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Viking Labeled Release Biology Experiment: Interim Results

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Abstract. *This report summarizes all results of the labeled release life detection experiment conducted on Mars prior to conjunction. Tests at both landing sites provide remarkably similar evolution of radioactive gas upon addition of a radioactive nutrient to the Mars sample. The "active" agent in the Mars sample is stable to 18°C, but is substantially inactivated by heat treatment for 3 hours at 50°C and completely inactivated at 160°C, as would be anticipated if the active response were caused by microorganisms. Results from test and heat-sterilized control Mars samples are compared to those obtained from terrestrial soils and from a lunar sample. Possible nonbiological explanations of the Mars data are reviewed along with plans for resolution of the Mars data. Although such explanations of the labeled release data depend on ultraviolet irradiation, the labeled release response does not appear to depend on recent direct ultraviolet activation of surface material. Available facts do not yet permit a conclusion regarding the existence of life on Mars. Plans for conclusion of the experiment are discussed.*

Prior descriptions of the labeled release (LR) Mars life detection experiment have indicated its scientific concepts (*1, 2*) and instrumentation (*2, 3*) and have presented data obtained from terrestrial soils (*1, 2*). Recently, preliminary data from the first two Mars samples have been reported (*4*). Briefly, the radiorespirometric LR experiment seeks to detect metabolism with or without growth by monitoring the evolution of radioactive gas from a 0.5 cm^3 surface sample after the addition of 0.115 ml of a nutrient (*2, 4*) containing seven organic substrates (formate, glycolate, glycine, DL-alanine, DL-lactate) uniformly labeled with ^{14}C. Total oxidation of any one of the 17 carbon positions would produce gas containing approximately 15,000 counts per minute (cpm) (based on instrument counting efficiency), whereas total utilization of the nutrient would produce gas containing approximately 257,000 cpm if we assume complete conversion of all carbon atoms to gas.

Each of the two Viking LR instruments, one aboard each lander, has now conducted three analyses of Mars surface material ("soil") between the time of touchdown and the communication blackout period, which occurs during conjunction of Mars with the sun between early November and mid-December 1976. As with many of the other Viking investigations, the LR experiment is not yet complete and four additional analyses are anticipated after conjunction. Thus, conclusions must he regarded as tentative pending completion of the Mars experiments, detailed analysis of the LR data as well as data of other related Viking experiments, and extensive laboratory tests now under way to help interpret the results. Because of the general interest in the subject, this interim report has been prepared to present results of all LR data obtained on Mars prior to conjunction. Also presented are views of possible chemical explanations of the data as well as tentative plans for the conclusion and analysis of the experiment.

Sample sites and sample acquisitions. Analyses on the first lander were conducted at Chryse Planitia (22.46°N, 48.01°W), an area of relatively low elevation dominated by previous, large-scale fluvial activity, selected as a possible niche for past or present life. The immediate vicinity visually resembles the deserts in southwestern United States, except for the vivid orange-red color of the surface. The area is heavily strewn with modest-sized rocks although the site to which the sampling arm was directed was a smooth patch of fine-grained material named "Sandy Flats." The sampling arm acquired the top 4 cm of the material and delivered it to the lander sample processor where it was mixed and sieved to provide all three biology experiments with uniform portions consisting of particles less than 1.5 mm in diameter. Fresh samples were acquired for the first and third analyses, whereas the second analysis used the same sample acquired for the first analysis and stored in the soil processor for approximately 20 sols (one martian sol = 24 hours and 40 minutes), the duration of the first cycle.

The second landing site. Utopia Planitia (47.97ºN, 225.67ºW, is approximately 4000 miles (1 mile = 1.6 km) from the first landing site, but closely resembles that of the first lander in appearance. On Viking lander 2 (VL2), a fresh soil sample was acquired for each experimental cycle. For the first two cycles, the sample was acquired from a pebble-strewn area, 'Beta," whereas the third cycle sample was acquired from an area exposed by pushing aside a rock, "Notch Rock," with the sampling arm. The rock pushing and sample acquisition event was conducted approximately 1 hour after sunrise when the sample was exposed to low angle sunlight for approximately 37 minutes prior to placement in the soil processor (*5*). It is estimated (*5*) that this sample contained at least 90 percent of material from under the rock and thus had been protected from ultraviolet (UV) light for a long period.

One important difference between the two landing sites was the amount of water vapor present in the local atmosphere. At Utopia, the average atmospheric moisture content was approximately 25 precipitable micrometers, whereas at Chryse, the average was about 10 precipitable micrometers (*6*). A second difference between the two sites is that, at the time of landing at Utopia (3 September 1976), the season was early summer and the average surface temperature was 222°K (7). This temperature is gradually and continually decreasing such that, by April 1977, the average surface temperature is anticipated to be 170°K. At this point, the lander thermal limits will probably be exceeded, and all operations are expected to cease. Average surface temperatures at Chryse, however. have been relatively constant at 210º to 220°K since touchdown and should remain so throughout the Martian year, allowing data to be collected for the lifetime of the lander (*7*).

LR analyses of Mars surface material. A summary of the six analytical cycles so far conducted on Mars is shown in Table 1. The LR results obtained from the first lander are shown in Figs. 1-3. For each cycle, three types of measurements were periodically obtained: test cell incubation temperature, detector temperature, and evolved radioactivity in the headspace gas. Temperature fluctuations with the test cell and detector chambers are caused by heaters and thermoelectric coolers responding to martian diurnal temperature variations to maintain test cell incubation temperatures at approximately 10°C. Diurnal fluctuations in evolved radioactivity appear to follow test cell temperature fluctuations and are more apparent in the linear plots that we now present than in the semilogarithm plots of cycles 1 and 2 presented earlier (*4*). These fluctuations must result, at least in part, from physically and chemically governed gas movement between the detector and test cell chambers caused by temperature differences.

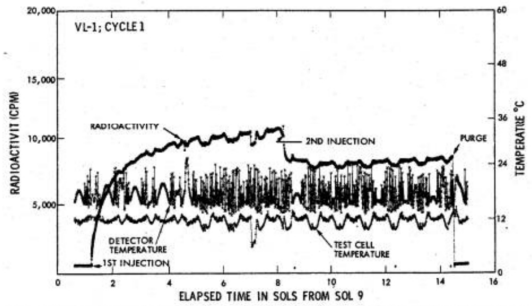
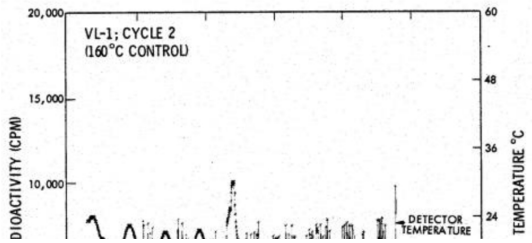


Fig. 1. Plot of LR data from first sample analysis on VL1. An active sequence was used on a fresh surface sample. Radioactivity was measured at 16-minute intervals throughout the cycle except for the first 2 hours after the first nutrient injection when readings were taken every 4 minutes. Radioactivity data include a background count of 490 cpm prior to the onset of the cycle. Detector and test cell temperatures were monitored every 16 minutes.



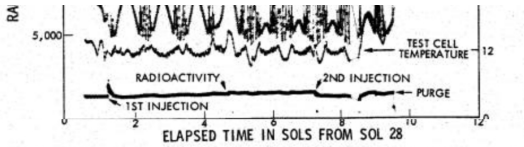


Fig. 2. Plot of LR data from second sample analysis on VL1. A control sequence was used in which a stored portion of the sample used for cycle 1 (Fig. 1) was heat-sterilized for 3 hours at 160°C approximately 21 hours prior to nutrient injection. Radioactivity was measured at 16-minute intervals throughout the cycle, except for the first 2 hours after each nutrient injection when readings were taken every 4 minutes. Radioactivity data include a background count of 508 cpm prior to sterilization. Detector and test cell temperatures were monitored every 16 minutes.

On cycle 1 of VL1 (Fig. 1), a rapid evolution of radioactive gas began immediately upon nutrient injection. For the first 10 hours, the magnitude and kinetics of gas evolution closely followed those obtained from terrestrial soils tested under terrestrial conditions (Fig. 4) in the test standards module (TSM), an instrument closely resembling the flight instrument *(2)*. After 10 hours, however, the radioactivity evolved from the Mars sample leveled, so that a near-plateau of approximately 10,000 cpm (net) was attained. The magnitude of the response plateau is about tenfold less than that obtained from terrestrial soils with moderately high microbial populations (Fig. 4). In contrast, the magnitude of the Mars response is not unlike that obtained from low population Antarctic soils, such as No. 664 *(8)* tested in the TSM.

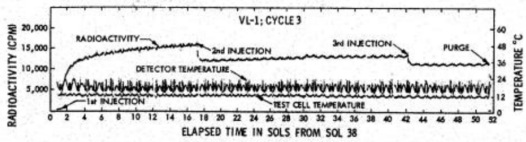


Fig. 3. Plot of LR data from third sample analysis on VL1. An active sequence with an extended incubation lasting 51 sols was used on a fresh sample of surface material. Radioactivity was measured at 16-minute intervals throughout the cycle, except for the first 2 hours after each injection when data were taken every 4 minutes. Radioactivity data include a background count of 519 cpm prior to the onset of the cycle. Detector and test cell temperatures were monitored every 16 minutes.

With terrestrial soils, heating at 160°C for 3 hours dramatically reduces the evolved radioactivity and constitutes a control to demonstrate the biological nature of the unheated soil response. As shown in Fig. 4 for Aiken soil (the Viking biology standard test soil, which contains approximately 10^5 aerobic microorganisms per gram), after heat sterilization and nutrient injection, radioactive gas evolution is greatly attenuated and attains a plateau approximately only 500 cpm over background. Typically, plateaus from heat-sterilized or from naturally sterile soils range between 300 and 800 cpm over background for a variety of soils tested *(2)*. The large difference between active and sterilized responses (~200-fold for Aiken soil) confirms a positive response from the "active," or nonsterilized, sample.

Table 1. Summary of LR sample analyses of Mars surface material. For each analytical cycle, the site for sample acquisition is indicated along with surface temperature at the time of acquisition and the sol on which collection occurred. The sols for each nutrient injection and for purging to terminate each incubation cycle are also shown. For each lander, sols are counted from the day of touchdown.

Analysis cycle	Sample collection			Type experimental sequence	Sol for injection No.:			Purge
	Site	°C*	Sol		1	2	3	
				VL1 (Chryse Planitia)				
1	Sandy Flats	−83	8 (fresh)	Active	10	17		23
2	Sandy Flats	−83	8 (stored)	160°C control	29	35		37
3	Sandy Flats	−21	36 (fresh)	Active, long incubation	39	55	80	89
				VL2 (Utopia Planitia)				
1	Beta	−23±5	8 (fresh)	Active	11	18		24
2	Beta	−23±5	28 (fresh)	50°C control	34	38		47
3	Under Rock	−66	51 (fresh)	Active, long incubation	53	60		†

*Surface temperature. †Purge will occur after conjunction.

For the Mars surface sample, heat treatment similarly resulted in a significant attenuation of evolved radioactivity. In cycle 2 (Fig. 2), a baseline of 1300 cpm was observed after sterilization, of which approximately 500 cpm is attributable to background and the remaining 800 cpm represents residual contamination from the first cycle. Upon nutrient injection, the count rose to approximately 2100 cpm, then fell to 1300 cpm and slowly rose to 1500 over the subsequent 6-sol period when nutrient was injected a second time. Upon purging at the end of the cycle, the count dropped to the initial background of 516 cpm, proving that the additional 800 cpm present following sterilization were attributable to gas and that the kinetics during the cycle did not reflect a test cell leak. Details of this kinetic response have been presented (*4*).

The major attenuation in the Mars heat-sterilized control meets one of the previously established criteria *(2)* to demonstrate the biological nature of the active positive response from a duplicate portion of the same sample. Responses from soils known to be naturally sterile are compared in Fig. 5 with initial kinetics obtained from the "active" and control cycles of the Mars sample. Experiments with lunar soil and the reportedly sterile Antarctic soil No. 542 (*9*) were conducted in the LR TSM. The difference in evolved radioactivity between "active" and control cycles is seen in Fig. 5 to be negligible for both of these samples. Further, when corrected for background, the evolved counts from the active cycles of lunar and Antarctic No. 542 soils are less than 1000 cpm, within the limit of responses from all heat-sterilized soils.

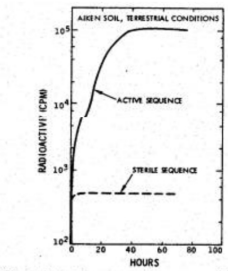
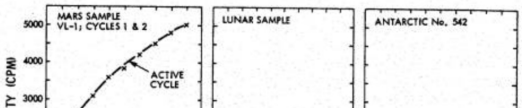


Fig. 4. The LR response from terrestrial soil in TSM. A 0.5-cm³ sample of Aiken soil was added to the TSM under terrestrial atmospheric conditions at room temperature and 0.115 ml of nutrient was injected according to a flight sequence. Radioactivity evolved after nutrient injection to either an active (_____) or control (– – – –) sample is shown as a function of time. The control soil was heat-sterilized in the TSM test cell for 3 hours at 160°C prior to nutrient injection. Background radioactivity has been substracted from all data.

The heat-treated portions of lunar and Antarctic soils both exhibit decreases in radioactivity after the initial gas evolution resulting from nutrient injection. Extensive testing established that neither of these decreases was caused by a leak in the TSM test cell. Gradual "gettering" of the evolved radioactive gas was exhibited by the basic lunar sample (*p*H 9.4) over extended time, but did not resemble the kinetics of the Mars control. Heat-treated samples of Antarctic soil No. 542, on the other hand, do resemble (Fig. 5) data obtained from the control cycle of the Mars sample. Similar results have also been obtained in the TSM with heat-sterilized samples of Antarctic soil No. 664. Other than with these three soils, no decreases in gas levels have been observed after nutrient injection on a wide variety of soils tested in the TSM. The two Antarctic soils have a high carbonate content (0.168 and 0.25 percent by weight, respectively) and *p*H values of 7.5 and 8.1, respectively *(10)*. The possible relation of these soils to the Mars data is under investigation.



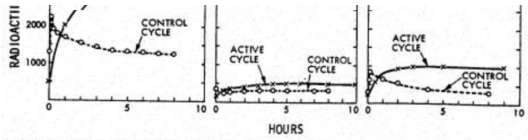


Fig. 5. Comparison of VL1 Mars data with data obtained from lunar and Antarctic soils. Initial kinetics following nutrient injection are compared for Mars data obtained from the first two cycles on VL1, for a lunar sample examined in the TSM, and for the naturally sterile Antarctic soil No. 542 examined in the TSM, as indicated. Both active (——) and control (– – –) sequences were conducted for each soil. Control samples were heat-sterilized for 3 hours at 160°C in the test cell prior to nutrient injection. Radioactivity evolved after nutrient injection is shown as a function of time.

The third cycle on the Chryse lander was an extended active incubation lasting 50 sols. Three injections of nutrient were added, on sols 39, 55, and 80, to the sample collected on sol 36. It was hoped that the extended incubation would permit evidence for growth to be observed, thereby unequivocally demonstrating a biological response by exponential kinetics. As is shown in Fig. 3, however. the kinetics after the first nutrient injection closely resemble those obtained from cycle 1, and no evidence is seen throughout the entire cycle for an exponential response. The magnitude of the initial response is higher than in cycle 1, reaching a near-plateau of approximately 13,900 cpm over background during the same time interval in which 10,100 cpm over background were evolved during cycle 1. For cycle 3. the net counts evolved before the second injection (15,500 cpm over background) correspond closely to total utilization of 1 of the 17 carbons in the nutrient. Although it is not excluded that the radioactivity could have been derived from more than one of the substrates, the gas evolution kinetics appear to be first order, suggesting that only one substrate participated. The difference in response magnitude between the first and third cycles probably results largely from radioactive gas contamination which was approximately 1200 cpm at the onset of cycle 3. This is evident in the fine structure in the early portion of the curve which is not discernible on the scale of Fig. 3.

One significant difference in the sample acquisition for the two active cycles on VL1 is that the cycle 1 sample was collected when the surface temperature was -83°C and maintained in the soil processor at approximately -40°C for 1 hour and 14 minutes before delivery to the test cell. The cycle 3 acquisition, on the other hand, was collected when the surface temperature was -23°C and maintained in the processor for 1 hour and 40 minutes at +18°C before delivery. Once in the test cell, incubation temperatures for both cycles were maintained at an average temperature of approximately 10.5° ± 3°C. These data suggest that the agent responsible for the active Mars response is stable to temperatures of +18°C. Further, since the cycle 1 and cycle 3 samples were collected and maintained at approximately 10°C for 2 and 3 sols respectively, the data suggest that "aging" has no effect on stability at this temperature.

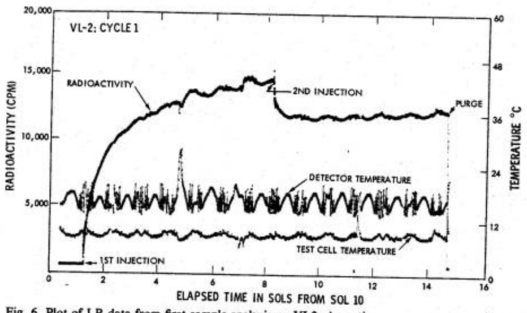


Fig. 6. Plot of LR data from first sample analysis on VL2. An active sequence was used on a fresh surface sample. Radioactivity was measured at 16-minute intervals throughout the cycle except for the first 2 hours after each nutrient injection when readings were taken every 4 minutes. Radioactivity data include a background count of 541 cpm prior to the onset of the cycle. Detector and test cell temperatures were measured every 16 minutes.

Sample analyses from the second lander are shown in Figs. 6-8. Data from cycle 1 (Fig. 6) and cycle 3 (Fig. 7), which are active sequences, are remarkably similar both in kinetics and magnitude to those obtained from the two active cycles on VL1 (Figs. 1 and 3). The response from the Beta sample in cycle 1 (Fig. 6) attained a near-plateau of approximately 14,000 cpm over background after the first injection. Cycle 3 (Fig. 7), conducted on the sample acquired from under Notch Rock, obtained a near-plateau of approximately 10,200 cpm (net) after the first injection. The data from the under-the-rock sample are, in fact, so similar to those obtained from VL1, cycle 1 (Fig. 1), that the curves are essentially superimposable. They differ somewhat only in detailed diurnal temperature fluctuations, probably because of the different temperature patterns between VL1 and VL2. On VL2, average test cell temperatures were about 3°C lower than on VL1, whereas maximum detector temperatures were about 3°C lower for the first 4 sols and about the same as those for VL1 for the remaining sols. Comparing the two samples collected for VL2, surface temperatures during the acquisitions were approximately -23°C for cycle 1 and -66°C for cycle 3. The cycle 1 sample also had a longer residence time in the test cell (3 sols versus 2 sols for cycle 3) before nutrient injection. As with VL1, the higher surface temperatures during collection and longer residence before injection correlate with the higher response. The reason for this apparent correlation is not currently known.

The data from the sample acquired from under Notch Rock indicate that direct ultraviolet irradiation of the surface material is not responsible for the Mars active response. It seems highly unlikely that the brief sample exposure at low sun angle between the time of rock movement and soil acquisition could allow sufficient ultraviolet activation of surface material to produce the LR response. Further, approximately only 10 percent of the total sample was derived from an area not covered by the rock *(5),* and the material under the rock had probably been there for at least a few thousand years *(11).* Thus, the agent responsible for the LR activity is apparently stable to long periods in the dark and is not dependent on recent direct ultraviolet activation. Alternatively, ultraviolet activation could have occurred millions of years ago and, in the absence of a deactivation mechanism, the active material might have remained stable. Another possibility that cannot be excluded is activation of atmospheric material in direct sunlight with subsequent aeolian or other permeation into the soil.

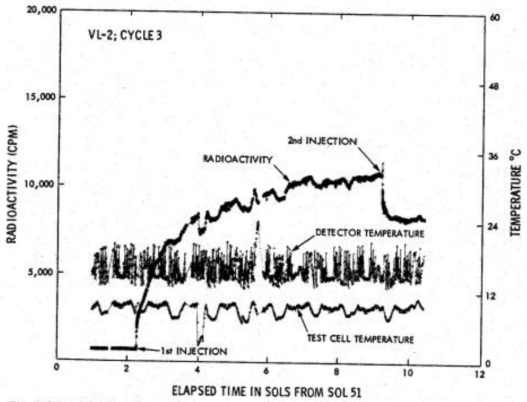


Fig. 7. Plot of LR data from third sample analysis on VL2. An active sequence was used on a fresh sample that was acquired from surface material exposed by pushing aside a rock. Radioactivity was measured at 16-minute intervals throughout the cycle, except for the first 2 hours after each injection when readings were taken every 4 minutes. Radioactivity data include a background count of 659 cpm prior to the onset of the cycle. Data obtained in the single channel counting mode between sols 53 and 60 have been corrected to the dual channel mode of operation for comparison with the remainder of the cycle and with data from previous cycles. Detector and test cell temperatures were measured every 16 minutes. This sample analysis has

The sequence used in cycle 2 on VL2 heated a freshly acquired Beta sample for 3 hours at a temperature of approximately 50°C. Although such a "cold sterilization" had never before been performed on a flight instrument, the experiment was conducted in an attempt to distinguish between biology and chemistry as the cause of the LR response. Thus, if the response had resulted from martian organisms, these organisms would not have previously experienced temperatures as high as 50°C and would probably be damaged or killed by such exposure. After the treatment at 50°C, a response similar to those obtained in active cycles would strongly favor a

chemical explanation, whereas a materially reduced response would be consistent with a biological agent. At the very least, a dramatically reduced response would narrow the range of possible chemical reactants to those stable at 18°C but unstable at 50°C. By activating only one of the two heaters used to attain sterilization temperatures of 160°C, the desired 50°C was, in fact, achieved and maintained for 3 hours in the sample used for cycle 2. The sample was allowed to cool and nutrient was injected immediately upon cooling.

The results of cycle 2 (Fig. 8) show that the 50ºC preliminary treatment caused a significantly attenuated LR response. Further, the kinetics of gas evolution seen in Fig. 8 reveal several unusual features. As is shown, the radioactive gas evolved indicates cyclic increases and decreases that are especially pronounced during the first few sols after the first nutrient injection. After the fourth sol, however, the periodicity becomes regular and a frequency of 1 cycle per sol is evident. Because this extraordinary behavior was not understood, several diagnostic experimental sequences were uplinked to the LR module during the last few sols of the incubation cycle in an attempt to detect an instrumental anomaly. These diagnostics showed no indication of a hardware malfunction, including leaks or faulty electronics. Thus, although the reason for the unusual kinetics is not understood, it can he concluded that treatment of the martian soil at 50°C greatly reduces the rapid evolution of radioactive gas after nutrient injection.

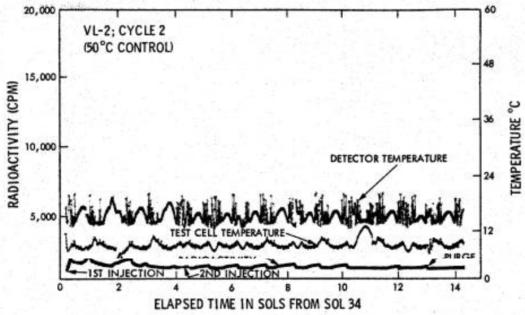


Fig. 8. Plot of LR data from second sample analysis on VL2. A control sequence was used in which a fresh surface sample was heat-sterilized for 3 hours at 50ºC approximately 2 hours before nutrient injection. Radioactivity was measured at 16-minute intervals throughout the cycle, except for the first 2 hours after each nutrient injection when readings were taken every 4 minutes. Radioactivity data include a background count of 590 cpm prior to sterilization of the soil. Detector and test cell temperatures were measured every 16 minutes.

Each complete LR cycle administered two nutrient injections to each sample except for the sample in cycle 3, VL1, which also received a third nutrient injection. The time of each injection is listed in Table 1. For all cycles except cycle 2, VL2, addition of a second nutrient injection resulted in a sharp spike of evolved radioactivity followed immediately by a drop of 30 to 35 percent in total radioactive gas. An additional drop of approximately 23 percent was observed after the third injection in cycle 3 of VL1. These changes, at least in part, must reflect shifts in the carbon dioxide-carbonate solution equilibrium.

In all cases, after the initial drop after the second injection, a small gradual rise (approximately 50 to 100 cpm per sol) in radioactive gas ensued over the subsequent incubation period. For the first analytical cycle examined (Fig. 1), this rise appeared to be exponential, although an exact slope is difficult to determine because of the interference of the temperature-induced fluctuations in radioactivity and because only 6 sols of data were acquired prior to purge. To show the exponential rise, progressive determinations of slopes were made with 3 sols of data for each calculation. The resulting slopes showed a small but significant progressive increase with time, whereas the corresponding slopes attained from a similar analysis of the test cell temperature showed no change with time. However, the biology of instrument-mounting-plate temperature showed a rise of 2º to 3ºC over the same period. When the data after second injection were examined over the longer incubation period of cycle 3, VL1, however, the corresponding rise was clearly linear. Thus, in cycle 1, VL1, there may not have been sufficient data to distinguish linear from exponential kinetics or the rise may somehow have been associated with increasing, mounting-plate temperatures. Alternatively, the “exponential” rise may be absent in cycle 3 because the second injection occurred 16 sols after the first injection and martian organisms may not have survived the period between injections. For the first cycle, the second injection occurred 7 sols after the first. This possibility will be explored on VL2, cycle 3, where the time between the two injections has been preserved at 7 sols and a long extended incubation follows the second injection. These data will be acquired during the conjunction period and will be available early in 1977.

In summary, the results to date of the first six LR analyses of the Mars surface material have demonstrated the following:

1) Addition of nutrient to surface material results in a rapid evolution of counts until a level of 10,000 to 15.000 cpm is achieved, possibly corresponding to utilization of only one of the carbon substrates offered.

2) The active responses attained at both landing sites are remarkably similar in kinetics and magnitude.

3) The active response does not appear to depend on direct or recent ultraviolet activation of the surface material tested.

4) The active response is stable to 18°C but is greatly reduced by heat treatment for 3 hours at 50°C and is obliterated by 160ºC treatment. The kinetics of gas evolution after the treatment at 50°C are unaccountably peculiar and differ significantly from those after 160°C treatment and from those of unheated samples.

5) Second injection in all cycles except the 50°C cycle result in a sharp spike of evolved radioactivity, then an immediate 30 to 35 percent decrease in gas level, followed by a gradual linear rise during subsequent incubation.

Possible nonbiological explanations. The responses obtained from the Mars sample indicate biology by virtue of the major difference in radioactivity evolved from test and control sequences. However, the circumstances on Mars are sufficiently different from those on Earth to warrant extreme caution in reaching a conclusion concerning the existence of life. The fact that no organics have yet been detected from the Mars surface sample (*12*) is in contrast to any terrestrial soil containing life. Further, while not a necessary criterion for the detection of life, exponential gas evolution that would accompany growth or reproduction and provide unequivocal evidence for life has not developed. Finally, the environmental conditions on Mars include a high ultraviolet flux striking the surface, which could be responsible for the presence of highly reactive inorganic compounds.

The possibility of radiation-induced catalysis or the production of reactive compounds on the surface of Mars had been considered previously. In 1970, it was proposed (*13*) on the basis of energy calculations that ultraviolet radiation striking silica molecules on Mars surfaces could cause atom point or electron point defects (“splits” or “disjunctions”) in silica crystals, thereby trapping atoms in interstitial lattice positions or trapping electrons away from their orbits and creating positive holes. The resulting material would he highly reactive with organic substrates. After extensive review of these radiation-induced reactions, however, they were considered sufficiently unlikely and received low program priorities at that time. A similar theory involving siliceous surface material (*14*) proposes that solar flux protons, or harder ionizing radiation, create similar defects. Hydroxyl radicals and water. created at the points of adsorption of the radiant energy, could react to cause chemical degradations directly or indirectly.

The surprising nature of the Viking biology data has now attracted wide interest to the possibility that ultraviolet radiation of the Mars surface material may, in some way, be responsible for the Viking biology results and has made imperative a study of such effects. Several additional theories have been advanced to account for the LR results chemically, all centering upon the high UV flux striking the martian surface material with subsequent production of highly reactive compounds. These could react rapidly with one or more of the LR substrates, formate being the most likely candidate, to produce the observed gas.

One theory recently advanced (*15*) to explain the LR responses hypothesizes the formation of hydrogen peroxide or a metal peroxide by ultraviolet photolysis of water tightly adsorbed onto the Mars surface material. The reaction could be catalyzed by Fe_2O_3, FeO, or TiO. Using Fe_2O_3 again as a catalyst, the peroxide formed then oxidizes formate to CO_2. In the presence of additional water vapor and the warmer temperature in the test cell, hydrogen peroxide could also decompose to water and oxygen, possibly accounting for the oxygen evolution seen in the gas exchange experiment (*4*). Also on the basis of the formation of hydrogen peroxide, it has been proposed (*16*) that ferrous ions become oxidized to the ferric state through the action of ultraviolet light on clean mineral surfaces. In this theory, production of the peroxide from water bound to ferric ion is dependent on the provision of fresh, unweathered surface material. In addition, it has been suggested (*17*) that one or several of a variety of peroxides. superoxides, or ozonides created in the Mars surface material could account for the decomposition of formate in the LR experiment. Other investigators (*18*) have suggested chemical explanations of the LR data, which rely on metals to decompose formate. Rare metals known to react with formate to produce CO_2 include rhodium, iridium, and rubidium.

Because most chemical theories proposed to account for the labeled release data with Mars surface material involve formate, it was imperative to determine whether the formate in the LR nutrient arrived on Mars intact. In anticipation of such questions, the flight nutrient was prepared 2 years ago (*19*) in excess, and a portion

was stored during the interim at room temperature in sealed glass flight ampoules. These ampoules received essentially the same heat treatments as ampoules incorporated into flight instruments received during instrument and lander heat sterilizations. One of these spare flight ampoules was recently broken in the TSM reservoir, and a nutrient portion was removed by injection into a glass vial substituted for the TSM test cell. After the nutrient was degassed in the reservoir according to the flight regime, another portion was similarly removed. The formate concentration was then determined (*20*) on both portions according to an enzymatic procedure (*21*). The results showed formate present at 3.1 x $10^{-4}M$ before degassing and 3.5 x $10^{-4}M$ after degassing. In a second ampoule, the concentration after degassing was 3.0 x $10^{-4}M$. These concentrations are somewhat greater than the original 2.6 x $10^{-4}M$, as anticipated from the evacuation and filtration procedures used for loading flight ampoules and from water loss through evaporation during degassing. The results nonetheless demonstrate that the formate in the LR nutrient arrived on Mars intact. Thus, formate decomposition by the Mars surface sample could account for the 15,000 cpm evolved in the Mars assay.

Although it is possible that inorganic reactants could account for the LR data, sufficient analyses have now been conducted on Mars to place a considerable number of constraints on the nature of such oxidants. First, the reactants must be widely distributed on Mars. They must react with the LR nutrient in the dark at test cell temperatures of 10°C. Their presence on Mars does not depend on recent ultraviolet activation, and they are apparently stable in the dark for extended periods of time. Further, if present on the very surface, they must not be destroyed by ultraviolet. The surprising finding that a strong LR response is obtained from material collected under a rock puts serious doubt on those theories requiring direct and recent ultraviolet activation of surface reactants. That the reactants are stable at 18°C in the dark but inactivated at 50°C greatly limits the number of candidate chemical oxidants. Finally, any theory must account for the peculiar emissions and readsorptions of radioactive gas seen in the 50°C control experiment.

Plans for conclusion of the LR experiment. Each LR instrument on Mars still has one unused test cell. In addition, each instrument can conduct one “soil on soil” experiment in which a fresh sample is added to a test cell containing a previously tested, but dried, soil. Assuming continued good health of these two remarkably performing spacecraft and their communications systems, additional Mars tests will be conducted after conjunction during the extended mission. Of the limited experimental options possible, a high priority is verification of the 50°C response. As another candidate experiment, the first and second nutrient injections can be administered closely together early in the cycle in an attempt to determine whether the limiting factor for gas production is in the soil or in the nutrient. Samples sequestered for weeks in the soil hopper may also be tested for effects of time and temperature. Finally, a long, cold incubation, in which the test cell incubation temperature is permitted to approach martian ambient conditions, will be conducted on VL2 for a period of up to several months. For some of these experiments, Mars samples may be obtained from the darker-hued material seen ed from the darker-hued material seen near some of the rocks, from a “deep hole” dug 8 or 9 inches (1 inch = 2.54 cm) into friable material, or from essentially the original sampling sites (Sandy Flats or Beta). Final selection will, within the experimental limitations, be designed to optimize our understanding of the nature of the LR response.

In addition to the future experiments on board the Mars landers, a considerable laboratory effort has been initiated to provide tests of the various chemical hypotheses advanced to explain the Mars results. Soils, now being prepared under stringent conditions to replicate the Mars composition and environmental conditions, are being irradiated or mixed with peroxides, superoxides, and other oxidants. These soils are now being examined in our laboratory and in the LR TSM. If all theories that remain operative within the constraints of the Mars data are considered, the laboratory program may verify or eliminate chemistry as a possible cause of the LR results. As yet, however, no chemical experiment has quantitatively reproduced the LR Mars data. Thus, despite all hypotheses to the contrary, the distinct possibility remains that biological activity has been observed on Mars.

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