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LIFE ON MARS? THE VIKING LABELED RELEASE EXPERIMENT*

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Viking radiorespirometry ("Labeled Release" [LR]) experiments conducted on surface material obtained at two sites on Mars have produced results which on Earth would clearly establish the presence of microbial activity in the soil. However, two factors on Mars keep the question open. First, the intense UV flux striking Mars has given rise to several theories postulating the production of highly oxidative compounds. Such compounds might be responsible for the observed results. Second, the molecular analysis experiment has not found organic matter in the Mars surface material, and therefore, does not support the presence of organisms. However, sensitivity limitations of the organic analysis instrument could permit as many as one million terrestrial type bacteria to go undetected.

Terrestrial experiments with UV irradiation of Mars Analog Soil did not produce Mars type LR results. Gamma irradiation of silica gel did produce positive results, but not mimicking those on Mars. The life question remains open.

The Viking Labeled Release (LR) Experiment (Levin and Straat, 1976) has obtained results consistent with the presence of microbial life on Mars. In the LR experiment, a dilute aqueous solution of simple, uniformly labeled carbon compounds is applied to a sample of soil in a sealed test cell. The headspace atmosphere in the chamber is monitored for the appearance of radioactive gas. A positive response is tested for biological lability by repeating the experiment after heating a duplicate portion of the sample to 160°C to sterilize it. Tests conducted with terrestrial soils containing viable microorganisms invariably produce positive responses and, under favorable environmental conditions, gas evolution plotted as a function of time reveals the classic microbial growth curve with lag, exponential and stationary phases clearly defined.

Prior to Viking launch, a carefully controlled LR experiment was conducted in a sister instrument to those flown to Mars. The atmospheric composition and pressure, the water vapor content and the temperature of the test cell containing a California soil with a rich microbial population were controlled to simulate conditions anticipated within the test cell on Mars. The results of the active and control cycles are presented in Fig. 1 as the cumulative evolution of radioactive gas over Martian sols (1 sol = 24.6 h). At the point indicated in Fig. 1, a second aliquot of the radioactive nutrient was injected onto the soil which immediately produced a fresh radiorespirometric response. In the control sequence, a duplicate portion of this soil was heated to 160°C for 3 h and permitted to cool prior to nutrient injection. As seen in Fig. 1, essentially no gas evolved.

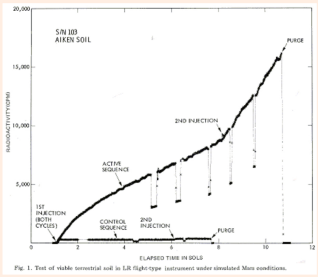

Fig. 1. Test of viable terrestrial soil in LR flight-type instrument under simulated Mars conditions.

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As of this writing, two active cycles of the LR experiment have been performed at each of the two Mars lander sites some 4000 miles apart. All four active cycles have yielded positive, and surprisingly similar, results upon their first injections. However, rather than producing additional gas, subsequent injections in each cycle have all produced diminutions in the accumulated quantity of radioactive gas already evolved. Typical results, those for VL-1, Cycle 3, are presented in Fig. 2. During this long incubation, a total of three nutrient injections were made. The first produced a strong positive response, but the second and third were followed by a decline in the amount of radioactive gas evolved by the first, indicating a drastic change or loss of the active agent in the soil sometime prior to the second injection.

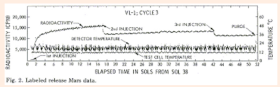

Fig. 2. Labeled release Mars data.

Fig. 3 summarizes the current first injection data at Viking Lander 1. Two active cycles and one control cycle are presented on a scale comparable to that used in Fig. 1 to permit ready comparison of the Mars and Earth results. Similarly, the current first injection results at Viking Lander 2 are summarized in Fig. 4 to the same scale.

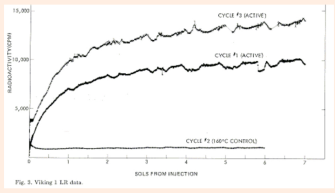

Fig. 3. Viking 1 LR data.

Comparison of the results from all four Mars active cycles to the results obtained from terrestrial soil under simulated Mars conditions, Fig. 1, shows that, with respect to the first injection, the responses are similar in magnitude over the time span measured. The shapes of the first injection portions of the active cycle curves on Mars and on Earth differ in that the Mars responses approach plateau earlier in the time course. Nonetheless, the similarity among all four active tests on Mars is striking. This is despite the fact that one of the experiments, VL-1, Cycle 3, was conducted on a sample obtained by moving a rock which had protected it from UV radiation for many thousands of years. It is interesting to note that, while the Mars data fail to show evidence of exponential gas evolution, from which growth might be inferred, neither do the terrestrial microorganisms under the imposed Martian conditions. The 160°C preheated control samples produced similar, low level results on Earth and Mars.

Although the LR results are consistent with the presence of life on Mars, other factors prevent the indicated conclusion at this time. Experimenters for the other two Viking life detection experiments, Pyrolytic Release and Gas Exchange, have stated (Horowitz, 1977) that their experiments have produced no confirming evidence. Also, the Molecular Analysis Experiment (Biemann, 1977) has found no evidence of organic matter in the Martian surface material. However, the sensitivity threshold for this experiment could permit 10^6 terrestrial type bacteria to go undetected provided they were not accompanied by organic debris many times their own mass as is generally, if not always, the case in terrestrial soils (Biemann, 1977).

Various hypotheses that exotic Martian conditions, principally the high UV flux, could produce active states or highly oxidative compounds in the Mars surface material led to speculations (Levin and Straat, 1976) that physical or chemical processes might falsely indicate the presence of life. The early receipt of a positive active cycle and a negative control from the LR experiment on Mars intensified speculation on these hypotheses.

Within the severe limitations of the spacecraft instrument, the authors attempted variations in the LR experiment soon after the positive results on Mars in an attempt to resolve the dilemma. The control pretreatment temperature was dropped to 50°C (actually approx. 51.5°C) (Levin and Straat, in press) on the theory that microorganisms on Mars would be severely damaged even at 50°C, a temperature beyond their experience, but that physical or chemical phenomenon causing the breakdown of the nutrient was far less likely to be degraded by this relatively mild treatment. The results shown in Fig. 4 for VL-2, Cycle 2, indicate severe attenuation of the LR reaction. However, the strange kinetics aroused suspicion that the LR instrument had malfunctioned. A series of engineering tests remotely conducted on the instrument failed to detect any malfunction and a subsequent active cycle, Cycle 3, Fig. 4, produced a "normal" active LR curve for Mars. Nonetheless, the significance of the reduced response following heating to 50°C required that the effect be confirmed. Accordingly, Cycle 4, in which a fresh sample was preheated to only 46°C (Levin and Straat, in press), was conducted. The results confirmed the major reduction in the LR response achieved by mild heating although, this time, the strange kinetics were not evident. (The relatively minor fluctuations in the radioactivity curves correlate with temperature changes imposed on the test cell by the lander's temperature control mechanism as it reacts to the diurnal temperature swing on Mars.)

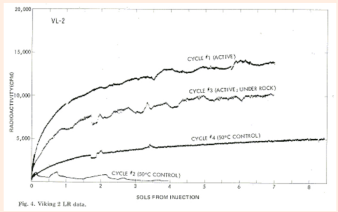

Fig. 4. Viking 2 LR data.

Simultaneous with the attempts to resolve the life issue on Mars, the authors undertook a series of laboratory simulation experiments. The experiments reported herein were complementary to the "under the rock" experiment conducted on Mars in that they sought to determine whether exposure of soil to radiation might mimic the LR results. A "worst case" test of the theories that ionizing radiation of oxygen-rich minerals might activate sites capable of degrading one or more of the organic substrates (Levin and Straat, 1976) comprising the LR medium was effected through the irradiation of pure silica gel with gamma rays emitted from a cobalt-60 source. Silica gel was selected because it was almost pure SiO_2 for which considerable evidence has been cited (Zeller et al., 1970; Zeller, 1976) concerning its activation by solar flux protons or harder, ionizing radiation. Moreover, SiO_2, in the form of silica gel, would provide enormous surface area to contact the LR medium. Davison silica gel, grade 950* was selected for use. Its typical chemical analysis on a percent dry weight basis is: 99.85% SiO_2; iron as Fe_2O_3, 0.005%; sodium as Na_2O, 0.004%; calcium as CaO, 0.018%; titanium as TiO_2, 0.058%; and zirconium as ZrO_2, 0.030%. Surface area is given as 700 m^2/g. Maximum total volatile content at 1750°F (954.4°C) is 6.5%.

Samples were prepared in the following manner. Samples (0.5 g) of the silica gel were placed into either 7 ml glass ampoules or 1.5 ml quartz ampoules. All ampoules were then heat treated at 160°C for three or more hours under continuously maintained vacuum. One atmosphere of $C0_2$ was introduced upon cooling and the ampoules were sealed. The ampoules were then heat sterilized at 160°C for a minimum of 2.5 h. The glass ampoules were then packed in dry ice and received 0.83 Mrad of cobalt-60 gamma irradiation. They were maintained in dry ice during shipment to the Biospherics laboratory where they were immediately placed in a cold room maintained at 4°C. The purpose of the cold treatment was to minimize hypothesized (Danielli and Plumb) temperature induced annealings of any disjunctions or defects in the silica gel produced by the irradiation.

The silica gel in quartz ampoules was treated in the same manner as just described for the glass ampoules. These samples were then Subjected to UV irradiation. The UV source consisted of 10 Rayonett, 2,537 Å lamps (15 watts per lamp) arranged in a 10 inch diameter circle. The ampoules were placed on their sides for maximum surface exposure of the silica gel and were stationed at the bottom center of the circle of lamps. They were irradiated continuously for 14 days at approx. 25°C. The physical constraints made it impossible to maintain these samples in dry ice during irradiation. However, immediately after irradiation, they were packed in dry ice where they were maintained during shipment to Biospherics and introduction into the 4°C cold room.

*Davison Silica Gel Selective Adsorption Grades, Technical Bulletin, Adsorbents Department, Davison Chemical Grade Co.

Prior to conducting LR experiments, one set each of duplicate ampoules exposed to gamma and UV radiation were given the following respective heat treatments for 3 h: 4°C, 50°C, and 160°C. All heated samples were then returned to the cold room where Labeled Release experiments were conducted. Each ampoule was broken and the silica gel contents transferred aseptically to sterilized glass vials of 25 cc capacity. 0.1 ml of VMI flight-type Labeled Release medium was pipetted onto each portion of silica gel. Absorbent pads (20 mm diameter, No. 470, Schleicher and Schuell) placed inside the glass vial screw cap, were quickly moistened with two drops of a freshly prepared, saturated solution of $Ba(OH)_2$. These caps were immediately screwed on to collect evolved $^{14}CO_2$. Incubation was maintained at the 4°C temperature of the room. As a control against contamination and a check on the noise level of the medium, duplicate portions of the sterile VMI alone were incubated. At intervals ranging from 15 min initially to a maximum of once per day toward the end of the experiment, the glass vial caps were removed and immediately replaced with fresh ones containing $Ba(OH)_2$ moistened pads. All pads exposed for the collection of radioactive $^{14}CO_2$ were transferred to planchets, dried and counted for radioactivity in a gas flow counter.

The results, adjusted for instrument sensitivity and scaled to permit direct comparison of the Mars and Earth results, are presented in Fig. 5. Fig. 5A presents the data on evolved radioactivity from non-irradiated silica gel subjected to the various heat treatments. All samples of silica gel evolved an amount of gas within the typical sterile control range for the Labeled Release experiment. No significant differences are attributed to the heat treatments. The results of the gamma irradiated samples, however, Fig. 5B, show that gamma irradiation has a pronounced effect on the non-biological activity of the silica gel. The samples approach the activity levels seen for terrestrial organisms under Martian conditions in Fig. 1 and for the Mars data presented in Figs. 3 and 4. All figures have been drawn to approximately the same scale to facilitate visual comparison. The 160°C heating of the silica gel significantly attenuated its response. However, the magnitude of the effect does not approach that on Mars where the 160°C preheat treatment virtually eradicated the response. Although the duplicates for the non-heated samples and the samples heated to 50°C overlap, there may be a slight (approx. 10%) reduction in silica gel reactivity following heating at 50°C. Again, however, the effect does not approach the magnitude of the response attenuation caused by heating the Mars sample to 50°C.

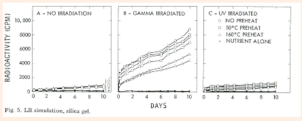

Fig. 5. LR simulation, silica gel.

Responses from the UV irradiated samples are presented in Fig. 5C. All are essentially within the range of control responses characterizing heat sterilized soils tested in the LR experiment.

TABLE 1

pH of UV and gamma irradiated and non-irradiated silica gel after heat treatment and LR incubation.

Pretreatment temp. (°C)	pH		
	UV	Gamma	Non-irradiated
4	5.55	5.75	5.70
4	6.05	5.75	—
50	5.90	5.45	6.00
50	6.10	5.50	—
160	5.90	5.65	5.90
160	—	5.90	—

pH VM1 alone after incubation: 8.30, 7.55.

Upon the conclusion of the experiment, 0.5 ml of distilled water (pH 6.3, unbuffered) was then added to each remaining portion of the sample and its pH determined. The results are presented in Table 1. The remaining half of each sample was plated on trypticase-soy agar to check for sterility. The material was shaken out of the glass vial in which it had been incubated and dispersed on the agar plate by agitating the plate. After incubating at room temperature for four days, all plates were negative for colonies.

TABLE 2

Mars analog soil composition wt.%.

Minerals		Elements	
Montronite	51.1	Na	0.35
Montmorillonite	25.5	Mg	2.65
Kieserite	9.4	Al	3.84
Calcite	6.0	Si	19.15
Hematite	5.0	P	0.01
Magnetite	3.0	S	2.26
		Cl	0.00
		Ar	0.00
		K	0.15
		Ca	5.17
		Ti	0.19
		Fe	15.71
		C	0.75
		O	49.78
	100.0		100.0

An identical set of experiments was simultaneously performed on a portion of the Mars analog soil (Clark et al.) prepared by the Viking Inorganic Analysis Team in accordance with X-ray fluorescence analyses performed on Mars. The composition of the Mars analog soil is presented in Table 2. The results of this experiment are presented in Fig. 6. In its non-irradiated condition, the Mars analog soil, Fig. 6, produced gas evolution in significantly greater quantities than did non-irradiated silica gel. There is essentially no difference between the non-heated Mars analog soil sample and the sample preheated to 50°C. The magnitude of the responses approach 50% of those obtained from Mars. The apparent diminution in response of the sample preheated to 160°C may be an artifact in that the very early portion of the curve shows this response exceeding the others prior to a sharp break in the slope which may reflect a gas leak or other loss of that day's collection. Fig. 6B shows that exposure of the Mars analog soil to gamma irradiation did not increase its reactivity with VM1. Nor is any significant attenuation in response attributable to the heat treatment indicated. However, all responses are above normal control levels for typical sterilized soils examined in the LR experiment. UV irradiation of the Mars analog soil, as shown in Fig. 6C, resulted in a diminution in all responses producing results at, or near, the normal sterilized soil control levels.

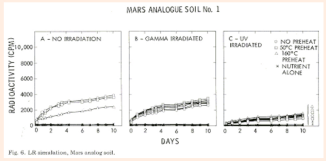

Fig. 6. LR simulation, Mars analog soil.

The pH of each portion of the Mars analog soil was determined in the same manner as for the silica gel samples. The results are shown in Table 3. Sterility tests were performed on all portions of Mars analog soil at the termination of the LR experiment identically as described for the silica gel. All plates were negative.

TABLE 3

pH of UV and gamma irradiated mars analog soil after heat treatment and LR incubation.

Pretreatment temp. (°C)	pH		
	UV	Gamma	Non-irradiated
4	8.38	8.23	8.37
4	8.06	8.37	—
50	8.07	8.30	8.20
50	8.07	8.23	—
160	8.23	8.23	8.20
160	8.17	8.36	—

pH VM1 alone after incubation: 8.30, 7.55.

Discussion

The gamma radiation levels in the vicinities of the Viking spacecraft on Mars have not yet been measured. However, they must be low in that the background levels monitored by the LR radioactivity detectors correspond extremely closely to the gamma levels anticipated from the radioisotopic thermoelectric generators (RTGs) carried out by the Viking landers. Any external gamma contribution to the background readings must, therefore, be minor. The background level for the flight Labeled Release instrument on Earth, without the influence of the RTGs, is approx. 10 cpm. Thus, the 0.83 Mrad gamma dose administered to the silica gel and Mars analog soil samples represents an accumulated dose over a long period of time on Mars. This combination of a severe gamma dose administered to a substance highly susceptible to sustaining defects did cause a significant release of radioactive gas from the VMI approximating that of the LR Mars response in magnitude, but not in kinetics. Pretreatment of the irradiated silica gel at elevated temperatures resulted in decreased Labeled Release activity following nutrient injection. This is evocative of the LR Mars data, but the temperature effect on silica gel is greatly muted with respect to that on Mars.

The UV flux incident to the surface of Mars has been estimated (Glasston, 1968) at 2 X 10^{-4} W cm^{-2}. Current estimates (Shorthill, pers. comm.) indicate that only 50% of this UV flux reaches the Martian surface, yielding a flux at the surface of 10^{-4} watts or 10^{3} ergs sec^{-1}. Based on an estimated UV dose of 10,000 μW cm^{-2}, or 10^{5} ergs sec^{-1}, incident to the samples from the irradiation array, the UV irradiation given the samples exceeds that on Mars, by approximately two orders of magnitude. The LR response produced by UV irradiated Mars analog soil is even less than that produced by non-irradiated Mars analog soil. Thus, the results of these two tests of the Mars analog soil make it unlikely, if the Mars analog soil is a faithful representation, that ultraviolet irradiation is responsible for the results obtained on Mars in the LR instrument. While the positive response of the non-irradiated Mars analog soil and its possible pretreatment temperature dependency are of interest, the fact that its capability to produce gas from VMI is UV-labile makes it an unlikely explanation of the Mars results. The analog soil tested contained alpha-Fe_2O_3. Recently, it has been suggested (Oyama, Viking Biology Team) that epsilon-Fe_2O_3 might be the form on Mars and that it might react with organic compounds. Accordingly, the Viking Inorganic Analysis Team is presently preparing another Mars analog soil, similar to the first, but containing epsilon-Fe_2O_3. The authors plan to test this material for possible activity in the LR experiment.

Conclusions

1. The Viking Labeled Release Experiment has produced evidence for life on Mars. However, non-terrestrial soil chemistry may be mimicking a biological response. All hypotheses require further study before a conclusion can be reached.
2. Intense gamma irradiation of silica gel causes evolution of gas when the LR VMI medium is applied to it. The amount of gas evolved approaches that observed in the LR experiment on Mars. However, while some pretreatment temperature dependency was observed in the silica gel, the effects are not as great as those observed in the Mars experiments. This "worst case" test is extreme in that the gamma levels on Mars do not approach the dosage applied in the test. However, the reaction is of sufficient interest that it will be explored further and its possible relevancy, to the LR Mars results studied, perhaps as an analog of long-term exposure to the solar wind.
3. No significant effect was observed in the LR experiment when silica gel was irradiated by UV light.
4. Non-irradiated Mars analog soil produces a response in the LR experiment. The response may show some slight pretreatment temperature dependency, but not of the magnitude observed on Mars.
5. Gamma irradiation of the Mars analog soil had no apparent effect in the LR experiment.
6. UV irradiation of the Mars analog soil reduced the LR response to well within those normally observed from sterilized soils. It is thus unlikely that the Mars results can be attributed to a factor in the Mars analog soil.

Conclusions 3 and 6 in conjunction with the LR Mars data obtained from the "under the rock" sample tend to eliminate UV radiation as causative of the Labeled Release response.

Updated versions of the Mars analog soil (including epsilon-Fe_2O_3) will be tested with and without gamma irradiation in the Labeled Release Test Standards Module (TSM) (Levin and Straat, 1976) where they can be maintained under simulated Mars conditions throughout the incubation period. The effect of additional injections of nutrient can be studied in the TSM for comparison with the Mars results.

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