Corrections

ECOLOGY. For the article "Water-borne cues induce chemical defense in a marine alga (*Ascophyllum nodosum*)" by Gunilla B. Toth and Henrik Pavia, which appeared in number 26, December 19, 2000, of *Proc. Natl. Acad. Sci. USA* (**97,** 14418–14420; First Published December 5, 2000; 10.1073/pnas.250226997), the authors note the following correction. The sentence on lines 8 and 9 in the second column on page 14419 should read ". . . and the wet-weight change (*WWC*) of each agar dis**c** was calculated by subtracting the **start** weight from the **stop** weight."

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PERSPECTIVE. For the article "Planetary exploration in the time of astrobiology: Protecting against biological contamination" by John D. Rummel, which appeared in number 5, February 27, 2001, of *Proc. Natl. Acad. Sci. USA* (**98,** 2128–2131), the author notes the following correction. On page 2129, the article stated that astronauts from *Apollo* 11 and 12 were quarantined, but it should have stated that astronauts from *Apollo* missions 11, 12, and 14 were quarantined. The period of quarantine for the crew was 21 days from their last lunar-surface exposure, whereas the 30 days mentioned in the article was the designed time-period for completion of lunar sample safety protocols.

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GENETICS. For the article "Targeted modification and transportation of cellular proteins" by Pierre Colas, Barak Cohen, Paul Ko Ferrigno, Pamela A. Silver, and Roger Brent, which appeared in number 25, December 5, 2000, of *Proc. Natl. Acad. Sci. USA* (**97,** 13720–13725), the authors note the following correction to the acknowledgments section. "P.C. is grateful to Brian B. Rudkin for hosting the completion of this work, initiated in the laboratory of R.B. at Massachusetts General Hospital. We are grateful to Sandrine Mouradian for the flow cytometry experiments, Ron Geyer and Alejandro Colman-Lerner for anti-Ste5 aptamers, Fred Winston for RSP5, John McCoy for rabbit anti-TrxA antiserum, Rosine Haguenauer-Tsapis for Yep105, Stan Tabor for advice about TrxA mutants, and Mark Stahl for advice with the evanescent wave experiments. We thank Ron Geyer, Alejandro Colman-Lerner, and Jeffrey C. Way for comments on the manuscript. These experiments were supported by grants from the Association pour la Recherche sur le Cancer (to B.B.R.), the Ligue Nationale contre le Cancer (Rhône and Drôme committees; to B.B.R.), the Fondation pour la Recherche Médicale (to P.C.), and by a grant from the National Institute of General Medical Sciences (to R.B.)."

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Perspective

Planetary exploration in the time of astrobiology: Protecting against biological contamination

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These are intriguing times in the exploration of other solar-system bodies. Continuing discoveries about life on Earth and the return of data suggesting the presence of liquid water environments on or under the surfaces of other planets and moons have combined to suggest the significant possibility that extraterrestrial life may exist in this solar system. Similarly, not since the Viking missions of the mid-1970s has there been as great an appreciation for the potential for Earth life to contaminate other worlds. Current plans for the exploration of the solar system include constraints intended to prevent biological contamination from being spread by solar-system exploration missions.

The United States landed a pair of spacecraft on the surface of the planet Mars in 1976. The Viking landers were the first spacecraft successfully operated on the surface of another planet, and to many their primary purpose was to search for indications of Martian life. During the eight and one-half months after landing, the Viking spacecraft examined Martian samples by using their three different life-detection instruments, each of which carried a gas chromatograph/mass spectrometer (GC/MS). Together, the landers made 26 attempts to test for putative Mars microorganisms in the Martian soil material (1). These attempts, initially thought to be quite encouraging, because of the reactivity of the soil material when mixed with water, were considered eventually to be disappointing or equivocal by most of those hoping to find life—and it was the lack of organic compounds detectable by the GC/MS that was considered to be definitive. Without evidence of organics, the majority view of the Biology Team was that no organisms were detected by the two Viking landers. Henceforth, and despite the fact that the Vikings' sampling equipment never penetrated more than 10 cm below the surface of the planet, Mars was considered by many to be dead (*cf.* ref. 2)—much deader than even the deep-sea bottoms on Earth, which in the minds of some biologists were thought to be known quite well (*cf.* refs. 3 and 4).

There was a related irony then when only 7 months after the first Viking landing, the submersible *Alvin* discovered a previously unknown profusion of life on the deep-sea bottom ($\approx$2,500 m below the surface) in an "oasis" of hydrothermal vents along the Galápagos Rift in the Pacific Ocean (5, 6). Not only was this environment rich with macroorganisms previously unknown to science, but the vent ecosystem derived its existence from chemoautotrophic bacteria that used the sulfides and other materials venting from the subsurface as a source of energy (7). As a means of putting the question of life on Mars in perspective, it is significant that the vent ecosystems were not discovered on Earth until more than 100 years after the modern era of oceanographic exploration had begun with the voyage of *H.M.S. Challenger* (1872–1876). And the existence of these ecosystems had not been predicted, even though hydrothermal venting at midocean ridges was considered to be likely.

Perhaps Mars, too, still holds some surprises. Certainly the Earth continues to do so. Summit and Baross, elsewhere in this issue (32), discuss the nature of some of the organisms that have been found in extreme environments on Earth. In fact, the hardiness of life "as we know it" and as the Earth has likely known it for over 3 billion years (*cf.* ref. 8), stretches the imagination. Recent discoveries from elsewhere in the solar system suggest that environments exist on nearby worlds that might be capable of supporting some forms of Earth life. Mars, for example, has sites at which subsurface fluid flows (likely water) may be reaching the surface in the present day (9), whereas Jupiter's moon Europa almost certainly harbors a liquid water ocean below its icy surface (10, 11). Whether life exists on Mars or Europa is still an open question—a question that future missions would like to address.

But the search for life on other worlds is fraught with two concerns other than any sociological issues that might be brought forward by the discovery of life elsewhere. The first concern relates to the difficulty of discovering (possibly rare) life elsewhere, without Earth life confounding the measurements or masquerading as alien life. Part of the solution is undertaking the exploration of other worlds in a manner that does not export Earth life to places where it could grow and thrive. Such an act would threaten both science and possibly an alien ecosystem. Restrictions on "forward" contamination in solar-system exploration seek to prevent this exportation of Earth life. The second concern pertains to the potential difficulties of dealing with alien life that could be discovered on other worlds or in samples returned to the Earth from space. Will we know when we have found it? Is it harmful to humans? Is it harmful to ecosystems on Earth? Restrictions on the possible importation of alien life into the Earth's biosphere seek to avoid the problems of "back" contamination. Together the restrictions imposed on biological contamination in solar-system exploration have been known as "planetary quarantine," or more recently, "planetary protection."

Planetary-protection Heritage

The concepts involved in planetary protection are not unfamiliar to anyone who has studied the history of human exploration, whether through episodes like the introduction of the rat to Hawaii by the Polynesians, the more recent spread of the zebra mussel into the North American Great Lakes by bilgewater from ships returning from Europe, or the more-widespread exchange of microbes by sea-going vessels (*cf.* ref. 12). On Earth, the list of examples both forward and backward is extensive, although it is H. G. Wells (with the help of that other Welles—Orson) who was most successful in popularizing interplanetary considerations in the exchange of dangerous organisms. His *War of the Worlds* featured the invading Martians being killed off by Earth germs—the

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result of an encounter of the sort that the National Aeronautics and Space Administration (NASA) and others are pledged to avoid.

The introduction of planetary-protection principles into spaceflight practices was done early on—a product of the Sputnik era. In the international arena, quarantine standards were adopted by the International Council of Scientific Unions (ICSU) in 1958 (13, 14). With the strong urging of individuals such as Joshua Lederberg, the U.S. National Academy of Sciences made specific recommendations for the practice of planetary quarantine in their 1958–1960 studies (*cf.* ref. 15). Although the successful implementation of this practice was not realized instantly (16), by the early 1970s NASA had reached a robust state of capability in both its policy and practice. The United Nations Outer Space Treaty of 1967 had incorporated an agreement that space missions to other solar-system bodies would "conduct exploration of them so as to avoid their harmful contamination and also adverse changes in the environment of the Earth resulting from the introduction of extraterrestrial matter" (17), thus affirming the earlier ICSU position. In response, NASA established a Planetary Quarantine Office, which continues now as the Planetary Protection Office and has responsibility for the overall NASA program in this area. And ICSU, through its interdisciplinary Committee on Space Research (COSPAR), continues to provide a venue for international scientific discussions of planetary-protection questions and policies.

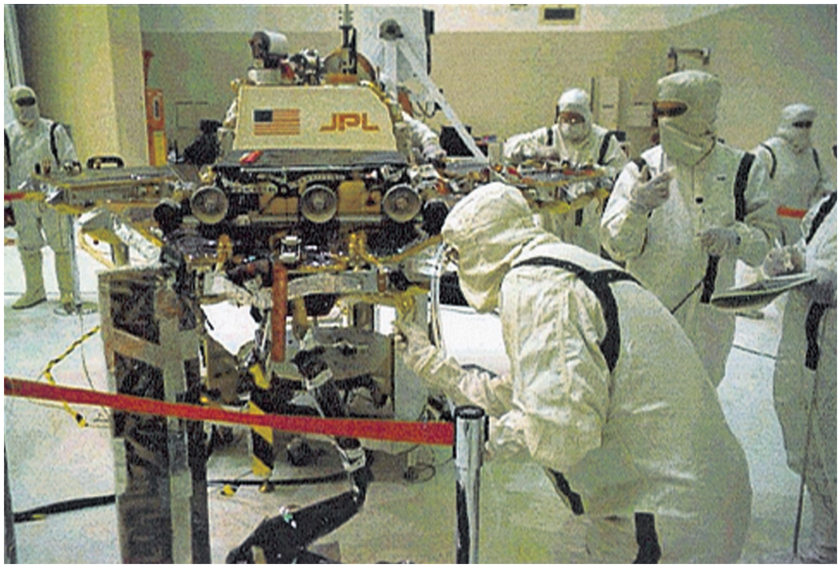

Fig. 1. Before the mission's launch to Mars in 1996, microbiological assays are conducted on the Pathfinder lander spacecraft and the Sojourner rover at NASA's Kennedy Space Center. Although conditions on most of the Martian surface are no longer thought to warrant heat sterilization for the prevention of forward contamination, the prelaunch cleanliness requirements are strictly monitored nonetheless. Photo by Robert C. Koukol, Jet Propulsion Laboratory.

Two Examples

The prevention of forward and backward contamination is the goal of planetary protection as stated in the NASA policy,[†] which focuses on the protection of science and the Earth. The prevention of backward contamination has been of practical concern only once during the history of the U.S. space program—during the initial Apollo missions to the Moon. At that time, the implementation of steps to avoid back contamination was handled by the manned-spaceflight organization, separately from the activities of the nascent Planetary Quarantine Office, which was concerned chiefly with robotic missions. A recent review of this activity is given by Allton *et al.* (19). Although many scientists at the time had come to the conclusion that the Moon was a very unlikely place to encounter extraterrestrial life, NASA determined to be cautious and to provide for a quarantine of the returning samples and astronauts. One of the most obvious lessons of this activity, however, was the difficulty of ensuring the protection of Earth from an unknown and low-probability threat while ensuring the safety of three very real and at-risk astronauts during the process. This juxtaposition inevitably led to compromises that were considered by some to have reduced the effectiveness of the lunar quarantine. Nonetheless, the astronauts from both Apollo 11 and 12 were quarantined for 30 days after their return to Earth, and the samples were subjected to an extensive life-detection and biohazard protocol (20). These analyses, however, detected nothing alive in materials returned by the early Apollo missions (19), and the quarantine was not continued for Apollos 14–17 (Apollo 13 did not land on the lunar surface). Under current policy, the Moon is considered to be effectively a part of the Earth.

Given the pervasive nature of life on Earth, it has been easier to envision the tradeoffs inherent in implementing forward contamination controls, and the arguments against these controls have been judged within a less-charged (if not always certain) framework. Under NASA's planetary-protection policy, the prevention of forward contamination has been practiced on all outgoing spacecraft but has been most notable when applied to spacecraft traveling to solar-system bodies of interest to the study of chemical evolution and the origin of life and where Earth life might survive. To date, Mars has been the only such body on which the United States has landed spacecraft. The first landings on Mars by the Viking missions, mentioned earlier, involved extensive design and implementation procedures intended to reduce greatly the biological load carried by the two Viking landers. In what was a heroic effort, each aspect of spacecraft assembly and test was focused on allowing the most stringent precautions to be used. In the process, each of the Viking landers were cleaned thoroughly and then heat treated—baked in an oven for 30 h after the coldest contaminated point reached a temperature of at least 110°C—both to protect Mars and to safeguard the spacecraft's biology package from contamination by Earth organisms. Results from Viking have indicated that most of the surface of Mars is less likely to support Earth life than once was thought (21). With the Space Studies Board's recommendation and COSPAR affirmation, these results have allowed for the deletion of the heat-treatment step for subsequent Mars landers (such as 1996's Pathfinder mission) that do not seek to detect life on Mars. Nonetheless, the Viking cleaning procedures still are considered to be the standard preparation for landings on Mars (Fig. 1), whereas missions seeking to detect life (e.g., by cultivation techniques)

†NASA's current planetary-protection policy statement (18): The conduct of scientific investigations of possible extraterrestrial life forms, precursors, and remnants must not be jeopardized. In addition, the Earth must be protected from the potential hazard posed by extraterrestrial matter carried by a spacecraft returning from another planet or other extraterrestrial sources. Therefore, for certain space-mission/target-planet combinations, controls on organic and biological contamination carried by spacecraft shall be imposed in accordance with directives implementing this policy.

Table 1. Summary of Space Studies Board recommendations on Mars sample return (25)

- Samples returned from Mars should be contained and treated as though potentially hazardous until proven otherwise.
- If sample containment can not be verified en route to Earth, the sample and spacecraft should either be sterilized in space or not returned to Earth.
- Integrity of sample containment should be maintained through reentry and transfer to a receiving facility.
- Controlled distribution of unsterilized materials should occur only if analyses determine the sample not to contain a biological hazard.
- Planetary protection measures adopted for the first sample return should not be relaxed for subsequent missions without thorough scientific review and concurrence by an appropriate independent body.

are subject still to full heat-treatment procedures or their equivalent.

Future Planetary-Protection Challenges

Since the time of Viking, the solar system appears to have become more rather than less interesting as a potential abode for extraterrestrial life, at least of the microbial sort. We also have a much more extensive appreciation of the widespread distribution and hardiness of Earth microbes, whether they are challenged by the extremes of heat, cold, desiccation, or radiation. The practice of planetary protection has become correspondingly more challenging as a result.

With respect to forward-contamination control, issues include the effective characterization and/or control of the load of Earth organisms carried by spacecraft and how to accomplish these tasks in the face of increasingly complex computerized systems and sensors. In facing the decontamination of complex electronics and machinery, however, NASA is not alone, and it is thought that many of the contamination-control solutions being developed for the bioengineering world will be adaptable to spaceflight missions. More esoteric questions involve the potential for survival and transport of organisms deposited on another world—whether it be a place like Mars, with blowing winds and dust but little apparent surface turnover, or a place like the ice-covered moon Europa, where the specific processes that reshape its surface and allow surface communication and mixing with the subsurface material are not well understood. Both the likely liquid-water ocean under the Europan surface and the deep subsurface of Mars (or any near-surface aquifers that still may exist) seem potentially to be conducive environments for some Earth microbes. Practices and procedures to avoid the contamination of these environments during upcoming missions are under development. Additionally, there is an ongoing debate about the ethical considerations associated with the risks involved in solar-system exploration (*cf.* refs. 22 and 23).

Currently announced plans for sample-return missions and their planned return dates include Genesis (2003), Stardust (2006), the Japanese mission MUSES-C (≈2006), and the first Mars Sample Return mission (≈2011–2013). On the basis of the expectation for life to exist on the other solar-system bodies to be sampled, before launch such missions are examined for their potential for back contamination (24) and their potential to present a hazard to the Earth's biosphere. Of the currently planned missions, only the Mars Sample Return mission is thought to have any potential to introduce biological contamination, although even in the case of Mars the prospects for extraterrestrial life to be encountered on the surface are considered to be small (25). Nonetheless, the probability that a mission returning samples from Mars will return a living entity is considered to be nonzero, and the potential for such an entity to cause damage to the Earth's biosphere cannot be discounted, because even organisms from other terrestrial continents may be the cause of major ecological disturbances (*cf.* ref. 26).

Balancing the benefits of a sample-return mission against its potential risks is not strictly a task for planetary protection, but it is clear that avoiding the risks from such a mission carries no ethical quandary of the sort that accompanies forward contamination considerations—rather it is a question of simple prudence. To that end, the Space Studies Board (25) has provided a series of recommendations to NASA on how to approach such a mission (Table 1). NASA is proceeding to plan a sample return from Mars with those considerations in mind.

Currently, the analyses that will be used to determine that a Mars sample does not contain a biological hazard are under development, with a wide variety of participants and expertise being represented. Questions to be addressed in designing these analyses are listed in Table 2.

Additional considerations for a Mars sample-return mission include the need to reduce and/or characterize spacecraft bioload to accomplish forward-contamination goals and minimize the potential for Earth organisms to make the round trip and be misidentified as Mars organisms. Work such as that of Gladman *et al.* (27) and the evidence that the Earth is the target of a natural influx of material from Mars (e.g., ref. 28) suggests that Earth

Table 2. Questions on returned sample analysis and testing

- What criteria must be satisfied to show that the samples do not present a biohazard?
- What will constitute a representative sample for testing?
- What is the minimum allocation of sample material required for analyses exclusive to the protocol, and what physical/chemical analyses are required to complement biochemical or biological screening of sample material?
- Which analyses must be done within containment, and which can be accomplished using sterilized material outside of containment?
- What would comprise an effective sterilization method for martian samples?
- What facility capabilities are required to complete the protocol?
- What is the minimum amount of time required to complete the protocol?
- How are these estimates likely to be affected by technologies brought to practice by two years before sample is returned?

organisms may have been transported to Mars in the course of the last 4 billion years or so, and some of them may have survived there. Conversely, organisms that may have originated on Mars may have come to Earth in the past. One goal of the exobiological study of Mars will be to examine this issue, and round-trip contamination certainly would obscure the ability to address these questions. Other, more-mundane considerations include the selection of a safe landing site, the location and capabilities of a sample-receiving facility to accomplish the required planetary-protection analyses, and the means of moving a returned sample from the landing site to the receiving facility.

A far more interesting question, of course, will address the means for proceeding if life *is* ever detected in a Mars sample or in a sample returned from Europa or some other solar-system location.

The Role of the Academies

The NASA planetary-protection policy (18) requires that NASA "take into account current scientific knowledge about the target bodies through recommendations from both internal and external advisory groups, but most notably from the Space Studies Board of the National Academy of Sciences." In this role, the National Research Council's (NRC) Space Studies Board has been the principal advisory group for NASA in this area since the time of Sputnik. A number of the NRC's reports are listed below (29, 21, 25, 24, 30), covering forward-contamination questions for Mars through the outer planets and their satellites and back-contamination concerns associated with Mars and a variety of moons and other small bodies of the solar system. Additionally, other reports from the NRC on similar issues (e.g., ref. 31) may have valuable guidance in addressing planetary-protection issues.

At the recommendation of the Space Studies Board, NASA also is establishing a Planetary Protection Advisory Committee within the NASA Advisory Council. This group will provide advice to NASA on a near-real-time basis and is expected to provide a valuable service in addressing both forward-contamination issues and the more widely sensitive issue of returning samples from other worlds that may harbor life. With the help of both of these groups—and other activities such as workshops that tap the broad community of life and planetary scientists—NASA is planning to continue its policy of safe solar-system exploration and its successful implementation.

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