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Laboratory Simulations of the Viking Labeled Release Experiment:: Kinetics Following Second Nutrient Injection and the Nature of the Gaseous End Product

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Summary. Injection of ^{14}C-labeled nutrient onto Mars soil produced an evolution of ^{14}C gas in the Viking Labeled Release (LR) experiment. However, a second injection of nutrient seven days later was followed by an abrupt diminution of the amount of radioactive gas in the test cell. Simulation experiments performed in the LR Test Standards Module (TSM) have yielded a plausible explanation for this diminution. Radioactive carbon gases were injected into the TSM test cell in the presence and absence of two Mars analog soils. After equilibration, water was injected and its effect observed. The results indicate that the flight data following second nutrient injection can be explained on a physico-chemical basis involving a carbon dioxide/water/soil equilibrium in the test cell. The results also suggest that the gaseous end product of the Labeled Release reaction on Mars is more likely carbon dioxide than carbon monoxide.

Key words: Viking biology ¾ Extraterrrestrial life detection ¾ Labeled release experiment

Introduction

The Viking Labeled Release (LR) life detection experiment (Levin and Straat, 1976a) has obtained results consistent with the presence of microbial life on Mars (Levin and Straat, 1977b, 1979a). However, failure of corroboration from two other life detection experiments and the organic compound analysis renders the issue doubtful. Our laboratory has undertaken a continuing investigation of the Viking LR data with the goal of assessing whether or not the LR Mars results could have been caused by inorganic reactions between the LR nutrient solution and the Mars surface material.

The flight data (Levin and Straat, 1976b, 1977b) show that, upon injection of the radioactive nutrient onto samples of Mars soil, a rapid evolution of ^{14}C-labeled gas occurred for several sols (1 sol = 24.6 h). When duplicate samples of the Mars soil were heated to 160ºC prior to conducting the experiment, no significant evolution of gas occurred after nutrient injection (Levin and Straat, 1976b, 1977b, 1979a). To date, laboratory simulation experiments seeking to duplicate these results chemically or physically have not succeeded (Levin and Straat, 1977a, 1979b, in prep.). However, a second component of the LR experiment also requires explanation for an overall understanding of the nature of the surface of Mars. This component of the active Mars response was seen after 7 sols when a second nutrient injection was added to the test cell. A brief spike of radioactivity was observed followed immediately by a 30-35% decrease (calculated from the apex of the spike) in the amount of radioactive gas present in the headspace. This response is unique and has never been observed in tests with variable or sterile terrestrial soils. This paper presents laboratory evidence for a physico-chemical explanation of this portion of the LR data.

To examine the second component of the LR response, the LR Test Standards Module (TSM, a flight-like laboratory instrument in which Mars atmospheric and experimental conditions can be closely simulated) was adapted to accommodate separate gas injections into the experimental test cell. After equilibration of a radioactive gas in the test cell with or without soil, water can be injected through the nutrient injection port (S/45) using the flight injection sequence. Because the liquid injection uses water rather than radioactive nutrient, no observable metabolic activity results from the liquid injection and the gas/water/soil equilibrium can be studied independently of biology.

The radioactive gas evolved in the LR experiment on Mars could be a carbon gas other than carbon dioxide; Plumb (1978) has suggested on theoretical grounds that the gas may in fact be carbon monoxide. To distinguish experimentally between carbon dioxide and carbon monoxide as possible end products, studies have been performed in the modified TSM with both ^{14}C-labeled carbon monoxide and ^{14}C-labeled carbon dioxide. The responses of each gas to liquid injections and to temperature cycle patterns similar to those observed in the LR test cell during the Mars mission were examined for possible matches to flight data. Evidence is presented that the ^{14}C-labeled gas evolved on Mars is more likely carbon dioxide than carbon monoxide.

Experimental

Test Standards Module (TSM). The LR TSM and its similarity to the flight instrument have been diagrammed and described in detail elsewhere (Levin and Straat, 1976a). In summary, the instrument consists of a 3.5 cc capacity stainless steel test cell connected via a 33 cm long 0.2 cm i.d. diameter 'swan neck' tube to a detector cell containing two solid state beta detectors. The total volume of the test cell detector assembly is approximately 8.5 cc and gas equilibrium between them is established within approximately 60 min. The labeled gas contained within this volume is counted with an efficiency of approximately 3%. Liquid (nutrient or water) is stored in an adjacent reservoir connected to the test cell by 0.1 cm i.d. diameter tubing. Upon appropriate opening of the solonoid valves separating the reservoir from the test cell, 0.115 cc liquid is introduced into the test cell. For the studies reported herein, the TSM was adapted to accommodate an injection of radioactive gas into the test cell through a separate injection port (located between S/52 and test cell).

Specific Radioactivity of Injected Gas. In the flight experiment, the radioactive gas which evolves following initial injection could only be derived from the supplied radioactive nutrient. In the 'active' test cycles, some 10,000-15,000 cpms evolved, corresponding to approximately 30 nanomoles of a one-carbon gas which mixed with the Martian atmosphere in the headspace of the detector and test cell assembly. Since the headspace volume is 8.25 cc, allowing for the 0.5 cc volume of soil, and since the Mars atmospheric pressure and composition are approximately 5 torr of carbon dioxide, approximately 2,500 nanomoles of unlabeled carbon dioxide were also present in the test cell. Assuming the end product of the Mars reaction is carbon dioxide, this means that at plateau, approximately 2,530 nanomoles were present and that the specific radioactivity of the evolved gas has been considerably diluted. Thus, the 30-35% decrease observed during the mission following second injection corresponds to an absorption of approximately 850 nanomoles of carbon dioxide, or 10 nanomoles if the gas evolved in the LR experiment is other than carbon dioxide.

In attempting to equate the TSM studies to these flight conditions, the radioactive gases utilized were selected to provide a count level of approximately 10,000 cpm at approximately 5−8 torr and 2,500−3,500 nanomoles, depending on the specific radioactivity of available commercial gases. The gas injection volume was 100 µl and the specific radioactivities of $^{14}CO_2$ (International Chemical and Nuclear Corp.) and ^{14}CO (New England Nuclear) were 0.041 and 0.0205 mCi/nmole, respectively.

Mars Analog Soils. The two Mars analog soils were prepared by the Viking Inorganic Analysis Team to match, as closely as possible, the analysis of the Mars sample obtained by the Viking x-ray fluorescence instrument. The first analog soil prepared, called Mars analog No. 1, was based on the tentative analysis of initial data from Chryse (the first landing site on Mars). As the Mars data became more refined, the second analog, called B2, was prepared by that Team. The composition of both soils (A. Baird, personal communication) is shown in Table I; both contain approximately 18% ferric oxide, have a pH of 7.2, and range in particle size from 10−100 µ. The most significant difference between them is that the iron in the B2 soil is approximately 11% gamma Fe_2O_3, a compound believed to be present on Mars (Hargraves et al., 1977) and hypothesized (Oyama et al., 1976) to be involved in the LR reaction. Prior to the addition of each test analog soil to the TSM, the soil was dried extensively (5 to 6 days) in a dessicator jar by pulling a vacuum through a cold trap; it was then sealed for a minimum of 3 days under one atmosphere of either nitrogen or carbon dioxide gas, as indicated.

Table 1. Chemical Composition of Mars Analog Soils No. 1 and B2 as Weight Percent[a]

A Mineral	No. 1	B2
Nontronite[b]	51.1	17.5
Bentonite[b]	25.5	29.8
Kieserite ($MgSO_4$)	9.4	18.5
Quartz (SiO_2)	–	14.4
Calcite ($CaCO_3$)	6.0	7.0
Maghemite (γ-Fe_2O_3)	–	11.1
Hematite (α-Fe_2O_3)	5.0	–
Magnetite(Fe_3O_4)	3.0	–
Rutile (TiO_2)	–	1.0
Halite (NaCl)	–	0.8

Nontronite is a naturally occurring clay mineral from a Riverside, California quarry, bentonite is a commercial grade material from Wyoming, maghemite was obtained from Memorex Corporation and is certified as pure gamma iron oxide. All other minerals are reagent grade chemicals.

Soils were prepared by mixing these materials for 24 h in homogenizing roller mills in proportions which yield X-ray-spectrographic results that closely approximate spectra obtained from Mars in flight-like Viking instruments.

B Oxide	No. 1	B2	Bentonite	Nontronite
SiO_2	39.5	42.2	63.5	50.5

Al_2O_3	7.0	5.9	17.3	4.0
Fe_2O_3	21.7	17.0	2.7	28.9
MgO	7.3	7.0	1.5	2.2
CaO	7.0	5.7	1.4	7.7
Na_2O	0.5	0.6	1.7	0.5
K_2O	0.2	0.2	0.6	0.1
TiO_2	0.3	1.1	0.2	0.5
P_2O_5	0.02	0.1	0.1	0.2
SO_3	5.5	12.5	0	0
Cl	–	0.5	0	0
CO_2	2.6	3.0	0	0

Values for Bentonite and Nontronite represent chemical analyses whereas values for Mars analog soils No. 1 and B2 have been calculated from the composition of the constituent minerals assuming stoichiometry of constituents. By convention, chemical analyses are reported as oxides, although the elements are not necessarily present as oxides. Total iron is reported as Fe_2O_3 but also includes Fe_3O_4. For each sample, the remaining weight is primarily water

[a] Courtesy of A.K. Baird, Viking Inorganic Analysis Team
[b] Chemical analyses presented below

Experimental Sequences. Experiments were conducted at 10ºC in the presence and absence of Mars analog soil in the test cell. When soil was to be present, an ampoule containing 0.5 cc of the prepared Mars analog soil was broken near the test cell and the soil added under a stream of dry carbon dioxide. After equilibrating in the sealed test cell at 10ºC and 5 torr carbon dioxide for 2.5 h, if desired, the soil was then sterilized according to the flight sequence by raising the temperature in the test cell to 160ºC for 3 h. After cooling for 9 h at room temperature, the test cell was vented for four minutes and the soil then incubated at 10ºC, the temperature of the Mars experiments, for an additional 1.5 h.

All experiments were initiated by the injection of 100 µl radioactive gas into the test cell which provided a total test cell pressure of approximately 7.5 torr. Subsequent injections of sterile distilled water were performed using the flight nutrient injection sequence (Levin and Straat, 1976a) in which the test cell was pressurized by two sequential additions of 18 torr helium prior to injection of 0.115 cc water and 18 torr helium. At the end of the injection, the total test cell pressure was approximately 69 torr, including 10 torr from the vapor pressure of water. At this point, the test cell liquid and gas contents became equivalent to that of a flight test cell following first nutrient injection. Addition of a second 0.115 cc water injection into the TSM test cell was performed as during the Viking mission without the two preceding helium pressurizations, but accompanied by 18 torr helium, giving a total test cell pressure of 87 torr. In some instances, helium pressurization was performed in the absence of a water injection for a total pressure increase of approximately 18 torr per pressurization.

Results and Discussion

Typical results obtained upon injection of ^{14}C-labeled carbon dioxide into the empty test cell are shown in Fig. 1. The magnitude of the gas level is approximately 9 ,500 cpm and was shown in a total of 14 runs to be highly repeatable with a range of only ±4%. Equilibrium between the test cell and the detector cell was reached within about 1 h. A later injection of water into the test cell resulted in a spike of radioactivity followed by a decrease to a new plateau level somewhat lower than that observed prior to the injection. A second water injection also caused a brief spike and also lowered the plateau level slightly below that observed prior to the second injection. These results show that physico-chemical changes in the observed gas plateau levels can be induced by injections of water and suggest that the changes relate to the carbon dioxide/water equilibrium.

With Mars analog soil No. 1 present in the test cell, injected ^{14}C-labeled carbon dioxide immediately began to disappear, reaching equilibrium with the soil at approximately 50 h (Fig. 1). This decrease probably resulted from an exchange between injected $^{14}CO_2$ and the gases contained within the soil matrix. In support of this hypothesis, more $^{14}CO_2$ (app. 85%) was adsorbed within 10 h by a soil pre-equilibrated with nitrogen than by a soil pre-equilibrated with carbon dioxide (50-60%). It should be noted that two runs were performed with soil pre-equilibrated with carbon dioxide because a leak was suspected in one of the run. When corrections are made for the estimated leak rate, the results of both runs are similar. For soil pre-equilibrated with carbon dioxide, sterilization in the test cell for three h at 160ºC did not affect the results.

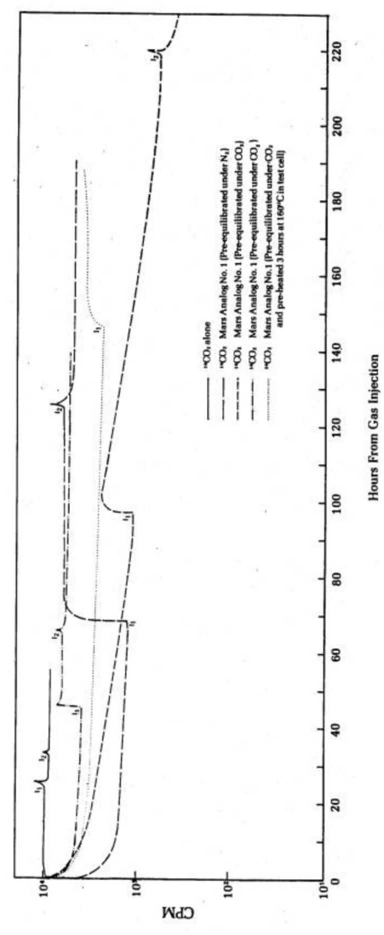


Fig. 1. Effects of water injections on $^{14}CO_2$ equilibrium in presence and absence of Mars analog soil No. 1. Mars analog soil was prepared by desiccating for 5 days and then sealing under either nitrogen or unlabeled carbon dioxide. Soil was then added to the TSM test cell and, if desired, heat sterilized for 3 h at 160ºC according to the flight sequence. Radioactive carbon dioxide (specific radioactivity = 0.041mCi/mmole) was then injected into the TSM test cell in the presence and absence of 0.5 cc Mars analog soil No. 1, as indicated, to provide approximately 2900 nanomoles and 9500 cpm at the onset of each run. The resulting test cell pressure was approximately 7 torr. After equilibration, 0.115 cc of sterile water was added to the test cell by the flight injection sequence. Injections (I_x) are indicated for each run, with x indicating the xth injection into the test cell during the run. Data from one of the duplicate runs with Mars analog soil No. 1 pre-equilibrated with CO_2 (—) has not been corrected for an apparent test cell leak

Upon injection of water into the test cell containing $^{14}CO_2$ in equilibrium with Mars analog soil No. 1, all soil samples released carbon dioxide such that the gas level in the headspace attained a new plateau level (Fig.1). In the case of soil of pre-equilibrated nitrogen, the new level was almost equal to that observed when radioactive gas was originally injected. In the case of the soil pre-equilibrated with carbon dioxide, the new level was above that prior to the water injection but not as high as the original level following injection of gas into the test cell. However, the new level cannot be precisely determined because the extent of dilution with unlabeled gas which had been adsorbed on the soil during pre-equilibration is unknown. Thus, the observed level is probably below that which would have been observed had no dilution occurred. Upon a second injection of water, all samples exhibited a brief spike followed by a decrease such that the final plateau level was below that observed prior to injection. The percent decrease (calculated from the apex of the spike) is 27, 42, and 50% for the three runs with Mars analog soil, comparable to the 30-35% decreases observed in LR flight data.

These results suggest that the decreases observed on flight following second nutrient injection could be due solely to physical equilibrium phenomenon related to the carbon dioxide-water equilibrium rather than to a biological or chemical reaction with the LR nutrient. Comparing the results in the presence and absence of soil, it can be seen that soil magnifies the effect, but changes are apparent even in the absence of soil. In addition, because an outgassing rather than a decrease is observed in the TSM studies following the first water injection onto soil, the results also suggest that the Viking LR results after first injection probably need not be corrected for a masked reaction involving adsorption of radioactive gas simultaneous with its evolution. (Note that in the TSM experiments, $^{14}CO_2$ evolution following first water injection is not equivalent to $^{14}CO_2$ evolution following the first nutrient injection on flight. In the TSM experiments, gas evolution results from an outgassing whereas on flight it must result from a biological or chemical reaction between the LR nutrient and the Mars soil.)

Two additional TSM experiments were conducted in which water was injected into the TSM test cell containing $^{14}CO_2$ in equilibrium with Mars analog soil B2. The results of duplicate runs are compared in Fig. 2 to results obtained in the presence and absence of Mars analog soil No. 1. As shown, with the B2 soil, both water injections resulted in a brief spike followed by an immediate decrease (ranging from 11-33%) in plateau level. Superimposed on this reaction, however, is a gradual adsorption of radioactive gas by the soil. The rate of adsorption is repeatable in both experiments and no test cell leak could be discerned in either. Thus, while the B2 soil containing γ-Fe_2O_3 amplifies changes in $^{14}CO_2$ levels, the changes differ from those obtained with the Mars analog soil No. 1.

A similar series of TSM experiments was next conducted with ^{14}C-labeled carbon monoxide (Fig. 3). After injection of water into the TSM test cell containing ^{14}CO (no soil), a brief spike is seen followed by a return of the gas level to essentially the same level observed prior to injection. The addition of Mars analog soil No. 1 has no impact on these results. The failure of ^{14}CO to show changes in plateau level in response to water injections suggests that carbon monoxide was not the gas evolved on Mars.

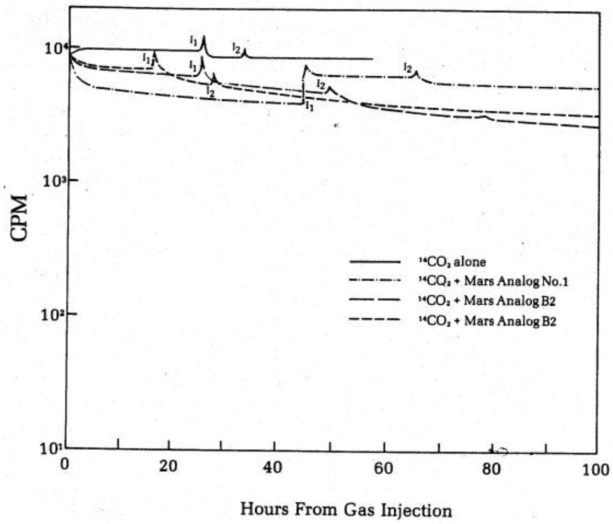
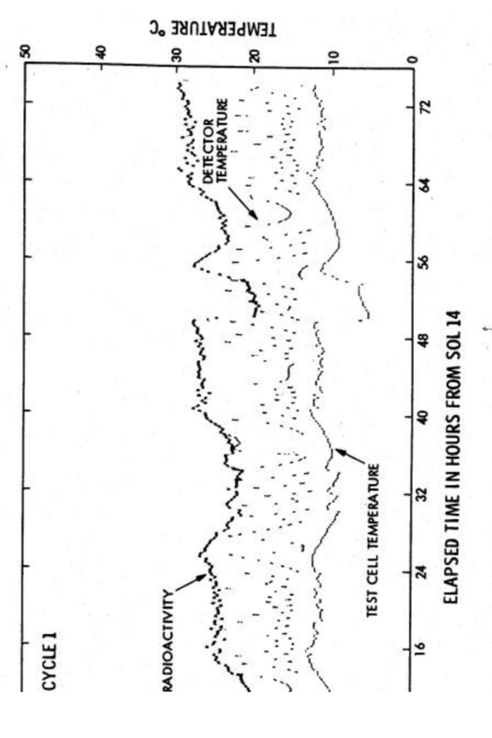


Fig. 2. Effects of water injections on $^{14}CO_2$ equilibrium in presence and absence of Mars analog soils. Mars analog soils were prepared by desiccating for 6 days, sealing under unlabeled carbon dioxide, and adding to the TSM test cell. Radioactive carbon dioxide (specific radioactivity = 0.041mCi/mmole) was then injected into the TSM test cell in the presence and absence of 0.5cc Mars analog soil No. 1 or B2, as indicated, to provide approximately 2900 nanomoles and 9500 cpm in each run. The resulting test cell pressure was approximately 7 torr. After equilibration, 0.115 cc of sterile water was added to the test cell by the flight sequence. Injections (I_x) are indicated for each run where x indicates the x^{th} injection into the test cell during the run

During the LR flight experiments, levels of radioactive gas present in the test cell headspace were observed to undergo a diurnal fluctuation. A detailed kinetic study has revealed that these fluctuations directly correlate with fluctuations in the test cell temperature (Fig. 4). To determine the influence of temperature on levels of $^{14}CO_2$ or ^{14}CO present at the end of the experiments shown in Fig. 1-3, the test cell temperature was cycled between 9ºC and 16ºC in a pattern approximating that on flight. With ^{14}CO present, no changes in gas level subsequently occurred either in the presence or absence of Mars analog soil. With $^{14}CO_2$ present, fluctuations in the gas levels in the detector cell were observed in the presence of both Mars analog soils but not in the absence of soil. These results eliminate the possibility that the gas fluctuations on flight were caused by temperature-driven gas distributions between the test cell and the detector cell since both gases would have been affected equally in the TSM experiments if this were the case. Rather, the temperature driven fluctuations in gas level appear related to changes in the gas/water/soil equilibrium in the test cell. The fact that they occur with $^{14}CO_2$ and not with ^{14}CO supports the conclusion that the end product of the reaction on Mars is not carbon monoxide and that it is probably carbon dioxide. It should be noted, however, that while the temperature-driven fluctuations in the TSM are similar in pattern to those in flight data, they are only about one-third the magnitude seen on flight.



fluctuations in radioactivity and in temperature. These flight data present observed radioactivity, test cell temperature over a 72 h period. Data are selected from Lander 1, cycle 1 during Sols 14 through 16

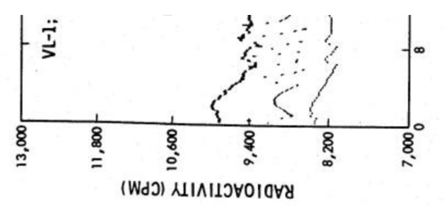


Fig. 4. Relationship between temperature and detector tem

The possibility was next examined that the spike associated with each injection is caused by a pressure surge since all liquid injections into the test cell are pressurized under helium (see Experimental). To test this, helium only (no liquid) was injected into a test cell containing $^{14}CO_2$. The results are compared in Fig. 5 to effects of water injections added to the TSM test cell containing either $^{14}CO_2$ or ^{14}CO. As shown, injection of helium alone provides a spike comparable to that obtained with a liquid injection. Further, the magnitude of the effect is independent of the type of gas present.

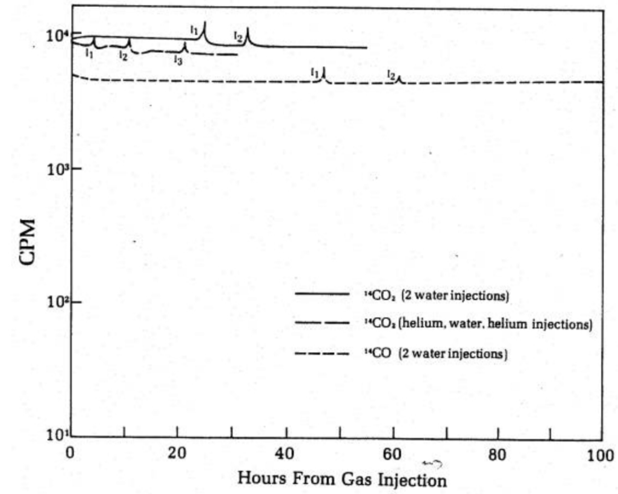


Fig. 5. Effects of helium versus water injections into test cell containing either $^{14}CO_2$ or ^{14}CO. Radioactive carbon dioxide (specific radioactivity = 0.041mCi/mmole) or carbon monoxide (specific radioactivity = 0.021mCi/mmole) was injected into the TSM test cell to provide approximately 3000 nanomoles and 9500 or 5000 cpm, respectively. The resulting test cell pressure was approximately 7 torr. After equilibrium, injections of either helium or 0.115 cc sterile water, as indicated, were added to the test cell by the flight injection sequence. Injections (I_x) are indicated for each run where x indicates the x^{th} injection into the test cell during the run

A summary of percent increase observed for each of the spikes in Fig. 5 is given in Table 2 along with similar data derived from other TSM experiments in which Mars analog soil was present with the labeled gas. In each case, a higher percent increase is observed for the first injection than for a second or third injection during a given run. This correlates with the higher percent pressure increase accompanying a first injection. The results suggest that test cell pressurization with helium causes a transient surge of radioactive gas into the detector chamber, thereby causing the observed spike. Further, all TSM spikes resulting from second water injections are similar in magnitude to the transient spikes observed during the Viking mission after second (but not first) nutrient injections. Thus, the flight spike following second injection appears to result from a pressure surge rather than from additional reactions between the LR nutrient and the Mars soil.

Table 2. Effect of injection into LR test cell on level of ^{14}C-gas

Test cell contents	Instrument	Type injection	Percent increase
$^{14}CO_2$	TSM	1 - Helium	44
		2 - H_2O	20
		3 - Helium	17
$^{14}CO_2$	TSM	1 - H_2O	32
		2 - H_2O	14
^{14}CO	TSM	1 - H_2O	26
		2 - H_2O	17
$^{14}CO_2$, Mars analog soil No. 1	TSM	1 - H_2O	83
		2 - H_2O	14
$^{14}CO_2$, Mars analog soil B2	TSM	1 - H_2O	49
		2 - H_2O	16
^{14}CO, Mars analog soil No. 1	TSM	1 - H_2O	33
		2 - H_2O	12
Mars soil, ^{14}C-gas	Lander 1, cycle 1	2 - VM1	15
	Lander 1, cycle 3	2 - VM1	8
	Lander 1, cycle 3	3 - VM1	5
	Lander 2, cycle 1	2 - VM1	15
	Lander 2, cycle 3	2 - VM1	14

Injections of either water, helium, or LR nutrient (VM1) were added into the LR test cell on either a Viking Lander or the TSM, as indicated. Each injection is identified as the 1st, 2nd, or 3rd injection in the indicated run. Prior to injection, the test cell pressure is approximately 5 torr. First injections of liquid increase the pressure by 64 torr whereas second and third liquid injections increase the pressure by an additional 18 torr each. Helium injections alone increase the pressure by 36 torr. The observed sharp increase in detected radioactivity following each injection is given relative to the preceding plateau level

In conclusion, that part of the LR flight data observed following second nutrient injection can be accounted for by physico-chemical explanations. Further, a carbon dioxide end-product for the Mars reaction can account for the observed changes in plateau level accompanying liquid injections and for the temperature driven diurnal fluctuations in gas level. However, it is of interest to note that the reaction with Mars analog soil No. 1 more closely simulates flight data than that with Mars analog soil B2. Future laboratory simulation experiments seeking chemical explanations of the LR flight data will focus on that part of the flight response following first nutrient injection.

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