STRATEGIES FOR INVESTIGATING MARTIAN MICROENVIRONMENTS FOR EVIDENCE OF LIFE:
THE EXPEDITION ONE EXPERIENCE

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While it is not known whether life ever existed on Mars, answering this question is a major rationale for human exploration. The search for extant or fossil life necessarily will be conducted in collaboration with geological analysis to identify microenvironments favorable to life forms that may have evolved a unique biochemistry. A proposed exploration strategy, assuming a base installation with extensive research and communication equipment, involves sorties of days to a few weeks by parties of three persons. Two types of vehicles will be needed: a pressurized research rover (PRR) with adequate living quarters and a small field laboratory, and one or more faster, non-pressurized 4-person vehicles (SRV) for scouting, re-supply, and rescue. A day on sortie would include one or more EVAs, analysis of samples and instrument data, communication with personnel at the base, and maintenance of all research and engineering systems. It is essential that some sample analysis take place during the sortie in order to guide further choice of sampling sites and to preserve raw data in the event of astronaut loss.

Analog research such as Expedition One is invaluable to assess exploration strategy and to indicate areas for future design. For example, field equipment and techniques must permit rapid sampling and measurement, because the air supply on EVA is limited. All collection components must be fully integrated with analysis systems on the PRR and at the base. Consumable items for analysis will be limited by the constraints of payload weight; thus each test performed must answer a particular question. While robotic exploration components augment human capabilities in some circumstances, our experience during Expedition One indicates that human effort will be required to study some sites of particular biological interest. A crucial requirement of human exploration, however, is the avoidance of contamination of sampling sites with Earth origin organisms or organic compounds.

INTRODUCTION

Orbiter and unmanned lander missions have provided extensive data on the topography and geology of Mars. All present evidence indicates that water has played a major role in shaping landforms, and still exists in at least the polar areas as ice or frost¹. Whether life has ever been present on Mars is unknown, and is the crucial question for all future explorations. Unless the lander expeditions currently underway produce conclusive evidence that life in some form exists,

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human explorers will answer the question during onsite analysis of likely Martian habitats, or of likely fossil sites. Clearly, the search for life will be coupled closely with geological exploration, since the search for promising habitats involves surveys of both landforms and local geochemistry.

In order to design a reasonable search, it is necessary to envision how an exploration mission might be structured. Analog research, such as that carried out by Expedition One, can then test whether the assumptions underlying the mission architecture would be workable under actual field conditions. In this paper, we describe a proposed mission configuration and the experience of Expedition One at the Mars Desert Research Station (MDRS) in Utah, USA, in finding and sampling unique microbial habitats. Future analog studies should build on the lessons learned during our initial trial.

GENERAL MISSION CONFIGURATION FOR BIOLOGICAL AND GEOLOGICAL EXPLORATION ON MARS

For purposes of designing a specific surface study, we need to consider the assumptions of the overall mission. A crew would have a base of operations, either in the lander vehicle, or in a structure built to house all crewmembers. The Mars Base (MB) would provide life support equipment for the duration of the stay, and scientific equipment. Further assumptions include:

1. The landing site will be chosen on the basis of extensive imaging and robotic lander data. The presence of water and specific mineral composition will be known to a scale of 100 m or smaller.

2. The crew will know something about the levels of toxicity and corrosiveness of the regolith. It is crucial to know this in advance, since precautions to avoid hazards to the crew and damage to equipment will affect the time available for the actual research.

3. Since all aspects of exploration on Mars carry significant risks, ongoing capture of raw data and communication between crewmembers will be essential. This will ensure a return on the mission, even if the crew is lost.

4. Much scientific analysis will take place during the actual exploration or at the lander. Only the samples of greatest significance, or that require analysis outside the capabilities of available equipment, will be returned to Earth.

Exploration Equipment

The Mars Base will include a compact but well equipped laboratory equipped for a broad range of spectral and chemical analysis, space for sample preparation and storage, light, electron, and scanning electron microscopes, reagents, supplies, and spare parts, and animal and plant habitats. The latter are to be used for toxicity studies, and are separate from any greenhouse facilities designed for crew food and environmental processing.

Actual exploration involves two types of vehicles, a three-person pressurized research rover (PRR), and one or more faster, non-pressurized rovers for scouting, re-supply, and rescue (SRV).
The PRR will be used by three crewmembers for a sortie away from the base for an extended period. Such a vehicle would be designed to travel over rough and sandy terrain, albeit slowly. Engineering trade-offs include a balance between carrying capacity for fuel and other consumable supplies, and weight. These constraints will determine the speed and total number of days for the sortie, and hence the total area that can be explored. The PRR will contain a pressurized cabin with life support equipment, and other facilities for living and working:

1. A very small laboratory (1-1.5 m width) with miniaturized instruments for immediate field study; e.g., a gas analyzer, microscope, spectroscopy equipment. It will also have a small area for supplies and sample storage. If prior data indicates that samples may be acutely toxic, a glove box configuration for a sterile sample preparation area will be included.

2. Storage for instruments to be deployed outside the PRR

3. A small area with tools and supplies for equipment repair and maintenance

4. Computer and communications equipment

5. Airlock and storage for three pressure suits

6. Common area with space for food storage and preparation, and a collapsible work table

7. Three bunks or hammocks, and a toilet and personal hygiene area

The SRV will be used for scouting from the Base, and for re-supply and rescue of the PRR crew if needed. It will be light, fast, and highly maneuverable, and be able to carry at least four persons in pressure suits.

**Sortie Design**

The landing party will make multiple three-person sorties while on the surface. The specific expertise among them may vary, depending on the goals of the sortie, but should include two persons with field science training, and a driver. Engineering, emergency medical, and communications expertise must be represented, preferably by each crewmember. An essential feature of this strategy is prior scouting to determine the most productive areas for exploration. The landing party will be spending a great deal of time ensuring their own safety and wellbeing. Distribution of effort between these activities and actual exploration and analysis is a question for serious analog study. A major consideration for the search for Martian biota is to determine how much effort should be expended in the investigation of a given site or feature. Preliminary large-scale \(^2\) and local scouting in the SRV will indicate areas of promise, and will enable the crew to select a direction for the initial sortie.

A typical sortie in the PRR might last for weeks, depending on fuel and other constraints. Personnel remaining at the Base will maintain constant communication with the exploration party, perform maintenance, and carry out scientific investigations. A "day on the road" for the sortie crew might look like this:
1. The sortie crew departs in the chosen direction at a speed that depends on the terrain and the distance to the first selected stop site. The PRR design should allow observation of the landscape by all members of the party, so features of interest can be noted that might have been missed during the initial scouting. Each day might include two EVAs and driving to total about ten hours.

2. Each EVA includes suit donning, equipment assembly and checking, and airlock cycling. The time outside will depend on the air capacity and must be monitored closely by each crewmember as well as by the person remaining aboard the PRR. As is the case for good SCUBA practice, both persons on EVA must return to the PRR at a prearranged air level, or sooner if the situation warrants.

3. Great care must be taken to avoid contaminating crew and laboratory areas with soil from the outside. Even if it is not toxic and corrosive, grit readily damages sensitive equipment.

4. The PRR is parked before dark at a safe spot, out of danger of rockslides, etc. After parking, the crew performs analyses on the samples returned to the rover, prepares a summary for the day, and transmits it together with raw and experimental data to the Base.

5. They then plan for the next day and perform maintenance on all systems.

6. The rest of the day is spent in eating, personal time, and sleep.

It is not known how long a crew could work productively under these conditions, and whether non-travel days need to be scheduled for rest, repairs, and planning. If a crew were to decide that long-term travel in that general direction was desirable, however, the SRV could bring supplies to extend the sortie, or perhaps lay a cache for an extended stay in one area. The potential for repeating this would depend on the range and carrying capacity of the SRV.

Return to the Base need not follow the exact same route. If features of interest are identified which have not been explored during the outward leg of the sortie, they can be examined on the way back. If instruments have been placed for data collection over a period of days, they can be retrieved.

**EXPLORATION DESIGN FOR MICROBIAL HABITATS AT MDRS**

Despite the barren, arid appearance of the landscape around MDRS (the Hab), which is analogous to the MB, microbial life is everywhere. The first step for exploring microbial diversity in this area is to identify geologically diverse areas likely to provide niches that supply nutrients for a wide range of microbial life. The basic problem is the considerable range of scale required to do this. For example, landforms relevant to the geochemistry of the area may be measured in kilometers, local features at 1-10 m, individual mineral deposits at a few cm, while a bacterial colony may be only 1 mm in diameter. Individual bacteria, which can occupy small crevices in particles of soil, are usually less than 1 mm in diameter. Thus, an explorer needs to recognize patterns with the unaided eye with dimensions which can vary by a factor of $10^6$. We collected material from several sites of interest, both on foot and with the aid of prototype rovers used in a PRR simulation.
Search for unique habitats

One site of particular interest was at the head of a box canyon, one of a series that occur in shallowly dipping Late Jurassic sediments on the north side of Route 24, about 4 km west of Hanksville. The canyon walls and floor are comprised of siltstones, shales, gypsum, and minor sandstone of the Summerville Formation, while resistant cap rock at the top of the canyon walls consist of the sandstones and conglomerate of the Salt Wash Member of the Morrison Formation. The box canyons have the characteristic vertical sides, theater head, and flat floors of canyons cut largely by groundwater sapping. The seeping groundwater weathers and loosens the Summerville Formation, resulting in particles dislodged by wind and gravity, undermining the Salt Wash Member. This process is common in many areas of flat lying geology on earth and is believed to be an important process in the formation of at least some of the valley networks on Mars.

The groundwater seeps in the theater-heads of these valleys are important from an astrobiological perspective for three reasons: First, they are areas of active water discharge at the surface. Second, they are sites of active weathering and nutrient release. Third, they are sites sheltered from cosmic radiation. Such theater-heads would be a prime target for any astrobiological research on Mars. However, they are difficult to access and sample. For Example, Figure 1 shows the authors preparing to survey and sample microhabitats underneath the sandstone overhang, seen in the upper left. They are wearing the MarsSkin simulation suits.

Figure 1: Cavity Showing Sappage in the Wall Underneath a Sandstone Cap
The authors also visited an unnamed uranium prospect to the west of North Caineville Mesa. This prospect has no known historical production\textsuperscript{12}. Samples were taken from spoil dumps adjacent to a small adit, sunk into steeply dipping carbonaceous conglomerate and sandstone of the Salt Wash Member of the Morrison Formation. Using an ultraviolet lamp, the mineralization was revealed to be primarily autunite, distributed along fractures and intimately associated with fossil wood fragments. As the sandstones are somewhat weathered, the autunite is interpreted to be the result of oxidation of primary uraninite. The presence of this phase was not confirmed visually, however the pitch-colored mineral would have been difficult to distinguish from the fine-grained fragments of carbonaceous matter.

We deem the site interesting because of radiation from the uranium mineralization, measured at 0.3 millirads, provides an interesting proxy for the high levels of cosmic radiation that is a feature of near surface Martian environments. Studies of areas with high natural radioactivity elsewhere in the world have shown the presence of microorganisms with specific radiation resistant abilities\textsuperscript{13,14}. Sampling this site provides the first step in testing for the presence of such organisms in the MDRS field area.

Other sites sampled on foot, and while wearing MarsSkin suits, include a region of cobbles of igneous origin, near White Rock canyon, and an area with lichen-rich boulders near Kent Reservoir. Further details of sampling procedures are given below.

**Rover-Based Studies**

Three sorties were carried out in prototype PRRs, as described in a companion paper in this volume\textsuperscript{6}. The authors served as field scientists to test the performance of the rovers, and to assess exploration and sampling operations under full simulation, and under a time-limited scenario. Each test was carried out during one day, with three sampling EVAs. For this study, we wore the MDRS simulation suits, which are considerably heavier and more cumbersome than the MarsSkin suits shown in Figure 1. Suit assembly and airlock cycling took place before and after each EVA, which lasted between 30 and 45 minutes. The following three sites were visited during each test:

Site 1 consisted of an eolian plain with uncemented small dunes of quartz sand partly mantling a deflated surface armored with pebbles. There were scattered boulders of Early Cretaceous Dakota Sandstone, composed of quartz sandstone and lithic conglomerate. The surfaces of the boulders were covered by thin desert varnish.

Site 2 consisted of smectitic clays and thin lenses and beds of quartz sandstone and ferricrete, gypcrete, and silcrete, these last three representing Jurassic paleosols. Two features were studied at this site. Feature a) was a small wash eroded into the clay, the surface of which was crumbly and subject to episodic wetting. Feature b) was an erosional rise capped by sandstone, the top of which had a loose sandy soil with a distinct cryptogamic crust.

Site 3 consisted of a low relief wash cut into a smooth plain of smectitic clay, of the Brushy Basin Member. A temporary puddle had formed in the wash where it ponded against the windrow at the edge of a graded track. Because the clay surface was either wet or had only just dried out, its surface was smooth and not crumbly, unlike the previous site.
At each site, the field team identified features of interest by walking to areas likely to provide a unique habitat, and collected samples and took photographs. It was frequently difficult to carry out both a survey and collection in the time allotted. The crewmember remaining in the rover kept track of the time and alerted the field team to return. This was a significant simulation of what a day on the road would be like on Mars, and indicates how operations and technology will need to be improved.

**SAMPLING METHODS AND ANALYSIS OF COLLECTED MATERIALS**

The first step in designing a sampling strategy is to decide how large a sample is required, which depends on the questions the sample is required to answer. If the source is abundant, it is nevertheless not desirable to collect excessive quantities, for several reasons: First, the material must be put into a container, which will then be stored. In both analog and Martian situations, space is severely limited. Secondly, supplies for analysis are limited, so that reduced reaction volumes and small culture devices are favored. All supplies and collection equipment must be carried by the field scientist, who may be required to navigate difficult terrain. If the material is not abundant, then it will be necessary to have highly sensitive, miniaturized experimental procedures, with priorities for use of a limited sample. Finally, removal of all of a possibly unique sample must be avoided.

**Investigations at MDRS**

For the reasons stated above, sampling of diverse microbial habitats during Expedition One was carried out on a minimal scale. The techniques employed are shown in Table 1. Every effort was made to collect samples under sterile conditions. All supplies, such as toothpicks and tubes, were autoclaved before beginning the sortie. Metal tools, such as the spatula ("scoopula") were sterilized with 95% ethanol on site, just before use. Other personnel on the EVA were cautioned to stand back and avoid trampling collection sites.

All of the techniques employed here are standard for microbiological fieldwork. Swabbing was carried out with dry Q-tips and dry wooden toothpicks against dry surfaces. If the surface of interest is hard, and does not lend itself to breaking off a representative sample, then surface removal or *in situ* analysis are the only choices. During the rover studies, sterilized glass microscope slides, equipped with brightly colored plastic telltales, were left in place for 24 hr and retrieved during the next sortie. Instrument placement for later collection is an example of the type of field activity likely to be employed during Martian exploration. We were able to find the samplers by returning the rover to the previously established GPS coordinates, recognizing general surface features from the previous day, and then spotting the telltale after walking to the site. Given the time constraints for the EVA, it is essential to carry out this operation quickly and efficiently.
Table 1

SAMPLING TECHNIQUES

<table>
<thead>
<tr>
<th>Method</th>
<th>Collection Tool/Container</th>
<th>Sample Type</th>
<th>Examples</th>
</tr>
</thead>
<tbody>
<tr>
<td>Surface scraping</td>
<td>Curved metal spatula 1.5 ml capped tube</td>
<td>Materials on rock or soil surface</td>
<td>Lichens, exfoliating crust</td>
</tr>
<tr>
<td>Bulk samples</td>
<td>Curved metal spatula 1.5 ml capped tube</td>
<td>Clay, sand, small pebbles</td>
<td>Gypsum crystals, soil under rocks</td>
</tr>
<tr>
<td>Swabbing</td>
<td>Sterile cotton swabs, toothpicks</td>
<td>Rock surfaces</td>
<td>Igneous rocks, radioactive deposits</td>
</tr>
<tr>
<td>In situ collector</td>
<td>Glass microscope slide 50 ml plastic tube</td>
<td>Organisms and molecules attracted to glass</td>
<td>Dry sand under rock, damp puddle</td>
</tr>
</tbody>
</table>

All of the samples collected were returned to the Hab for further workup. As an example of in situ analysis, detailed examination of some features was carried out with a hand lens. This operation was made more difficult by the need to view the area through the simulation suit face plate, and could compromise the sterility of the area under examination. A Geiger counter was used to detect radioactivity at the uranium prospect area.

Preliminary growth experiments were carried out on most of the samples collected. A complete enumeration of all microorganisms in a given sample challenges present technology; for example, studies have estimated that at most a few percent of microorganisms present in soil can be cultured in organic media. Despite the presence of dormant and viable nonculturable organisms, it is helpful to determine whether anything can be cultured. Accordingly, both rich (Luria Broth, LB) and minimal (filtered water from Kent Reservoir) agar plates were prepared. The rationale for the latter is that minerals adequate for the growth of some organisms should be present in standing water from a local source.

Very small portions (2-3 mm³) of dry samples were applied to marked areas of the plates using the back ends of sterile plastic micropipetor tips. Plates were incubated at approximately 30°C and observed daily. Abundant outgrowth was observed from all dry samples; while organisms have not been characterized, rapidly spreading, mucoid colonies were present in most of them.

The most unusual outgrowth was associated with a sample collected at the uranium prospect. Several large chunks of fossilized wood were observed, which showed radioactivity at a level of 0.3 millirads. Upon splitting a lump of this material with a hammer, clumps of a black, coal-like material were revealed in the interior. These areas were likewise radioactive. After dry plating a small fragment of the black material on rich medium, a luxuriant, fluffy white aerial mycelium from an unidentified microorganism appeared within a day. No such mycelium was observed in any of the other samples. Since extensive laboratory work was not possible at MDRS, due to minimal
equipment and insufficient time, all samples have been stored for further analysis using appropriate techniques:

**Sampling Techniques for Mars Exploration**

The biological field and analytical methods employed during Expedition One are completely inadequate for a real Mars mission. Despite the use of small scale sampling at MDRS, the entire collection cycle is inefficient, may not permit the most significant discoveries, and is doubtless incompletely sterile. The following changes could be developed incrementally in future analog studies.

1. Instrumental analysis in the field, such as provided by a Portable Infrared Mineral Analyser (PIMA), and temperature and humidity measurements could be used to characterize sites at the 1-10 cm scale. While these and other instruments were available at MDRS, their use as currently configured would be awkward in situations such as the overhang shown in Figure 1. Field instruments must be free of organisms and organic compounds, and repaired in an area of the PRR completely separate from crew activities.

2. Each small area of interest in a feature must be photographed and assigned a unique identifying code. This information should be transmitted to the PRR. A prototype Datalogger\textsuperscript{18} was developed and used on Expedition One and, in more advanced form, could be used for this purpose.

3. Samples should be collected in specialized, sterile, single-use collection devices which collect, store, and insert the specimen into analytical instruments. Different configurations could include sharp tips for scraping and inorganic, nonporous tips for swabbing. Each should be identified uniquely using a barcode or embedded magnetic tag. The field scientist need only remove the collector, take the sample, close a cover, and store it for return to the PRR. Ideally, this operation should be performed with one hand, and must be convenient to carry out using pressure gloves. It will require some thought to estimate how many devices will be required for the mission.

4. A gas-trapping collector could be developed for use in confined areas where volatile byproducts of any actively metabolizing colony might accumulate. Such a device would be left in place for a period, and collected later. The time required might be substantial, depending on the sensitivity of the detector and the yield of metabolites in the gas phase at Martian temperatures.

5. All tools, instruments, and collection devices should be carried in an instrument package on the astronaut’s person. It should be easy to see each item, and to remove and replace it. Since a field scientist will need to sit, kneel, and stretch to reach overhead sites, the carrier must fit closely against the body in order to avoid interfering with these operations. It is unknown how much weight a human can carry on the surface of Mars, but a carrier should be as light as possible. Wearing a carrier is preferable to holding a container of some kind like a suitcase, since walking would be much easier and placing a carrier on the ground is undesirable.

6. Materials capable of maintaining properties such as transparency and flexibility under Martian conditions should be examined for use in equipment components.
Science Work Products

Exploration is risky business. It is important to preserve raw data and the increasingly detailed analyses from the beginning, in case the sortie or the mission is lost. The work will vary, depending on the kind of information a particular sample is intended to provide, as shown in Table 2.

<table>
<thead>
<tr>
<th>Step</th>
<th>Location</th>
<th>Action</th>
<th>Product</th>
<th>Decision</th>
</tr>
</thead>
<tbody>
<tr>
<td>Identify EVA sampling/instrument deployment site</td>
<td>Base, PRR</td>
<td>Prepare sampling materials and instruments</td>
<td>Site location and description</td>
<td>Instrument/sampler retrieval. Revisit site?</td>
</tr>
<tr>
<td>Preliminary EVA analysis</td>
<td>PRR</td>
<td>Chemistry and mineral analysis</td>
<td>Descriptive analysis of each sample</td>
<td>Set priorities for further analysis on selected samples</td>
</tr>
<tr>
<td>Preliminary sortie summary</td>
<td>PRR</td>
<td>Comparisons among samples; Changes with local terrain</td>
<td>All raw data; results of laboratory studies</td>
<td>Identify features of greatest interest</td>
</tr>
<tr>
<td>Extensive chemical and biological sample workup</td>
<td>Base</td>
<td>Analysis of selected samples; attempt culture; toxicity studies</td>
<td>Geochemical and biology analysis for sortie; consult Earth</td>
<td>Identify samples requiring further study</td>
</tr>
<tr>
<td>Select next sortie direction</td>
<td>Base</td>
<td>Evaluate all results</td>
<td>Refined or redirected questions</td>
<td>Scientific merit and engineering feasibility</td>
</tr>
</tbody>
</table>

Only some samples will be returned to Earth. Those samples whose analysis is complete, or duplicate samples, will be left behind at the Base, labeled and identified, for analysis by future missions if deemed appropriate.

CONCLUSIONS

Can we do this? Will human and technical factors be reliable enough to answer real questions safely? Only by testing every aspect of exploration strategy under close simulation on Earth will we be able to identify and correct shortcomings. Two things we will not be able to simulate are the lesser Martian gravity and the profound isolation of the crew. All other
components, from scientific analysis systems to training, can be developed and tested at analog research facilities.

**Use of Robots as Adjuncts to Human Investigation**

Previous and ongoing studies of the Martian surface have been carried out by robotic landers. While instrument packages and autonomous rover explorations will provide invaluable information regarding, for example, the composition of the regolith at various sites, there will be some areas which will require human investigation. For example, it is difficult to imagine how a robot could both investigate and sample the overhang depicted in Figure 1. We stood on loose and shifting eroded rock fragments above a steep slope while reaching overhead to sample areas of the overhang roof. While a robot could have been lowered from the top to photograph the cavity, it is unreasonable to foretell the dimensions of every site of interest in order to configure a set of extendable sampling arms or other appendages. Furthermore, it isn't obvious how a robot could have arrived at this site; small position changes might well have sent it tumbling down to the valley floor. Robots probably are most useful in more forgiving terrain as mobile mineral analysis tools, and for exploration of narrow crevices where humans cannot enter readily.

**Contamination of Study Sites**

Will it be possible to prevent the entry of Earth-origin microorganisms and organic material into the Martian environment? While the species we bring with us would seem ill suited to proliferation on Mars, even cell debris could confound a search for organic compounds. Survival of bacteria under the most extreme conditions on Earth is known to occur. It seems inevitable that such material will be released from human habitation sites. Transport of microorganisms over long distances is known to occur on Earth19. In view of the planet-wide dust storms known to take place, it is just a matter of time before organic material is distributed everywhere on Mars. We must both take precautions at particular sampling sites and devise the means to distinguish native Martian organic material, if it exists, from that introduced from Earth.

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