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The Viking Labeled Release Experiment and Life on Mars

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ABSTRACT

The Labeled Release (LR) life detection experiment aboard NASA's 1976 Viking Mission reported results which met the established criteria for the detection of living microorganisms in the soil of Mars. However, a variety of reasons led to the consensus of involved scientists that the positive responses at both Lander sites were caused by a chemical agent in the soil and not by microorganisms. In the years since Viking, new information from Mars and Earth has come to bear on this issue. Perhaps most spectacular are the analyses of SNC meteorites ALH84001 and EETA79001. The Viking LR experiment and each of the major chemical theories that have been proposed to explain it are reviewed in the context of these post-Viking developments, together with some Viking data hitherto unapplied to this important issue. Each of the theories attributing the LR results to chemistry is shown to have one or more key defects. *It is concluded that the Viking LR experiment detected living microorganisms in the soil of Mars.* Recommendations for confirming this conclusion in the near future are given.

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1. INTRODUCTION

Fresh speculation about life on Mars was aroused by reports of possible biochemical and microbiological fossil evidence of life found in meteorites ALH84001¹ and EETA79001², generally accepted as of martian origin. Together with other developments concerning life on Mars, a re-examination of this major scientific issue is warranted.

In 1976, the Viking Mission Labeled Release life detection experiment (LR) returned data from Mars fully satisfying the pre-mission criteria for the detection of microbial metabolism. Nonetheless, the consensus of scientists interpreting the Viking results was that the surprising activity the LR detected in the martian surface material should be attributed to chemistry and not biology.

1.1 Principal challenges to biological interpretation of LR data

1. No organic compounds were found in martian soil analyzed by the Viking Gas Chromatograph Mass Spectrometer (GCMS).
2. H₂O₂, chemically formed in the upper atmosphere, was thought to descend to the soil and, directly or through forming complexes or compounds, to oxidize the LR substrates to evolve labeled gas.
3. It was assumed that there is no liquid water on Mars, and that its absence would make life impossible; and, as a corollary, the overall environment was believed too extreme to support life.
4. The amplitude and kinetics of the LR response from Mars was thought to be "too much too soon" for any putative martian biology.
5. Although not a pre-mission criterion for life, a second injection of LR nutrient onto positive samples failed to re-invigorate the evolution of gas, as generally occurs with terrestrial soils. Instead, some of the gas evolved after the first injection appeared to be reabsorbed into the soil.
6. No visual evidence for life was reported when the Viking camera images were examined.
7. UV light from the sun was thought to activate soil particles, which then disrupted the LR nutrient upon contact, releasing labeled gas. Also, UV light's destructive effect was held to account for the reported lack of any organic matter on Mars.
8. Clays on Mars were proposed to react with the LR nutrient to release labeled gas.
9. A non-biological explanation of the Mars LR response seemed far simpler than proposing a separate origin of life on Mars. Application of Ockham's Razor, therefore, indicated chemistry or physics over

biology.

For one or more of the reasons stated above, the majority of involved scientists believed that the LR response was non-biological. However, the bases for that belief have been weakened. Over the intervening years since Viking, many new facts have emerged. Re-examination of the data and relevant scientific discoveries, including the evidence for life in the martian meteorites, requires a new evaluation. Each of the reasons enumerated above to support the non-biological rationale will be examined under new light.

2. BACKGROUND

NASA selected three life detection experiments for Viking based on three different assumptions about possible martian life. Therefore, it was explained, were there life on Mars, it would be likely that only one of the experiments would detect it.

2.1 The LR life detection experiment

A brief review of the Viking LR experiment is needed for the background to this discussion. The LR applied a small drop of nutrient solution to the center of a soil sample. The liquid spread outward, providing a wet-to-moist gradient through the sample. Organic substrates in the nutrient were at very low concentrations to prevent possible toxicity. For the same reason, no co-factors, growth supplements, or inorganic compounds were added (it was presumed that the soil already would have the ingredients required to sustain any life present). No buffer was used so that the sample itself would determine the pH. A slight helium overpressure and a temperature of 10°C were maintained to assure liquidity of the water during the experiment. Phase separation by gravity provided a signal free from the noise of the radioactivity still retained in the liquid. The LR experiment was the simplest and the most sensitive to microorganisms by orders of magnitude. Unlike the Pyrolytic Release (PR) experiment, it required no intervention (an optical filter) to prevent a false positive; unlike the Gas Exchange (GEx) experiment, it did not adjust the raw data (factoring in presumed coefficients of the martian soil and adjustments for probable gas sample size) prior to interpretation.

The LR experiment^{3,4} is based on the widespread metabolism by microorganisms of Miller-Urey compounds⁵, which are presumed to have been available for pre-biological evolution on primitive Earth. Selection of a particular compound was made after its metabolism was confirmed in each of a wide variety of microbial species in pure and mixed cultures and in soils. Designated VM1, the LR nutrient solution was an aqueous solution of 2.5×10^{-4} molar concentrations each of formate, glycine, D-alanine, L-alanine, D-lactate, L-lactate, and glycolate, each uniformly labeled with ¹⁴C. The LR experiment moistens a 0.5 cc soil sample with 0.115 ml of VM1 for a total of 3.91 uCi per application. Evolution of ¹⁴C gas from the moistened soil indicates microbial activity. Following such a positive response, a duplicate of

the original sample is heated to impair or destroy any organisms present. After cooling, that sample is tested exactly as was the one that produced the positive result. A positive response from the second sample indicates that the response of the first was non-biological in nature, while a negative response, demonstrating that the causative agent had been deactivated by heat, completes the evidence for life. The temperature regimen selected by the Viking Biology Team to distinguish a biological response from a non-biological one was 160°C for 3 hr.

Thousands of soils and microbial cultures of a wide variety of species were tested. No sample with demonstrated living organisms failed to respond. The controls readily confirmed biological activity. (Only several of the hundreds of soils were sterile.) The metabolism of as few as 50 cells in lag phase was readily detected. In a final test of an LR flight instrument, 0.5 ml of a California soil was placed under the experimental conditions to prevail on Mars. After 3 days of the reduced atmospheric pressure, humidity and temperature, the LR substrate solution was injected. Radioactive gas immediately evolved and increased through the standard 8-day LR test period (see SN103, FIG. 6). A second injection of nutrient then produced an increased response. A duplicate of the test soil was heated to 160°C for 3 hours and, after cooling, was tested. Essentially no response from the heated sample occurred, completing the evidence that there had been living organisms in the first sample and that they had survived exposure to the martian test conditions.

2.2 The LR on Mars

The results from the first LR experiment at the Viking 1 Site on Mars are shown in FIG. 1. At the Viking 2 Site, some 4,000 miles away, the soil produced a similar positive response. After eight sols (one martian sol = 24.67 Earth hr) of continuing, but slowing, evolution of gas, the soil samples at both sites were injected with nutrient a second time. Each sample promptly reabsorbed approximately 20% of the gas evolved to that point. A duplicate of the active sample was heated at 160°C for 3 hr and, upon testing, gave a nil response. In additional controls, 60% less gas was evolved after heating a duplicate sample to 46.0°C. After another duplicate sample was heated to 51°C, the response became erratic and produced 90% less gas than did the active sample. Active Mars soils, enclosed in the Viking sample distribution box and held at approximately 10°C for 2 months at one site and 3 months at the other, showed no activity upon testing. In all, 9 tests were run. All of the Viking LR results⁶ at both sites are summarized in **Table 1**.

Taken alone, the LR results were a strong positive indication of living microorganisms in the soil of Mars. The lability of the active agent in the soil to mild temperatures is difficult to explain otherwise. The difference between the responses after the heatings at 46.0°C and 51.5°C are reminiscent of laboratory microbiology analyses, such as the standard method⁷ for distinguishing fecal coliforms from the coliform group, which relies on small temperature differences.

2.3 Defects in non-biological interpretations of LR results

Updating the last review⁸ with more recent information, each of the above enumerated arguments against a biological interpretation of the LR Mars data will be addressed.

1. No Organics: The Viking GCMS, designed to identify organic matter in the martian soil, failed to detect any at either Viking Lander site⁹. The presence of organic matter on Mars had been generally anticipated as a result of: photochemical synthesis from primitive atmospheric constituents¹⁰, synthesis from current martian atmospheric gases^{11,12} by UV, infall from comets and meteorites¹³ and from interplanetary dust particles¹⁴ (estimated¹⁵ at some 10^5 kg of organic carbon per year).

The report of organic matter, including amino acids, inside the two SNC meteorites presumed from Mars, if verified, would confirm this anticipation. A report¹⁶ on EETA79001 found L-amino acid concentrations exceeding those for D-amino acids. Such chiral preference is found only in biological material. The finding¹⁷ of low molecular weight organic compounds being solar-stripped from the Hale-Bopp comet supports the widespread formation of organic matter.

Nonetheless, the Viking GCMS negative results were widely accepted as persuasive "evidence" that the Viking samples contained some exotic chemistry, but no life^{18,19}.

However, the LR's finding²⁰ of living microorganisms, as incorporated into FIG. 6, in Antarctic soil #726, in which the GCMS concurrently had detected no organic matter, provided a direct comparison of the two instruments. Moreover, a wet-chemistry analysis of Antarctic #726, performed as a control, found²¹ organic matter. Recently, the same soil, maintained in bonded storage by NASA, was re-examined²². The sample was subjected to prolonged digestion in concentrated HF/HCl and then pyrolyzed at 500°C (the highest pyrolysis temperature applied by the Viking GCMS). While the actual data were not presented, it was stated that the spectrum showed "weak bands in the 2850-3000 cm^{-1} characteristic of the C-H stretch of methylene groups (-CH₂-). Consistent with this, (the) C2 (fraction) gave hydrocarbon fragments on pyrolysis and showed aliphatic (sp^3) carbon in its ¹³C CP-MAS NMR spectrum." Thus, organic compounds surviving this rugged digestion were detected in the soil. They were attributed to kerogen and coal refractory to the GCMS pyrolysis. Biological possibilities were discounted although viable microorganisms have been reported²³ within anthracite coal taken from deep underground. The purpose of the intensive digestion of the Antarctic #726 was not stated. One must wonder what variety of labile organic compounds were present prior to the digestion.

When asked how the report of organic matter in ALH84001 could be reconciled with the negative results of the Viking GCMS, a NASA spokesman²⁴ said that the GCMS had been sent to Mars long ago and may not have been sensitive enough to detect the amounts of organic matter in the low microorganism population in the meteorite. The Viking GCMS was reported²⁵ to require organic content equivalent to 10^6 bacterial cells to obtain a response in its 100 mg soil sample. This is in sharp contrast to the 50-cell

sensitivity of the LR. The difference in sensitivities can readily explain the disparate results reported by the two instruments.

A fresh examination of the Viking Pyrolytic Release Experiment (PR) data²⁶ supplies direct support for the formation and persistence of organic matter on Mars. In the experiment, a sample of soil is taken into a chamber containing simulated martian atmosphere with its CO and CO₂ labeled with ¹⁴C. The sample is then exposed to simulated martian sunlight to permit any phototrophic microorganisms to photosynthesize and incorporate the label. After an incubation period, the non-incorporated gases are exhausted from the chamber, and the sample is pyrolyzed to release any labeled gas that had been fixed by the microorganisms. The detection of the gas released by the pyrolysis step is evidence for life.

During the development of the PR instrument, the formation and accumulation of organic matter from CO and CO₂ split by the UV of the simulated sunlight posed the serious threat of obtaining a false positive on Mars. The experimenters reported, "About 14 nmoles of ¹⁴C organics per gram of volcanic ash shale are formed within 70 hours of irradiation under a realistic simulation of martian conditions."²⁷ This formation of organic matter from simulated martian atmosphere under simulated sunlight was independently confirmed²⁸. The "solution" to the PR false positive problem was to incorporate an optical filter to screen out the UV below 320 nm.

In its first experiment on Mars, the PR produced a net response of 81 cpm upon pyrolysis of the sample²⁹. Extensive counting rendered a statistical significance exceeding 3 sigma above background and noise for this response. The response, nonetheless, was far short of the pre-mission criteria for life. The PR Experimenter concluded that the signal was of chemical origin.

Seeking phototrophs, the PR was the most Mars-like experiment (no water was added). This made it difficult to get a response from terrestrial soils. Cultured microorganisms were frequently added to test soils to get a response. The PR operated on the presumption that martian organisms, under their native environment, would produce a response.

Overlooked until now is the obvious fact that the low, but significant, PR response was from pyrolysis of the organic matter that had been created within the instrument on Mars. The UV filter did not completely eliminate the formation of organic matter. Pre-mission tests^{30,31} of the PR had shown that, even with the optical filter, organic matter accumulated on sterile test soils. The PR results on Mars indicate that the formation of organic matter is proceeding.

2. H₂O₂: The atmosphere of Mars produces H₂O₂^{32,33}. It was theorized^{34,35} that the H₂O₂ precipitates onto the Mars soil. The H₂O₂ and other oxidants produced from it were assumed to be in the soil sample and to have oxidized the LR organic substrates to release radioactive gas. It was proposed³⁶ that the oxidant

(s) also released the oxygen detected in the Viking GEx experiment. This oxidative chemistry also was credited with having cleansed the surface of Mars of any organic material, including microorganisms.

The most telling evidence against the H₂O₂ theory is in a just-published report³⁷ of very sensitive Earthbased IR telescopic measurements made through the entire column of the martian atmosphere. No spectrographic feature for H₂O₂ was found. The sensitivity of the instrument reduces the upper limit for H₂O₂ in the atmosphere of Mars to 30 ppb. **Table 2** presents the amounts of martian H₂O₂ predicted by current leading H₂O₂ theorists. Sensitive to, or near, these projected amounts, the new experiment seriously weakens the H₂O₂ theories.

Other unresolved difficulties have been reported³⁸ for all theories attributing the LR Mars results to H₂O₂ under martian conditions. This chemical will oxidize the LR compounds to produce CO₂. However relatively strong concentrations (approximately 2%) of H₂O₂ are required to emulate the amplitude and kinetics of the LR Mars positive responses. The most significant fact countering the H₂O₂ theory is that H₂O₂ and any of its proposed derivatives do not approximate the thermal sensitivity of the martian agent causing the LR responses. At 50°C, 90% H₂O₂ decomposes at only 0.001% per hour³⁹. Of numerous attempts to simulate those results with H₂O₂, none reported has succeeded under conditions consistent with those on Mars. H₂O₂, highly sensitive to destruction by solar UV, has an expected lifetime in the martian atmosphere of less than one day. Any deposition of H₂O₂ from the atmosphere to the soil is likely too small to account for the LR results⁴⁰. Based on the UV lability of H₂O₂, it is difficult for it to account for the LR activity. In addition, experiments^{41,42} reporting the formation of organic matter from simulated sun-irradiated martian atmosphere demonstrated that the organic matter accumulated despite the continuous incidence of the UV light. Evidently, the H₂O₂ formed under these simulated martian conditions was not made in sufficient quantity or did not survive the UV to oxidize the organic matter as fast as it was synthesized. The lifetime of H₂O₂ would be further reduced by the catalytic effects of γ-Fe₂O₃ reported⁴³ as likely to be extensive on Mars.

The re-examination of the Mars PR data also has repercussions for the H₂O₂ theories. The theories propose that there is oxidant in the soil. Why did that oxidant not destroy the organics formed and detected within the PR?

A variant chemical model proposing UV formation of peroxonitrites⁴⁴ in the soil has been proposed since the review cited above. Aside from its failure to explain the result in the sample taken under the rock, this theory has not demonstrated the thermal sensitivity of the agent detected by the LR. Also, the formation of peroxonitrites would require amounts of nitrogen in the Mars soil in excess of the limit set by the Viking elemental analysis⁴⁵.

A general question about attributing the failure to find organics on Mars to peroxide is the finding⁴⁶ of H₂O₂ in ancient permafrost cores on Earth, where the oxidant did not eliminate organic matter or life.

Being nearer to the sun and having more water vapor than Mars does in its upper atmosphere, Earth experiences the formation of H_2O_2 in amounts greater than does Mars. Yet, H_2O_2 was not inimicable to the development of life and the accretion of organic matter on Earth. Life developed enzymes to protect against this oxidant, and many species came to utilize it in their energy-producing metabolic processes.

3. No Liquid Water; Extreme Environment: Next to the peroxide theories, the most vigorous defense of the chemical nature of the LR results stems from the beliefs that all life must have access to water in liquid form and that none is available on the surface of Mars. Moreover, it has been stated⁴⁷ that the atmospheric pressure at the surface of Mars is below the triple point of water and likely has been so for several billion years. This is not supported by data obtained by the Infrared Interferometer Spectrometer (IRIS) experiment⁴⁸ aboard the Mariner 9 Mars orbiter. It reported that "Extensive regions have been found where the surface pressure exceeds the triple-point pressure of water" and that "under temperature conditions which frequently occur on Mars, liquid water can exist in equilibrium with its vapor." A recent publication⁴⁹ of Viking data provides a graph, FIG. 2, showing that, over a full martian year, the surface atmospheric pressures at both Viking landing sites continuously exceeded the triple point pressure for water. Further, inspection of the Viking data shows⁵⁰ that the temperature of the top several millimeters of soil beneath the Viking Lander 2 sampling head rose to $273^\circ K$ (the temperature at which ice liquifies) where it remained for at least several minutes. This is evidence for the melting of ice into liquid water. The thermal absorption of the metallic sampling head may have been responsible, but its thermal adsorption is likely equaled, or exceeded, by that of martian rocks. Surface temperatures in the southern latitudes at the start of summer were seen to exceed $273^\circ K$ for several hours per day. Thermal inertias for large areas of Mars range above 6, and above 30 for bare rocks. Albedos of unfrosted martian ground are as low as 0.095.⁵¹ Viking orbital data⁵² also support the presence of liquid water in the upper few millimeters of the martian surface. FIG. 3⁵³ shows that, during the Viking primary mission period, large areas of the martian surface were above $273^\circ K$ when an albedo of 0.25 and a thermal inertia of 6.5 were applied. Although the maximum temperature contour drawn for the Viking landing sites is $270^\circ K$, that surface temperature would be exceeded where, because of rocks or dark colorations, the albedo was less than 0.25 and/or the thermal inertia was greater than 6.5. Under these conditions, water would be in liquid form. "Thermal observations made half a century ago^{54,55,56} established that midday equatorial temperatures rose above the melting point of water."⁵⁷ Within large geographic areas, the upper layer of the martian soil exceeds the triple point temperature and pressure for water which, thus, would occur in liquid form at least transitorily in diurnal and seasonal cycles.

LR tests performed on the Death Valley sand dunes, shown in FIG. 4, detected microorganisms in the top one or two millimeters of the sand within one hour, as did tests performed on bare rock. Antimetabolites applied to duplicate samples as controls were effective in preventing evolution of gas, confirming the biological nature of the response. Samples of the top 2 mm of the sand were taken and analyzed by a NASA Jet Propulsion Laboratory (JPL) soil microbiologist. The moisture content and microbial

populations reported⁵⁸ were 0.9%, and 5.10×10^3 aerobic cells per gram, respectively.

The above considerations address the presence of liquid water in terms of the more familiar terrestrial biological needs. Other factors concerning water and biology may apply. Even before the announcements of probable evidence for life in the martian meteorites, the possibility that life once existed on Mars was gaining favor.⁵⁹ It was generally believed, however, that any such early life forms would have become extinct when liquid water was thought to have become unavailable. Alternatively, it was proposed, some organisms might have retreated to discrete "oases" deep below the surface where liquid water might still exist. Recent findings question the presumption that water activity on Mars is too low to permit aqueous moiety reactions. For example, chlorofluorocarbons are adsorbed onto stratospheric ice crystals which provide a liquid-like moiety for the chlorine to react with ozone.⁶⁰ Such ice activation may accompany the diurnal frost depositions Viking observed on Mars, making biological reactions possible. Alternatively, water vapor rising from the Mars permafrost may be adsorbed on soil particles to form "double donor waters." These are reported to exist commonly in ice where they expose very large surfaces as thin films and clusters.^{61,62} An atomic force microscopy study⁶³ of thin films of water indicates a solvating monolayer of phase I ice on adsorbing surfaces. This water might be available for biological reactions. Duracrust formations on Mars are attributed⁶⁴ to the solvation and transportation of sulfate to the surface by the vapor flux from the Mars permafrost. This water of solvation might also accommodate biological reactions. Current availability of this solvating factor on Mars was indicated by the Viking GCMS results, which were cited⁶⁵ as evidence for the availability of water to form the duracrust.

Since Viking, life has been found thriving in extreme environments heretofore believed deadly, including high temperatures and pressures, and even in non-aqueous environments.⁶⁶ A variety of microorganisms, including synergistic combinations such as lichen, live inside rocks in the dry valleys of the Antarctic⁶⁷, where precipitation is rare and occurs only as light snow. Aseptically removed from within the rock, scrapings produced the positive LR response included in FIG. 6.

Under extraordinarily harsh environments, many species of bacteria can enter a state in which they catabolize slowly, but neither grow nor divide⁶⁸ until nutrient concentrations, temperature, and other conditions become favorable. A review⁶⁹ cites many mechanisms for survival, including some for completely anhydrobiotic cells. Examples^{70,71} of dormant microorganisms indicate survivals approaching geologically significant time periods. Indeed, bacteria have been reported to have been resuscitated⁷² after 30 million years of dormancy under highly desiccating conditions. One of the surest methods of preserving bacteria indefinitely is to freeze-dry (lyophilize) them under conditions similar to those on Mars. Recovery from the resting cell state can be extremely rapid. An *Oscillatoria* strain subjected to drying in a desiccator above silica gel showed instantaneous recovery of photosynthesis upon resuscitation, with 100 percent viability.⁷³

During the development of the Mars Oxidant Experiment (MOx)⁷⁴, a test⁷⁵ was conducted in which a

California soil was dried to constant weight at 70°C. The end of an optical fiber was dipped into a glucose solution and dried, then was put into the dried soil and monitored for changes in reflectivity of the coated end to laser pulses sent through it as evidence for reaction. FIG. 5 shows the very rapid response showing that the glucose was being removed. A separate portion of the same soil pre-heated to 130°C produced no change in reflectivity confirming that the glucose had been removed by microorganisms. (A duplicate glucose-coated fiber put into solid potassium superoxide produced no reaction.) These results are reminiscent of the Viking LR data. The possibility exists that martian organisms may metabolize under present conditions or, long dormant, may have been resuscitated by the LR experiment.

4. "Too Much Too Soon": When the first positive result from the LR was obtained, it was stated that the response was "too much too soon" for microorganisms living under the extreme conditions of Mars. The Mars response was erroneously portrayed as exceeding the LR responses obtained from terrestrial soils and, therefore, indicative of chemistry. Were microorganisms present on Mars, the reasoning ran, they would be in far lesser numbers than in terrestrial soils. Hence, their response would be less, especially considering the harsh Mars environment. Furthermore, it was said that the response kinetics were indicative of chemistry, not biology. FIG. 6 compiles data directly comparing LR responses from a variety of terrestrial soils to the VL1 positive LR Mars response. The LR Mars response is seen to be near the lower end of the range, close to the Antarctic responses and to that from the Aiken soil in an LR flight instrument sealed within a chamber under the martian experiment conditions. The kinetics are seen to be similar. Thus, the "too much too soon" reason against acceptance of the LR response as biological is not supported by the relevant data.

5. 2nd Injection: LR tests with terrestrial soils generally show that, after a plateau in gas evolution has been reached, a second injection of the labeled nutrient produces a sharp renewal in gas evolution. While this was not one of the criteria for a determination of life on Mars, it would have lent support to such a determination. As seen in FIG. 7, when a second injection of nutrient was applied following the standard eight-day test cycle on Mars, approximately 20% of the gas previously evolved disappeared from the headspace of the instrument. This happened at both Viking sites. The gas slowly re-evolved to its eight-day level after approximately two months. A recent publication⁷⁶ cites the lack of a vigorous evolution of gas following the second injection as the reason the LR results could not be from microorganisms. The second injection results seem to be the simple reabsorption of gas by the soil when wetted. In a laboratory simulation⁷⁷, a Viking-type LR instrument containing sterilized Mars analog soil reproduced the reabsorption of approximately 20% of the headspace gas, FIG. 7 (shown with FIG. 1 for comparison). Furthermore, a search of the Viking development files revealed active soils which demonstrated the same lack of response, or slight reabsorption of gas, upon a second injection. Apparently, the microorganisms died during the test and the addition of water promoted reabsorption of the CO₂ evolved after the first injection. Of particular interest are the results obtained when lichen were tested by the LR. The lichen responded very vigorously upon initial contact with the aqueous nutrient, but the rate of evolution quickly decreased and essentially ceased after only 24 hours. A comparison of the lichen incremental responses with those of the Mars LR is presented in FIG. 8. This suggests the possibility that too much water could

have been a factor in both experiments. The above cited publication argues that increased gas evolution upon the second injection would have supported the biological nature of the response. However, had a second evolution of gas occurred, might not the argument have been made that water had become the limiting factor following the first injection, and that its resupply allowed a chemical reaction to continue?

6. No Visual Evidence: It was reported⁷⁸ that a study made by the Viking Lander cameras showed no evidence for macro or micro forms of life. However, a detailed analysis of all the Viking Lander images⁷⁹ revealed colored spots on many of the rocks and colored areas of the surface ranging from ochre to yellow to olive and green. A digital spectral analysis of the spots on the rocks as seen in six imaging channels (RGB and three near-IR) comported closely to that of images of lichen-bearing rocks. These rocks were viewed by the Viking cameras in the simulated Viking 1 Lander site and processed through the Viking Imaging System. The greenest objects in both fields of view were spots on the martian and Earth rocks. The respective digital numbers for hue, saturation and color for the spots in both images were similar. Subsequently, the greenish colors were independently confirmed^{80,81} with the additional observation that the green spots on the rocks were elevated by 0.1 to 1 mm above the background surface of the rock. While unusual weathering might account for the elevation of the spots and the changes observed, it has been suggested⁸² that lichen, or similar colonial microorganisms, could more readily satisfy the observations. Called "the pioneers of vegetation", lichen are the first life forms to appear on bare rock. Lichen have also been reported⁸³ to subsist when the only source of water is atmospheric vapor. The atmosphere at both Viking Lander sites was at or near water vapor saturation diurnally, and frost was seen in many of the morning images.

FIG. 9 is an image of three near-field rocks at Viking site 1 which were selected for study. The image was calibrated by the Viking Imaging System to provide the truest Mars colors. FIG. 10 is a heretofore unpublished set of 3 images encompassing most of the same area and taken at intervals of one martian year (two Earth years). All three images are treated identically, and closely approximate the calibration of FIG 9. The pictures are taken within several degrees of the same sunangle in order to permit comparison without mistaking light or shadow effects for true differences between successive images. The field of view was limited because of power conservation during the later portion of the extended mission. It is apparent that changes have occurred over time. Some of the near-field differences between the first two images were caused by sampling of the soil that occurred after the first image was taken. However, changes in both the near and distant fields cannot be associated with the sampling activity. A comparison of the same near-field fine surface structures among the three images shows that aeolian effects were essentially nil (the only apparent changes being from slumping that occurred around the dug hole). In particular, attention is directed at the foreground rock, which exhibits a major change in its patterned colored areas, and to the distant background where extensive pattern and color changes are evident. In several ways, then, the Viking Lander images provide evidence consistent with a biological interpretation of the LR data.

7. UV Light: This early hypothesis sought to explain both the LR active response and the lack of organics reported by the GCMS. An experiment at Viking Landing Site 2 eliminated this possibility. Before dawn, the sampling arm moved a rock before the sun's rays could strike it. A sample of soil that had underlain the rock and thus was protected from UV for eons was taken and promptly delivered to the instrument. Despite the absence of any UV exposure, the sample produced a positive LR response, essentially identical in kinetics and in magnitude to those of the Viking Lander 1 positive response seen in FIG. 1. This direct experiment carried out on Mars eliminated the possibility that UV light was the cause of the activity detected by the LR experiment on Mars.

8. Clays: It was proposed^{84,85,86} that clays on Mars reacted with the LR nutrient to produce labeled CO₂. Various clays were proposed in sequence as experiments detected deficiencies in duplicating the LR results. The clays, in turn, ranged from montmorillonite to nontronite to palagonite to smectites. The experiments suffered from basic problems⁸⁷ including the failure to sterilize the samples tested or to use sterile procedures in the experiment in order to exclude biological effects from microorganisms in the clays. Extreme pHs were imposed to obtain responses and the responses did not have the kinetics of the LR positive responses. Data replicating the thermal sensitivities of the LR active agent at the lower levels of 50°C and 10°C were not forthcoming. Perhaps most important to the interpretations was the fact that the responses were not normalized to those from the Viking LR instrument. The counting efficiency used on the clays was 75% compared to 3% for the LR. Hence all responses for the clays should have been reduced by a factor of 25, which would have placed them in the control or noise levels. In the last work cited above, the claims of replicating the LR data were reduced to state that "iron-enriched smectites . . . were shown . . . to simulate many of the findings of the Viking Labeled Release Experiments on Mars." Considering that there is no direct evidence for clays on Mars, and the flawed nature of the experiments, the possibility that clays explain the LR Mars data seems vanishingly small.

9. Complexities of Independent Origin of Life: While listed last, this is really the penultimate reason responsible for discounting the LR Mars data as evidence for life. The origin of life is so poorly understood that the complexity posed by it alone would make the application of Ockham's Razor favor a non-biological explanation of the LR data. However, the presumed evidence of microbial life in the two martian meteorites raises the possibility that an independent origin of life on Mars was not necessary for life to exist on that planet. If the biological fossils are confirmed, the principle of panspermia will be established. EETA79001 is only 600,000 years old, in effect, of the "modern" era on Mars. The environmental changes deemed by many as inhospitable to life already had occurred. Were microorganisms alive so recently, their survival to the present would be readily inferred.

The presence of microorganism fossils in the martian meteorites would also make a case against the oases theory⁸⁸ which proposes that microorganisms may currently exist only in pools of liquid water postulated deep beneath the surface. Analysis⁸⁹ of the physics of ejection of planetary material by meteoric impact concludes that materials escaping planetary gravity originate from, or near, the surface of the impact area. Thus, any fossils with the meteorites that fell on Earth could not be from deep oases.

Furthermore, were there deep sites of living microorganisms, it is likely that volcanic action, frost heaving, or other mechanisms would eject such material to the surface. There any microorganisms could be widely distributed by the winds. The microorganisms might adapt to or become lyophilized by the surface conditions. Either way, they might still respond in the manner detected in the LR instrument upon being moistened with liquid nutrient under benign conditions. Regardless, the meteorites provide a now plausible vehicle for ejection into space, lyophilization by the space environment, survival of re-entry, and thus, interplanetary transportation of living microorganisms. The independent origin of life on Mars is no longer a barrier to acceptance of the LR data as evidence for life.

3. CONCLUSION

Many hypotheses have been advanced and tested in attempts to account for the well-characterized activity detected in the surface material of Mars by the LR experiment. As shown above, these hypotheses have themselves been found wanting. The demonstrated success of the LR and the exquisite sensitivity with which it has detected microorganisms during its extensive test program with its record of no false positives can no longer be denied. No non-biological approach published, or known to the author, has duplicated the LR Mars data. Some laboratory experiments have produced positive responses, but the detailed thermal sensitivity exhibited by the variety of controls conducted on Mars has remained elusive in all such tests compatible with martian conditions. On the other hand, a combination of known properties of microorganisms, perhaps even those possessed by single species, could reproduce all aspects of the LR data. The biological interpretation of the Mars LR results is left standing alone. Recent discoveries of life forms thriving in extraordinarily severe environments on Earth strongly indicate that any alien organisms arriving on Mars might well and widely adapt to their new home. Application of the scientific principle leads to a conclusion: *the Viking LR experiment detected living microorganisms in the soil of Mars.*

4. RECOMMENDATIONS

1. Add Life Detection Tests to Planned Missions. The above conclusion will require independent experimental confirmation before achieving general acceptance. No life detection experiments are planned for the NASA's 10 Mars landers scheduled over the next decade. However, it is still possible to add life detection capability to them within the new NASA paradigm of "cheaper, smaller, faster." Even without a dedicated life detection experiment, it is possible that confirmation of extant life may be achieved. Pathfinder is now enroute to Mars for a July 4, 1997 landing. Its lander is equipped with a camera having better color and spatial resolutions than did Viking's. It is possible that a close-up picture of a rock might show clear evidence of biological colonies. A search for such evidence should be a Pathfinder priority.

2. Use Hubble Telescope. The Hubble Telescope should be used to seek evidence of large areal changes in the color and patterns of the martian surface and seek to correlate them with atmospheric

water vapor and climatic seasons. Useful images that already may exist in the Hubble files should be compared.

3. Seek Chirality in Mars Samples. Perhaps the surest robotic means for unequivocal distinction of biological from chemical reactions is a test for chiral activity. For some yet unknown reason, or by chance, when the first living cells came into existence their enzymes were chiral specific. They catalyzed protein-building reactions with L-amino acids only. They had a similar preference for L-carbohydrates over D-forms. Throughout the evolution of all living forms, these preferences have been genetically transmitted. This peculiarity of living systems provides a ready means of distinguishing them from chemical reactions. Chemical reactions, without the intervention of man, cannot distinguish between L- and D-isomers. On the other hand, all known life forms utilize and make virtually only the L-form of amino acids. Thus, were a sample of martian soil to react with one chiral isomer of a compound and not the other, the biological nature of the reaction would be established. A simple, small, low-cost experiment has been proposed⁹⁰ which would separately add L-cysteine and D-cysteine (FIG. 11) to respective small ovens in the Surveyor '98 Thermal and Evolved Gas Analyzer (TEGA). TEGA will measure CO₂ evolved when samples of martian soil placed in them are heated. Were the amount of CO₂ evolved from the oven containing one isomer of cysteine to exceed the amount from the oven containing the other isomer significantly, the case for life would be confirmed.

4. Protect Health and Environment. Samples of martian material should not be returned to Earth before a thorough evaluation of possible hazards. The simple test for chirality could provide a warning of potential hazards to health and environment before a sample from Mars is returned to Earth. An upgraded version of the LR experiment might be added to an upcoming martian lander. This experiment would differ from the original in that the chiral isomers would be offered separately. The advantages of this approach are that it builds on the proven technology of Viking and that the Viking results provide relevant data for comparison. In addition, it could permit testing under controlled environmental conditions. Many other means of inquiry would follow a positive test to begin studies of comparative biology.

5. Continue SNC studies. The ongoing appraisals of the meteorites reported to contain evidence of martian microbial life may confirm the conclusion reached herein. In particular, if EETA79001 is validated as to both its Mars origin and its evidence of microbial life, this would constitute such confirmation.

ACKNOWLEDGMENTS

In the 45 years since I first developed the radioisotopic method for detecting microorganisms, I have been fortunate in my assistants. This variously composed team total nearly half a hundred people. I have acknowledged each in the more than 50 published papers many have co-authored with me, and I now thank them collectively. I do want to mention several individuals. Dr. Patricia Ann Straat, my Viking Co-Experimenter, was my right arm at Biospherics during the 10-year period covering the Viking mission. She was essential to the success of the LR experiment. Her predecessor was Mary-Frances Thompson

who did a splendid job overseeing our early laboratory efforts. Engineer George Perez was able to take my sketchy concepts for the early instruments and reduce them to excellently functioning metal. TRW, Inc. converted the early instruments into the faultlessly performing Viking LR instruments. I particularly want to thank my son, Dr. Ron Levin, a physicist, for his constant support of the LR life interpretation, his help with the water-on-Mars issue, and in extracting the Mars images from the JPL tapes.

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FIGURES

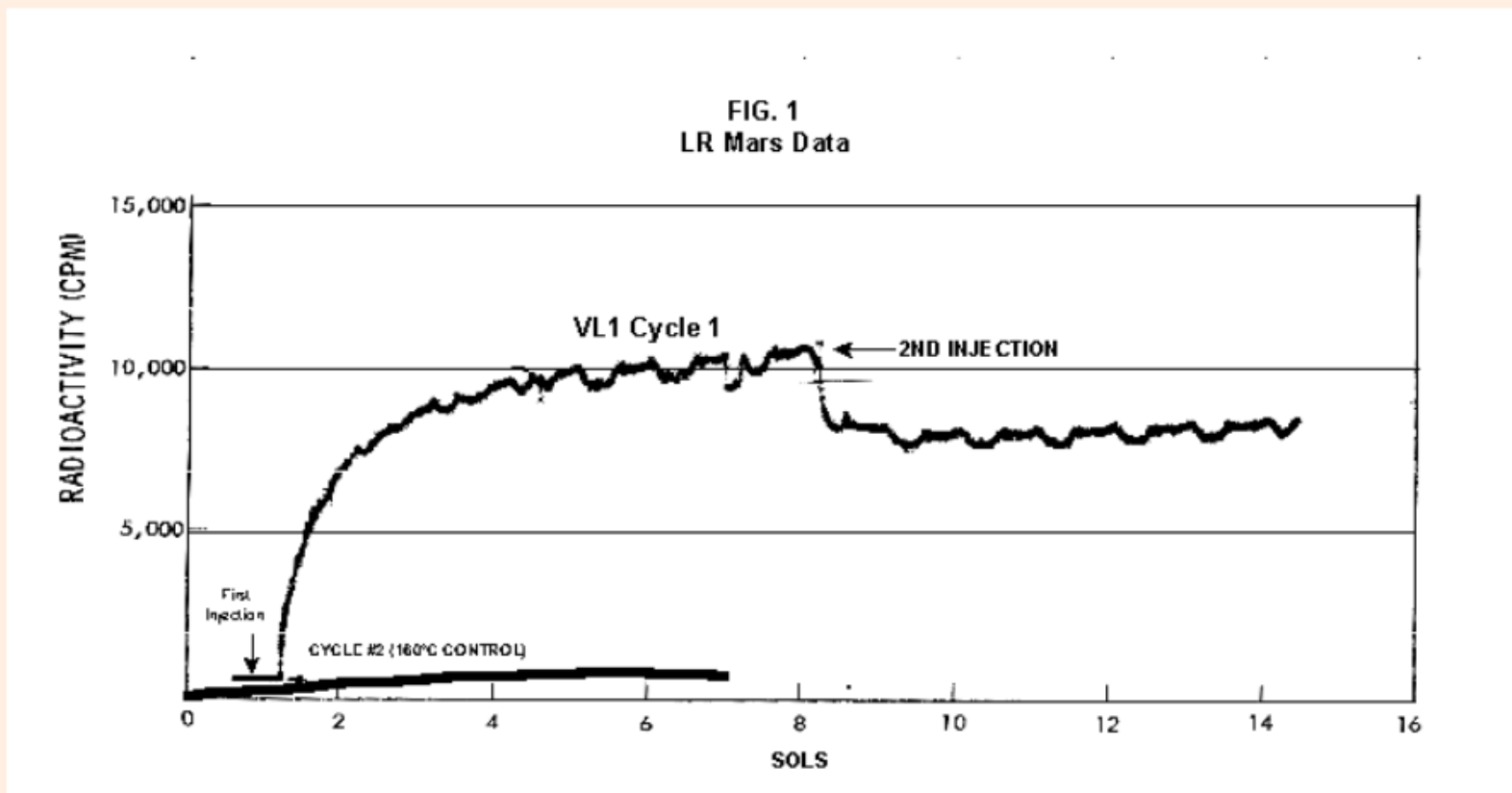
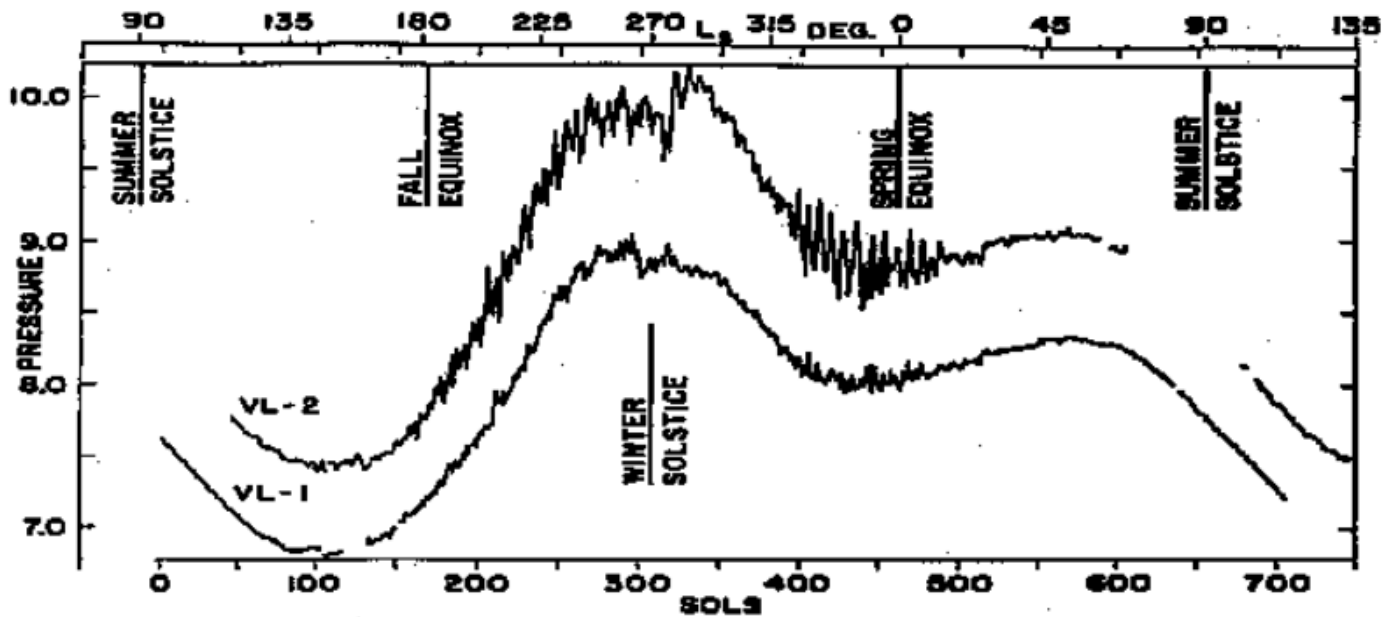
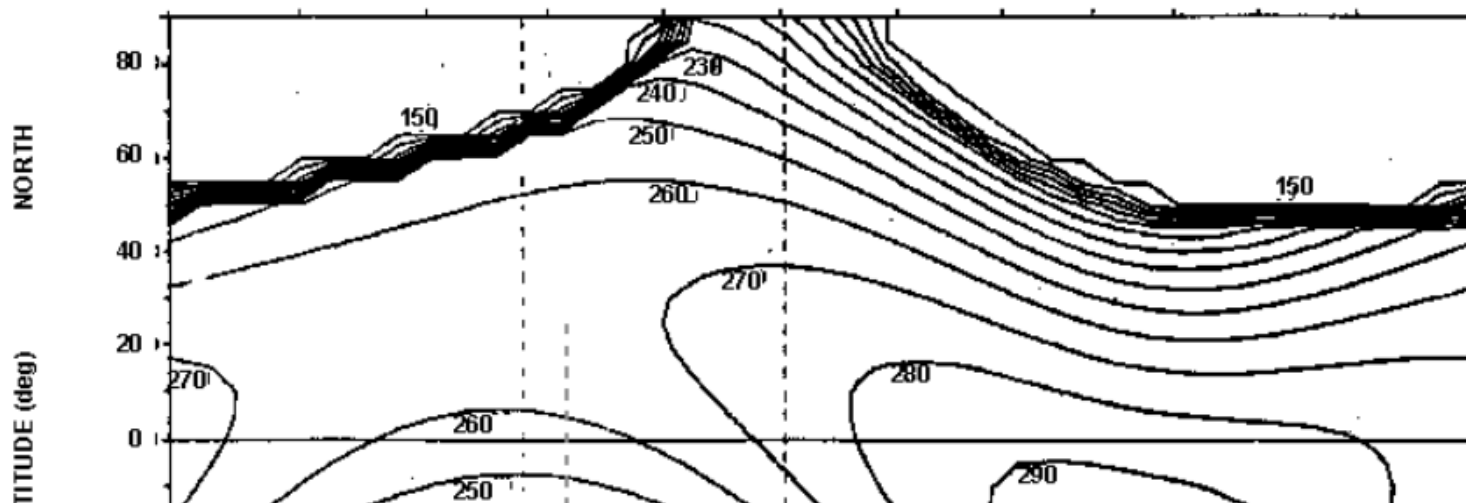


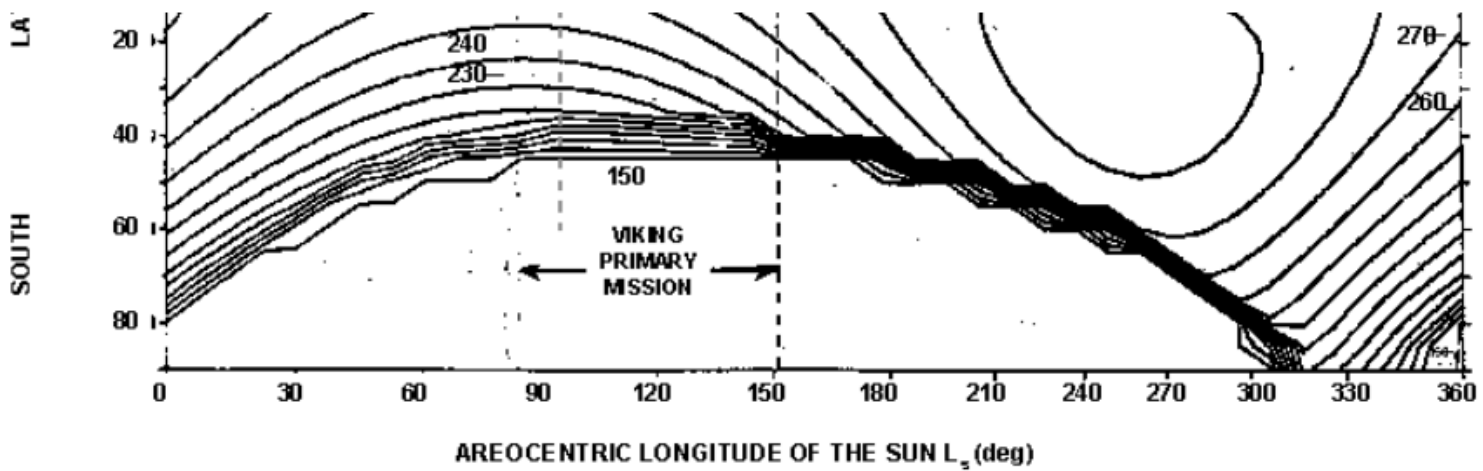
FIG 2
 Mean Daily Pressures at the Two Viking Landing Sites As a Function of Time of Year



From Carr, M.H., *Water on Mars*, Oxford University Press, New York, Oxford, 1996

FIG. 3
 Maximum Surface Temperature of Mars During Primary Viking Mission (°K)





from H.H. Kieffer, T.Z. Martin, A.R. Peterfreund, B.M. Jakosky,
E.D. Miner and F.D. Palluconi, *J.G.R.*, 82, No. 28, 1977.

FIG. 4.
Test of LR on Death Valley sand dune. Despite only
0.9% moisture in top 2 mm of sand, a strong
positive response was immediately obtained.



FIG. 5
Reactivity of Dry Glucose with Dried
California Soil in MOx Experiment

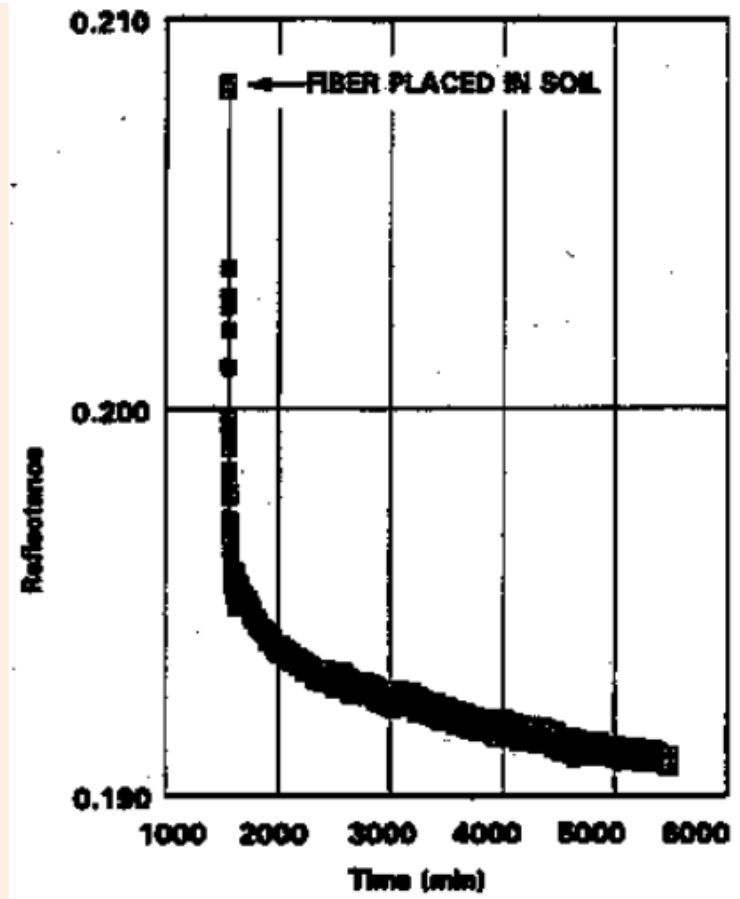
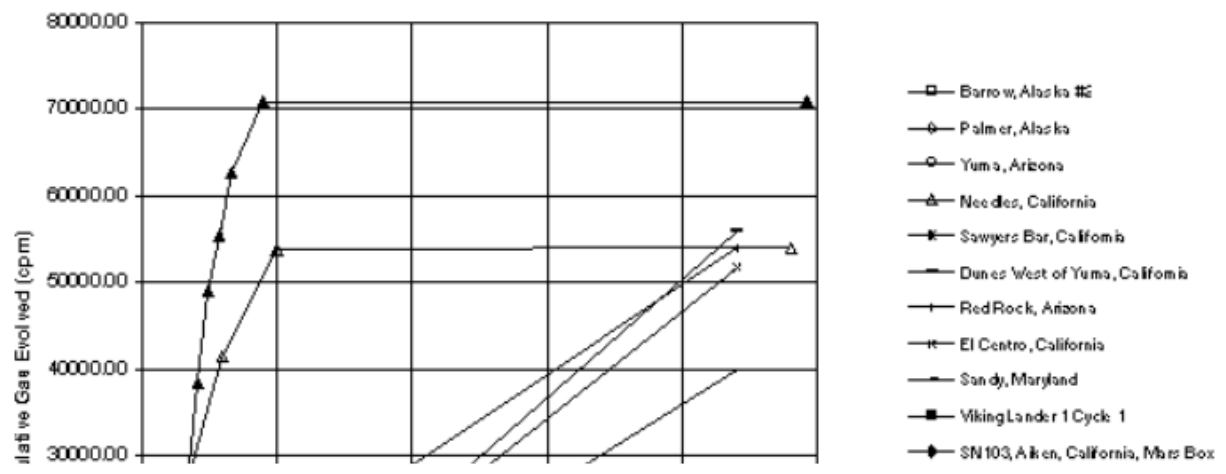


FIG. 6
Comparison of Terrestrial and MarsLR Active Responses



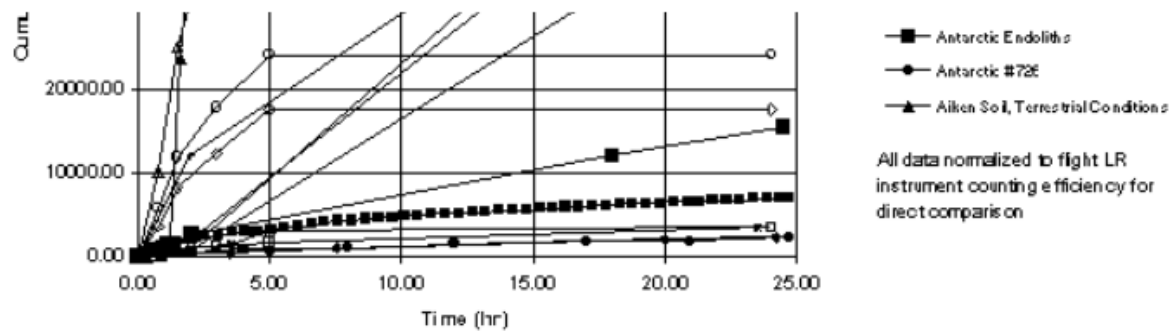


FIG. 7
Simulated 2nd Injection-TSM

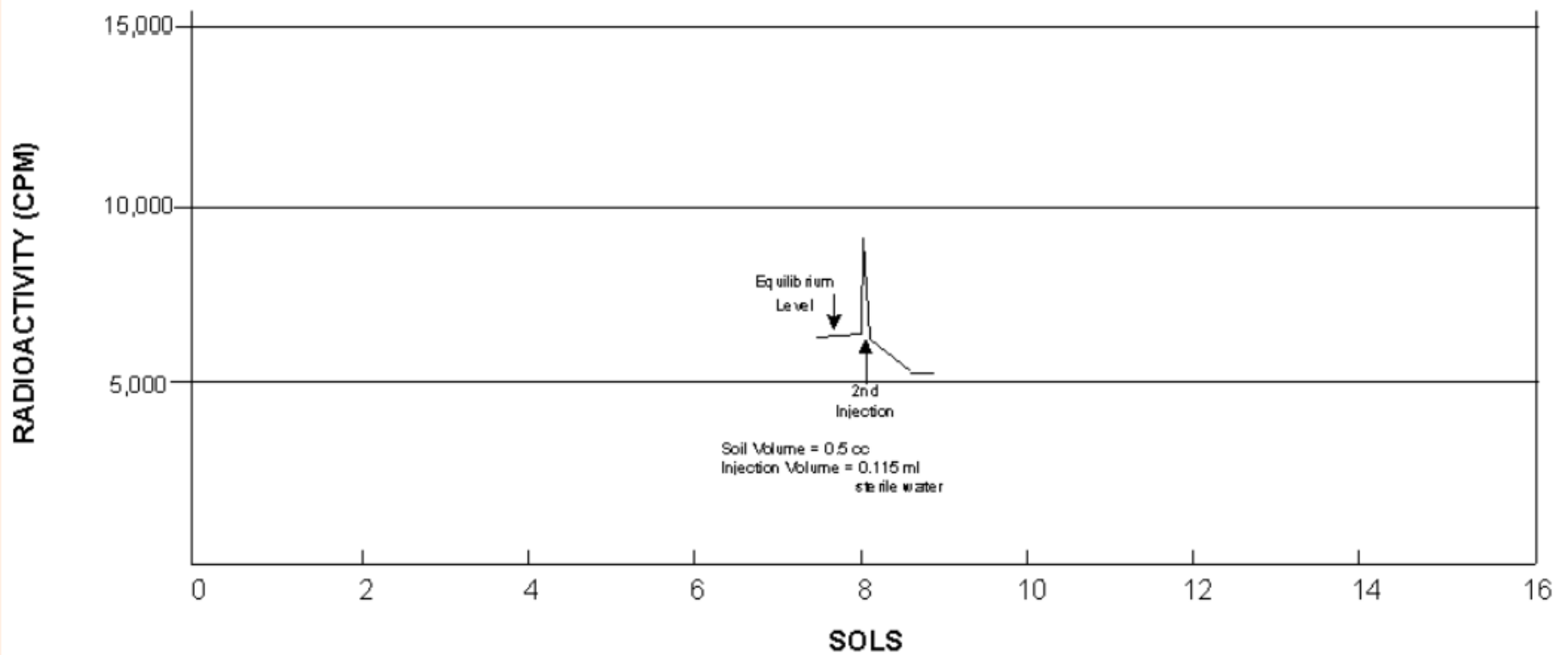


FIG. 8

Mars VL1 LR Incremental Responses
Compared to Lichen Incremental Response

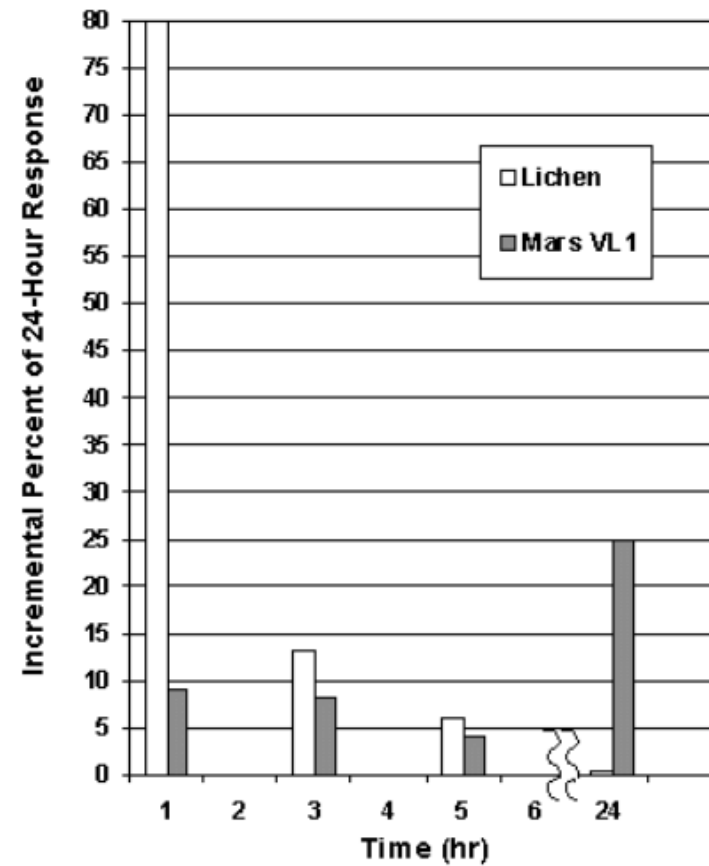


FIG. 9.

Radcam (truest color) image of rocks in near field of Viking Lander 1. Note coloration. Center rock is approximately 5 cm long.

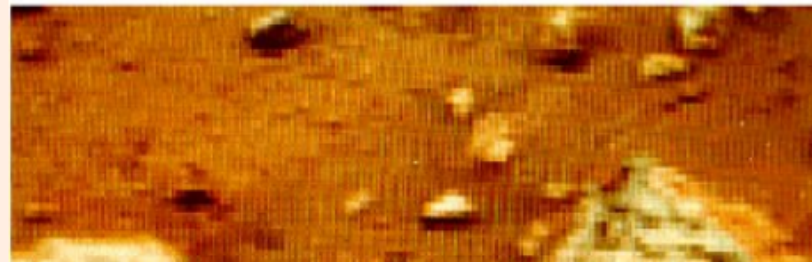




FIG. 10.

Three consecutive images taken at exactly martian yearly intervals to provide same sunangle. Lower center of 2nd and 3rd images from left show holes dug. Note color and pattern changes outside of area affected by digging. Persistence of fine structure of soil in foreground indicates little aeolian saltation occurred to account for changes.

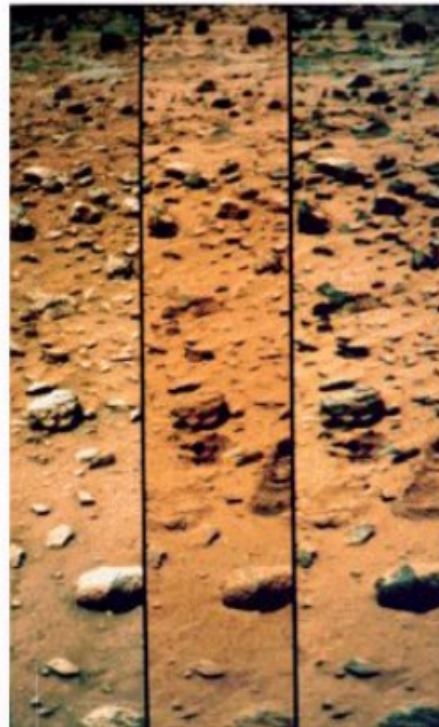
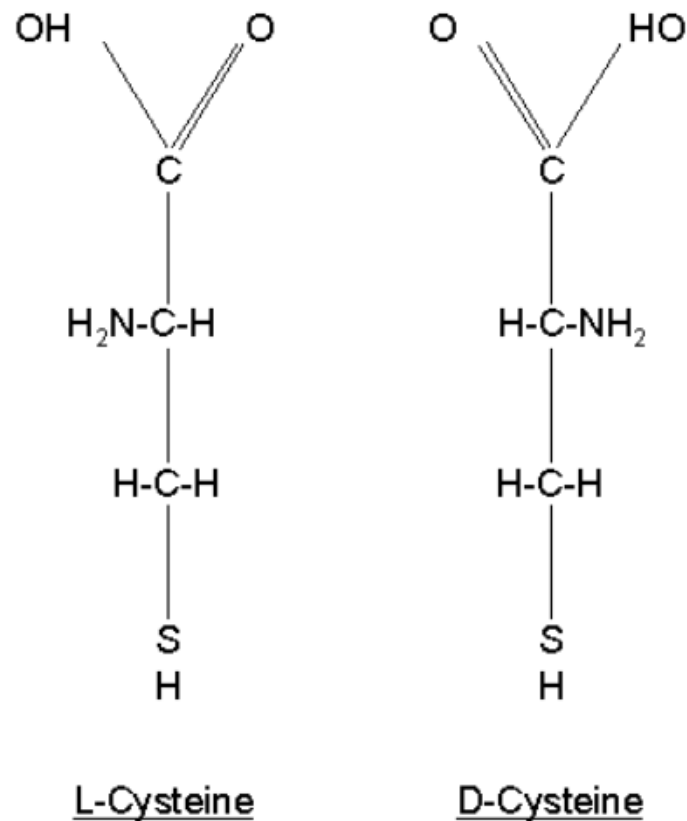


FIG. 11

Chiral Compounds Suggested for
Surveyor '98 TEGA Experiment



TABLES

TABLE 1
Summary of LR Mars Results

1	Soil maintained in LR 2-5 Sols produced results similar to that of positive response in FIG. 1.
2	Upon second injection of nutrient, approximately 20% of gas already evolved left detector volume (probably absorbed into soil) and gradually re-evolved over period of two months.
3	Soil* heated to 160° C for 3 hr produced nil response, similar to control in FIG. 1.
4	Soil** heated to 51° C for 3 hr prior to testing produced several small sporadic peaks (5%-10% of positive response) each of which was further reduced by approximately 90% prior to the start of the next peak.
5	Soil** heated to 46° C for 3 hr produced kinetics similar to positive response, but 70% reduced in amplitude.
6	Soils maintained 2 or 3 months in soil distribution box, in dark, at approximately 7-10° C, under ambient Mars atmosphere, pressure and humidity, produced nil responses after either 1 or 2 injections.
7	Soil** protected from UV by overlying rock produced typical active response.

* Run at VL1 site only.

** Run at VL2 site only.

TABLE 2
Upper Limits for Hydrogen Peroxide
in Mars Atmospheres

Experimental*: 7.5×10^{15} molecules cm^{-2}

Predictions by Current Theoretical Models:

Model	Predicted No. Molecules Cm^{-2}
Shimazaki (1989)	2.2×10^{15}
Krasnopolsky (1993)	3.8×10^{15}
Nair et al. (1994)	2.4×10^{15}
Atreya and Gu (1994)	10^{16}
Krasnopolsky (1995)	4×10^{15} (with N chemistry)
Krasnopolsky (1995)	6×10^{15} (without N chemistry)

*Data from Krasnopolsky, V.A., G.L. Bjoraker, M.J. Mumma, and D.E. Jennings, "High-resolution spectroscopy of Mars at 3.7 and 8 μm : A sensitive search for H_2O_2 , H_2CO , HCl and CH_4 , and detection of HDO ," *J. Geophys. Res.*, 102, No. E3, 6525-6534, 1997.

Viking 1 Mars picture of "Big Joe" shows same colorations found by Pathfinder.

