Table 1. Summary of carbon assimilation data from Mars. Column 2 indicates whether the lamp was on or off, whether or not water vapor was injected, and whether the soil sample was heatsterilized (control) or not. Counting rates are given with their standard errors. See text for further details.

Experi- ment No.	Conditions	Incubation temperature (°C)	Peak 1 (count/min)	Peak 2 (count/min)		
Chryse 1	Light, dry, active	$17 \pm 1$	$7,421 \pm 59$	96 ± 1.15		
Chryse 2	Light, dry, control	$15 \pm 1$	$7,649 \pm 60$	$15 \pm 1.29$		
Chryse 3	Light, dry, active	13 to 26	$6,713 \pm 58$	$27 \pm 0.98$		
Chryse 4	Light, dry, active	$16 \pm 2$	$2,040 \pm 42$	$35 \pm 1.6$		
Utopia ł	Dark, dry, active	$15 \pm 3$	$7,133 \pm 58$	$23 \pm 1.7$		
Utopia 2	Light, wet, active	$18 \pm 1.5$	$12,523 \pm 76$	$2.8 \pm 0.92$		
Utopia 3	Dark, dry, active	$10 \pm 2$	13,014 ± 44	$7.5~\pm~2.5$		

ticular interest is the similarity between the first peaks of Utopia 1 and the first three Chryse experiments, a finding consistent with other Viking data which show the surface fines at the two landing sites to be very much alike. A slow leak from the radiation counter was detected in the course of this experiment. To forestall a loss of data should the leak worsen in later experiments, the counting program was changed so that peak 2 was read out in both 1-minute and 16-minute segments instead of 16-minute segments only. (Peak 1 is normally counted in 1minute segments.)

The Utopia 2 sample was acquired from the same spot as Utopia 1. Approximately 80  $\mu$ g of water vapor was injected at the start of the incubation from a reservoir connected to the incubation chamber through a valve and flow restrictor. The near-doubling of the first peak confirms that the injection was successful. Peak 2 was very low, although the lamp was on, and was the first clearly negative result obtained in these experiments.

The sample for Utopia 3 was acquired from under Notch Rock, and it was incubated in the dark for this reason. No water vapor was injected, but the high first peak suggests a higher water content for this sample than for surface samples. This inference has been confirmed by direct determination of the water content of a different sub-rock sample from the Viking 2 landing site (3). Again, a low second peak, indistinguishable from those found in sterile or blank laboratory runs, was obtained.

Discussion. The findings to date from the PR instruments on Mars suggest that an organic synthesis from atmospheric CO or CO<sub>2</sub> occurs in the martian surface material. The synthesis is weak compared to that found in biologically active terrestrial soils (2) and, unlike the latter, is inhibited by small amounts of water. It resembles a biological reaction in being thermolabile, although there is room for doubt that the synthesis was completely

abolished in the Chryse 2 sample, which had been heated to 180°C for 3 hours. The second peak of Chryse 2 is higher than that expected for an inactive sample by  $105 \pm 30$  disintegrations per minute; that is, the difference is more than three times its standard deviation (Fig. 1). It would seem likely that some synthesis was occurring in Chryse 2, but this is uncertain owing to the fact that no lower peak has as yet been obtained in a Chryse sample. It seems reasonably clear, nevertheless, that the major part of the reaction is thermolabile, even at moderate temperatures. A nonbiological explanation for this thermolability could be the evaporation of traces of H<sub>2</sub>O, or possibly  $H_2O_2$ , required for the reaction. It is hoped that these and other ambiguities can be resolved by further experimentation on Mars and in the laboratory. N. H. HOROWITZ

G. L. HOBBY

Division of Biology,

California Institute of Technology, Pasadena 91125

J. S. HUBBARD

Department of Biology, Georgia Institute of Technology, Atlanta 30332

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## Viking Labeled Release Biology Experiment: Interim Results

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<sup>3</sup> 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 <sup>14</sup>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 dur-

ing 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 be 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 finegrained 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 (47.97°N, 225.67°W), 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 pebblestrewn 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 17 DECEMBER 1976

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



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.



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.

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 210° 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.

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.

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<sup>5</sup> aerobic micro-

organisms 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 ( $\sim$  200-fold for Aiken soil) confirms a positive response from the "active," or nonsterilized, sample.

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

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		Туре	Sol for injection No.:			D	
	Site	°C*	Sol	sequence	1	2	3	Purge
			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	$-23\pm5$	8 (fresh)	Active	11	18		24
2	Beta	$-23\pm5$	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.

"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.

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 (pH 9.4)over extended time, but did not resemble the kinetics of the Mars control. Heattreated 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 heatsterilized 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 pH values of 7.5 and 8.1, respectively (10). The possible relation of these soils to the Mars data is under investigation.

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 beAIKEN SOIL, TERRESTRIAL CONDITIONS

Fig. 4. The LR response from terrestrial soil in TSM. A 0.5-cm<sup>3</sup> 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 heatsterilized in the TSM test cell for 3 hours at 160°C prior to nutrient injection. Background radioactivity has been substracted from all data.

tween 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  $-83^{\circ}$ C and maintained in the soil processor at approximately  $-40^{\circ}$ 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  $-23^{\circ}$ 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  $10.5^{\circ} \pm 3^{\circ}$ 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.

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 nearplateau 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



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.

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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  $-23^{\circ}$ C for cycle 1 and  $-66^{\circ}$ 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 temper-

atures 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



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.



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 not yet terminated and will continue throughout conjunction.

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.

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 50°C 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 be concluded that treatment of the martian soil at 50°C greatly reduces the rapid evolution of radioactive gas after nutrient injection.

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 2° to 3°C 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 pe-



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 50°C 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.

riod 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  $18^{\circ}$ C but is greatly reduced by heat treatment for 3 hours at 50°C and is obliterated by 160°C 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 be 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<sub>2</sub>O<sub>3</sub>, FeO, or TiO. Using Fe<sub>2</sub>O<sub>3</sub> again as a catalyst, the peroxide formed then oxidizes formate to CO<sub>2</sub>. 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<sub>2</sub> 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  $\times$  10<sup>-4</sup>M before degassing and 3.5  $\times$  $10^{-4}M$  after degassing. In a second ampoule, the concentration after degassing was  $3.0 \times 10^{-4}M$ . These concentrations are somewhat greater than the original  $2.6 \times 10^{-4}M$ , 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 experi-

ment. 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 = 2.54cm) 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 vet, 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.

GILBERT V. LEVIN PATRICIA ANN STRAAT Biospherics Incorporated, 4928 Wyaconda Road, Rockville, Maryland 20852

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## North Polar Region of Mars: Imaging Results from Viking 2

Abstract. During October 1976, the Viking 2 orbiter acquired approximately 700 high-resolution images of the north polar region of Mars. These images confirm the existence at the north pole of extensive layered deposits largely covered over with deposits of perennial ice. An unconformity within the layered deposits suggests a complex history of climate change during their time of deposition. A pole-girdling accumulation of dunes composed of very dark materials is revealed for the first time by the Viking cameras. The entire region is devoid of fresh impact craters. Rapid rates of erosion or deposition are implied. A scenario for polar geological evolution, involving two types of climate change, is proposed.

On 30 September 1976, Viking 2 executed an orbital plane change maneuver modifying the inclination of the Viking 2 orbit from 52° to 75°. This maneuver made the entire north polar region of Mars visible to the Viking 2 spacecraft under favorable lighting conditions through an atmosphere of widely scattered clouds. This report presents some preliminary interpretations of the geological features in the north polar region gained from study of the orbital imaging data returned to Earth and processed by 1 November 1976.

Intense interest in observing the north polar region of Mars with the Viking orbiter cameras was stimulated in 1971-72 by high-resolution (200 m) Mariner 9 observations of the south polar region (1-5)as well as by moderate resolution (up to 600 m) observations of the north polar region (6). Briefly, these observations revealed a complex mass of eroded layered deposits near the martian south pole and strongly suggested the existence of a sim-17 DECEMBER 1976

ilar terrain near the north pole. The layered polar deposits provide the first persuasive geological evidence for cyclical climatic change on a planet other than Earth. Viking 2, with an improved imaging system relative to Mariner 9 (7) and a capability for extensive multiframe stereoscopic and colorimetric coverage, has provided an outstanding opportunity for examining this evidence in much more detail. This opportunity began at the time of the Viking 2 orbital plane change and will end, at latest, when the region passes over the terminator during the northern autumn (the fall equinox is 4 January 1977). It is quite possible, however, that the onset of the autumn-winter high latitude haze and cloud cover known as the polar "hood" may terminate surface visibility even earlier.

Geological framework. The polar terrains revealed so far by Viking 2 images can be conveniently classified into three types: layered deposits in the central polar region, a contiguous area covered by

dunes, and a cratered plains surface that appears to underlie stratigraphically both of these units. A sketch map delineating the distribution of layered terrains, dunes, and cratered plains appears in Fig. 1, which also illustrates the areal extent of the photographic coverage available for study. The materials of the perennial ice cap, identified as water ice by Viking 2 temperature measurements (8), occur primarily within the perimeter of the layered terrains, although isolated patches or outliers occur in physical contact with both the cratered surface and the dunes. The mosaic of long-range oblique Viking 2 images (Fig. 2) obtained before the plane change illustrates the pinwheel pattern of frost-free areas within the predominantly frost-covered layered deposits.

Characteristics of layered deposits. The layered deposits contribute more than any other feature to the geological distinctiveness and significance of the polar regions of Mars. We suspect that these layered deposits are exposed at the surface in all areas mapped in Fig. 1 where frost is absent. They are manifested on slopes by a parallel striping of the surface (Fig. 3) (9); however, in only a fairly small number of locations can the topographic and geological nature of the exposures be clearly discerned. In one of these areas (Fig. 3b), selected so that shading and shadowing dominates locally over brightness variations caused by albedo, a terraced slope can be recognized.

With the use of arguments presented previously for features viewed in the south polar region (2), a strong case can be made that erosion of a layered deposit accounts for the origin of the terraced slope. The lateral continuity of terraces and their uniformity in height suggest that the individual layers that gave rise to the terraces extend as continuous thin sheets over areas of several thousand square kilometers. Moreover, these individual sheets are not only quite constant in thickness, but the thicknesses of different sheets are rather similar to one another. The apparently unique occurrence of layered deposits in the polar region implies meteorological control over their formation. Direct deposition of dust from the atmosphere, perhaps influenced by the distribution of polar ice and modulated by climatic change, remains the most probable mechanism of accumulation. These climatic changes may have resulted from periodic, perturbation-induced changes in the orbital elements of Mars and the direction of its rotation axis (5)

Another distinctive attribute of the lay-