiation-counting laboratory, might be built for $2.5 million and staffed by 12 to 30 professional scientists.[11,12]

March 18, 1965

The Center for Disease Control (later the Centers for Disease Control and Prevention, CDC) publishes its recommended procedures for handling and quarantining the returned lunar samples.

June 1965

MSC management establishes a technical working committee for preliminary and final engineering design studies of LRL.[13]

July 1965

NASA Headquarters organizes a series of disciplinary working groups, which meet in Falmouth, Massachusetts, for the NASA Lunar Exploration and Science Summer Conference. Plans are made for soliciting and screening proposals by outside investigators. Participants consider and endorse the current plans for an LRL.[14,15] The group warns of the likelihood that lunar soil will contaminate the ocean upon return to Earth. Although the group makes recommendations on details of sample handling, nothing is said about doing so in a vacuum.

July 31, 1965

A meeting between representatives of NASA and the Public Health Service (PHS) recommends that construction of an LRL be made contingent on NASA accepting responsibility for adequate containment of astronauts and returned materials and equipment behind suitable biological barriers. The responsibilities of the Department of Agriculture and the Department of the Interior are recognized. The director of the Planetary Quarantine Bioscience Programs Division is specifically directed by the deputy administrator of NASA to establish informal discussions between PHS and the Office of Manned Space Flight (OMSF) on "planning for lunar return."[16]

August 9, 1965

Melpar Inc. issues a report, "Semidetailed Design Considerations Related to Functions and Sample Flow Through the Lunar Sample Receiving Laboratory." This plan includes a vacuum processing chamber (10^{-6}-mm pressure).[17,18]

September 27, 1965

Discussions between PHS officials and NASA at Houston reveal that the two groups differ greatly in the gravity they attach to the quarantine requirement. Arguments by MSC, that material near the lunar surface is surely sterile, are rejected. Even if it costs $50 million to implement an effective quarantine, argues the PHS

[11]Annexstad, 1978; see footnote 2.
[12]Compton, 1989; see footnote 3.
[13]Annexstad, 1978; see footnote 2.
[14]Annexstad, 1978; see footnote 2.
[15]Annexstad, 1978; see footnote 2.
[16]Bagby, J.R., Jr. 1975. Back contamination: Lessons learned during the Apollo lunar quarantine program. Prepared for JPL/CIT under Contract #560226.
[17]Annexstad, 1978; see footnote 2.
[18]Frondel, C. 1964-1970. Memos and papers from his participation in planning for lunar sample return and membership on the Lunar Sample Analysis Planning Team and Lunar Sample Preliminary Examination Team.

representative, the added expense is justified. The requirement for a splashdown procedure that prevents contamination of the ocean dates from this time.[19]

October 1965

The engineering study for LRL is completed and the projected facility cost is $9.1 million, up from an earlier estimate of $6.5 million because of the added cost of quarantine.[20,21]

November 1965

A survey of facilities in the United States that might serve to quarantine the Apollo crews finds none to be adequate.[22]

January 1966

The Interagency Committee on Back Contamination (ICBC) is established. It includes representatives from the Department of Agriculture, Department of the Interior, Department of Health, Education, and Welfare, National Academy of Sciences, and NASA.[23]

February 24, 1966

The House Subcommittee on Manned Space Flight rejects NASA's request for $9.1 million for the Lunar Receiving Laboratory.

March 9, 1966

A NASA Headquarters reorganization moves lunar science from the Manned Space Flight program to OSSA.

March 16-23, 1966

In response to congressional criticism, NASA carries out another survey of facilities external to MSC that might serve as quarantine sites for the crews and samples. NASA again concludes that none is suitable.

March 31, 1966

In a rehearing, NASA convinces the House Subcommittee on Manned Space Flight that $9.1 million is needed for LRL, and that amount is approved by the House of Representatives.[24]

May 1966

An LRL working group is established as part of the Planetology Subcommittee of NASA, composed of scientists from outside the agency. This group and ICBC establish the LRL requirements, and they review the design for construction.[25]

July 1966

Proposals have been received from prospective lunar sample investigators outside NASA.[26]

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[19]Compton, 1989; see footnote 3.
[20]Annexstad, 1978; see footnote 2.
[21]Compton, 1989; see footnote 3.
[22]Annexstad, 1978; see footnote 2.
[23]Annexstad, 1978; see footnote 2.
[24]Compton, 1989; see footnote 3.
[25]Annexstad, 1978; see footnote 2.
[26]Frondel, 1964-1970; see footnote 18.

August 1966

The Senate Committee on Aeronautical and Space Sciences approves an appropriation for LRL construction but reduces it to $8.1 million.[27] Construction of LRL begins.

October 1966

It is found that no aircraft carrier in the U.S. fleet has a deck crane rated to lift the Apollo Command Module (CM), with the astronauts inside, aboard during conditions of high seas. (The original plan has been to lift the CM onto a carrier and open it after it is connected to a shipboard containment facility.) The astronauts will have to exit the CM while it is floating in the ocean. Will this act contaminate the ocean? ICBC satisfies itself that it will not: The CM's environmental control system will effectively filter pathogens and dust out of the CM's air during the return trip. Thus the only concern is that the astronauts themselves might have become infected by hypothetical lunar pathogens, and they can wear biological isolation garments when they exit the CM. This is the basis for approving an ocean pickup.

However, this analysis discounts the presence of lunar dust in the CM.[28] The presence of the dust was acknowledged at the time, but not taken seriously: "The interior of the command module . . . may come in contact with a small amount of lunar material that has been tracked in by the astronauts; in any case it will contain the astronauts for more than 2 days after their lunar exposure. Thus it must be considered contaminated; after removal of the astronauts and sample containers, it will be sealed for the duration of the quarantine."[29]

January 10, 1967

The Science and Applications Directorate is established at MSC.[30]

January 13, 1967

LRL is placed in the Lunar and Earth Sciences Division at MSC, whose head reports to the director of the Science and Applications Directorate.[31]

February 1967

ICBC writes a quarantine protocol aimed at consuming 1.2 kg of material, 5% of the nominal sample collection, in testing the reactions of animals and plants to lunar material. The protocol and sample requirement have been simplified by a decision to search not for living organisms in general but instead only for infectious organisms.[32] The examinations are to include aerobic and anaerobic culturing; inoculation of plants, eggs, tissue cultures, amphibia, invertebrates, and normal and germ-free animals; and biochemical analyses.[33]

June 16, 1967

A formal protocol for operation of LRL and quarantining the samples and crew, 547 pages in length, is written by a team of biologists and physicians at Baylor University.[34]

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[27]Compton, 1989; see footnote 3.

[28]Compton, 1989; see footnote 3.

[29]McLane, J.C., King, E.A., Jr., Flory, D.A., Richardson, K.A., Dawson, J.P., Kemmerer, W.W., and Wooley, B.C. 1967. Lunar Receiving Laboratory. Science 155: 525-529.

[30]Annexstad, 1978; see footnote 2.

[31]Compton, 1989; see footnote 3.

[32]Compton, 1989; see footnote 3.

[33]McLane et al., 1967; see footnote 29.

[34]Comprehensive Biological Protocol for the Lunar Sample Receiving Laboratory, Manned Spacecraft Center, National Aeronautics and Space Administration, Houston, Texas. Prepared under Contract NAS 9-6157 to Baylor University College of Medicine, Houston, Texas. Submitted June 16, 1967.

June 29, 1967

The newly completed LRL is opened. It is directed by an interim manager.[35,36]

August 1, 1967

A physicist and expert in vacuum technology is named manager of LRL and chief of the Lunar and Earth Sciences Division.[37,38]

August 24, 1967

An agreement between ICBC and NASA on the protection of Earth's biosphere from lunar sources of contamination is signed.[39]

September 18-20, 1967

A meeting of approved outside sample investigators is held at MSC.[40]

September 1967

Two key committees are organized: the Lunar Sample Analysis Planning Team (LSAPT) and the Lunar Sample Preliminary Examination Team (LSPET).[41] LSAPT has 14 members, mostly physical scientists; its charge is stated in Chapter 6 of this report. LSPET also has 14 members, 11 physical and 3 biological scientists. It will have the responsibility of characterizing the various lunar samples before they are subdivided and sent to outside investigators.

October 5-7, 1967

The first meeting of LSAPT is held at MSC.[42]

November 1967

MSC estimates that the funding needed to support outside investigators in the United States will be $3.6 million in FY 1968 and $3.8 million in FY 1969. There are 110 approved investigators, including 27 (not funded by NASA) from other nations.[43]

December 1-2, 1968

The first meeting of LSPET is held. Meetings of LSAPT and LSPET soon come to be held at about 1-month intervals.[44]

February 23, 1968

At a second meeting of LSAPT, discussion centers on problems associated with the uncompleted vacuum chamber in which returned lunar sample containers are to be opened (Figure B.1). The LRL manager, who has

[35]Annexstad, 1978; see footnote 2.
[36]Compton, 1989; see footnote 3.
[37]Annexstad, 1978; see footnote 2.
[38]Compton, 1989; see footnote 3.
[39]Annexstad, 1978; see footnote 2.
[40]Frondel, 1964-1970; see footnote 18.
[41]Compton, 1989; see footnote 3.
[42]Frondel, 1964-1970; see footnote 18.
[43]Compton, 1989; see footnote 3.
[44]Frondel, 1964-1970; see footnote 18.

confidence in the system, believes it will be operational in 3 to 4 months. LSAPT members are more skeptical and are concerned about how samples will be processed if the vacuum system is still problematical when Apollo 11 returns from the Moon.[45]

April 1968

ICBC revives the question of whether the Apollo Command Module's environmental control system, by filtering microbes out of the CM's air during the return trip from the Moon, can really provide adequate planetary protection. ICBC raises the issue of the loose dust in the CM. However, by this time MSC is committed to the mission plan and can do little to change it. MSC institutes ineffectual plans for the astronauts to vacuum-clean the inside of the CM during their return to Earth.[46]

Another breach of planetary protection during the splashdown is identified: At an altitude of 10,000 feet the returning CM is to be vented to Earth's atmosphere. Reacting to ICBC's concern, MSC studies the problem but finds that a system to filter air being exhausted from the CM would be unacceptably heavy (30 lb), and bypasses the objection.[47]

In the final analysis, planetary protection is subordinated to other priorities. Quarantine of the crew was never intended to be absolute. One of the guidelines governing procedures states, "The preservation of human life should take precedence over the maintenance of quarantine." Thus if a command module begins to sink during recovery operations, or if a major fire breaks out in the crew quarters of the receiving laboratory, or if a quarantined astronaut suffers a medical emergency that cannot be handled within LRL, the plan is to break quarantine.[48,49]

October 22 - November 1, 1968

A simulation of the handling and preliminary examination of samples is held. At this time the F-201 vacuum chamber is still inoperable. Members of the biomedical component of the simulation find and critique many problems.

February 1969

NASA Headquarters asks experts from the regulatory agencies to evaluate MSC's preparations for receipt and quarantine of the lunar samples. These advisors find LRL far from ready to handle the mission: There are equipment problems, a shortage of technicians, incompletely trained personnel, and deficient protocols for biological testing.[50]

(Date uncertain)

An MSC contractor, Brown and Root Northrup (BRN), is given responsibility for carrying out the biological testing of returned samples. BRN and NASA personnel rewrite the Baylor protocol in time for the March simulation.

March 1969

A month-long simulation of sample processing is held. The F-201 breaks down repeatedly. Organic contamination in the system is found to emanate from the vacuum pumping system, reinforcing a feeling of LSAPT that

[45]Frondel, 1964-1970; see footnote 18.
[46]Compton, 1989; see footnote 3.
[47]Bagby, Jr., 1975; see footnote 16.
[48]Compton, 1989; see footnote 3.
[49]Johnston, R.S., Dietlein, L.F., MD, and Berry, C.A., MD, eds. 1975. Biomedical Results of Apollo, NASA SP-368, pp. 410-411, 418.
[50]Compton, 1989; see footnote 3.

FIGURE B.1 The vacuum chamber (the F-201) used for lunar sample processing in the Lunar Research Laboratory. Lunar Sample Curator E.A. King describes the chamber at a meeting of approved outside investigators in September 1967. The plate glass window through which the F-201 is visible is part of a "biological barrier" that envelopes the entire quarantined area, making it nominally a BSL-4 laboratory. SOURCE: Photograph by J.A. Wood.

processing in a vacuum is unworkable and should be abandoned. LSAPT recommends that sample containers be opened in a chamber filled with an inert gas, such as sterile nitrogen.[51,52]

April 4, 1969

NASA Policy Directive 8020.13 formally assigns responsibility for forward contamination to OSSA (meaning that an inventory of Earth organisms transported to the Moon should be maintained), and for back contamination to OMSF ("certifying to the Administrator that the recommendations and statutory requirements of the regulatory agencies have been fulfilled prior to the release of lunar astronauts or lunar exposed materials from quarantine"). Several years of warfare between OSSA and OMSF have preceded this.[53]

April 28-30, 1969

Another conference of approved outside investigators is held at MSC.[54]

June 1969

Another simulation of sample return is held.[55,56] For this occasion, the director of LRL is temporarily replaced by a member of top-level MSC management. Technical personnel in LRL feel the simulation is as far from

[51]Compton, 1989; see footnote 3.
[52]Frondel, 1964-1970; see footnote 18.
[53]Bagby, Jr., 1975; see footnote 16.
[54]Frondel, 1964-1970; see footnote 18.
[55]Annexstad, 1978; see footnote 2.
[56]Compton, 1989; see footnote 3.

satisfactory as the March 1969 simulation was. However, the interim director pronounces it a success and certifies the facility as ready to receive lunar samples.

July 24, 1969

Apollo 11 splashdown.

July 25 - August 10, 1969

The Apollo 11 samples are quarantined and subjected to preliminary examination. Sample containers are opened in the F-201, cursory preliminary examinations are made there, and individual samples are then packaged and placed in metal bolt-top vacuum containers and passed out of the F-201 for storage and distribution.

Few plans have been made for timely subdivision, packaging, and distribution of samples to outside investigators. No safeguards are in place against contamination of the samples that might compromise scientific investigations. Rocks are sawed or broken in open air, using ordinary, chemically impure tools. No plan is in place for the long-term curation of the samples, and this is an urgent problem because the Apollo 11 samples must be removed from the sample-handling facility, and the facility cleaned, before November 24, when the samples from the next mission (Apollo 12) will arrive.

Members of LSAPT are deeply concerned by the disarray of the system—the obstacles created by the vacuum chamber and also by quarantine protocols, the many potential sources of contamination of the samples, and the general inefficiency of operations. They take their case to the top, visiting the NASA administrator and the director of MSC. With the support of these managers, LSAPT members take personal responsibility for obtaining chemically clean chisels and other tools, and certifiably clean containers for sample distribution. Because of its effectiveness in improving LRL operations, and the outspoken nature of some of its members, LSAPT comes to enjoy an unprecedented degree of influence in the management of LRL.

November 6, 1969

After the Apollo 11 samples are found not to react with dry nitrogen, a consensus develops in LSAPT that handling the samples in pure nitrogen would be greatly preferable to relying further on the problematic F-201. However, the LRL director remains faithful to the vacuum system in which he has invested so much of his energy. In the end a compromise is reached: The mission plan for Apollo 12 is that one of the two sample return containers will be processed in the F-201 complex; the other, in a nitrogen-filled glove box.[57]

November 24, 1969

Apollo 12 splashdown.

December 2, 1969

During preliminary examination of the Apollo 12 samples a cut glove in the LRL vacuum chamber causes a "spill," sending 11 exposed people to the Crew Reception Area, which is filled to overflowing, for the duration of quarantine.[58] (Some personnel who also were exposed to the hypothetical spill evade quarantine by fleeing the area before guards charged with enforcing quarantine rules arrive.)

February 12, 1970

The manager of LRL is replaced.[59] The new manager also favors use of the F-201 for sample processing.

[57]Frondel, 1964-1970; see footnote 18.
[58]Compton, 1989; see footnote 3.
[59]Annexstad, 1978; see footnote 2.

FIGURE B.2 A portion of the sterile nitrogen atmospheric processing (SNAP) line, a system of glove boxes used for processing samples returned by the later Apollo missions. The cabinets contain purified dry N_2 at slightly more than atmospheric pressure, so leakage is outward, protecting the samples but not personnel (if the samples had contained pathogens). Gas in the cabinets is continuously monitored for contamination by organic compounds, water vapor, oxygen, and other trace gases. SOURCE: Photograph by J.A. Wood.

April 13, 1970

The explosion of an oxygen tank in the Service Module of Apollo 13 prevents that mission from sampling the Moon and delays the launch of the next mission, pending study of the failure. Where Apollo launches had been scheduled to occur at 4- or 5-month intervals, Apollo 13 opens a hiatus of 13 months between the return of Apollo 12 and Apollo 14 samples.

This "breather" is welcomed by LRL personnel and LSAPT as an opportunity to install equipment, and acquire tools, needed for clean sample handling and storage in LRL. Extensive glove-box facilities (Figure B.2) are constructed to facilitate processing of samples in a sterile nitrogen environment, tested, and cleaned.

November 1970

LSAPT succeeds in having the F-201 deactivated in favor of the use of nitrogen cabinets.

December 15, 1970

The manager of LRL is again replaced.[60]

[60]Annexstad, 1978; see footnote 2.

February 9, 1971

Apollo 14 splashdown.

April 28, 1971

After life-detection experiments on samples from three Apollo missions return negative results, NASA announces that ICBC will no longer require crew or sample quarantine.[61-63]

[61]King, 1989; see footnote 1.
[62]Annexstad, 1978; see footnote 2.
[63]Compton, 1989; see footnote 3.