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I. SUMMARY

The Viking Labeled Release (LR) life detection experiment on the surface of Mars has produced data consistent with a biological interpretation. In considering the plausibility of this interpretation, terrestrial life forms have been identified which could serve as models for Martian microbial life. Prominent among these models are lichens which are known to survive for years in a state of cryptobiosis, to grow in hostile polar environments, to exist on atmospheric nitrogen as sole nitrogen source, and to survive without liquid water by absorbing water directly from the atmosphere. Another model is derived from the endolithic bacteria found in the dry Antarctic valleys; preliminary experiments conducted with samples of these bacteria indicate that they produce positive LR responses approximating the Mars results.

However, because of the hostility of the Martian environment to life as we know it and the failure to find organics on the surface of Mars, a number of nonbiological explanations have been advanced to account for the Viking LR data. A reaction of the LR nutrient with putative surface hydrogen peroxide is the leading candidate. Other possibilities raised include reactions caused by or with ultraviolet irradiation, $\gamma$-Fe$_2$O$_3$, metalloperoxides or superoxides.
In our continuing effort to account for the LR flight data, we have this year performed extensive kinetic and thermal analyses of the flight data and conducted laboratory simulation experiments testing various chemical theories. Kinetic analyses have revealed that the positive flight response is composed of several separate reactions. Following first nutrient injection onto Mars soil, $^{14}C$-gas evolution occurs at a rapid rate which, in several days, approaches a plateau value. This evolution is ascribed to an initial outgassing from the nutrient, a reaction with a thermolabile agent in the Mars soil and a low level, constant reaction with a thermostabile component. Upon second nutrient injection, a brief spike is followed by rapid gas absorption and then by slow, linear gas evolution.

Data from the final two Viking LR cycles have been analyzed in detail and found to be consistent with, but not proof of, a biological interpretation. Each of the final two active cycles tested a surface sample that had been stored for several months at approximately $10^\circ$C. At each lander site, the resultant response was strongly diminished. At the end of one of these cycles, the incubation mixture was heated to $50^\circ$C to release any radioactive gas trapped in the sample matrix. These results suggest that more than one carbon substrate is involved in the LR reaction on Mars.

The thermal data from the stored samples, coupled with data from previous cycles, have formed the basis for evaluation of the thermal decomposition of the Mars active agent. The
The slope of the resulting Arrhenius plot has been used to test the fit of other flight data and to calculate the activation energy for thermal decomposition of the Mars agent. The results of the thermal analysis indicate that hydrogen peroxide cannot account for the flight data unless a catalyst significantly increases its thermal sensitivity.

Laboratory simulation experiments have tested the abilities of various nonbiological agents to yield the Mars LR response. Our results indicate that ultraviolet irradiation, $\gamma$-Fe$_2$O$_3$, and metalloperoxides and superoxides cannot account for the thermolabile active response following first nutrient injection. Further, $\gamma$-Fe$_2$O$_3$ cannot account for the thermally stable, linear, slow gas evolution accompanying the thermolabile reaction. However, $10^{-1}$ M hydrogen peroxide produces an active response in the Test Standards Module (TSM) strikingly similar to that obtained on Mars. Further getter-type laboratory experiments show that hydrogen peroxide reacts with all substrates contained in VM1, albeit at different rates. The magnitude of the reaction with VM1 is highly dependent on the hydrogen peroxide concentration with evolved $^{14}$CO$_2$ approximately doubling for each ten-fold increase in hydrogen peroxide concentration. Catalytic amounts of $\gamma$-Fe$_2$O$_3$ significantly (app. 5-fold) stimulate the reaction.
Thermal studies indicate that hydrogen peroxide is decomposed by heating for three hours at 160°C but not at 50°C unless a metal catalyst (e.g., $\gamma$-Fe$_2$O$_3$) is present. However, in the TSM, hydrogen peroxide is completely inactivated by three hours heating at 50°C. This may be caused by volatilization rather than decomposition removing the hydrogen peroxide from the reaction site to another part of the instrument and, therefore, presumably should also have occurred in the flight instrument on Mars. Further, studies in which hydrogen peroxide was added to certain candidate analog soils indicate that hydrogen peroxide is not stable on fine particulate matter typical of the Mars surface. Thus, in order for hydrogen peroxide to account for flight data, some complex with the Mars soil must be postulated to prevent volatilization in the flight instrument, to enhance thermal instability at 50°C, and to stabilize hydrogen peroxide on fine particulates and against photolysis by UV.

Additional simulation experiments have sought an explanation for the evolved gas spike and absorption phenomenon following second nutrient injection. These studies have been performed in the LR TSM by injecting $^{14}$C-labeled gas into the test cell in the presence and absence of Mars analog soil. After equilibration between the injected gas and the test cell, water was injected and the effect on the gas content
in the headspace 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 LR reaction on Mars is more likely carbon dioxide than carbon monoxide.

In conclusion, although the results following second nutrient injection can be explained by physico-chemical phenomenon, the results for the thermolabile reaction following first nutrient injection leave unresolved the question of whether the Mars LR data were generated by biological or chemical activity. Hydrogen peroxide mimicks the active response obtained on Mars but the stability of hydrogen peroxide to thermal treatment and to the Mars environmental conditions appears inconsistent with this theory. However, it is possible that complexes between the surface material and hydrogen peroxide may exist on Mars which could preserve hydrogen peroxide. It is recommended that further laboratory efforts explore the possible generation of such complexes and the determination of their stability relative to that of the active agent on Mars.
II. INTRODUCTION

The Viking Labeled Release (LR) life detection experiment monitors radioactive gas evolution following the addition of a radioactive nutrient containing $^{14}$C-labeled organic substrates to a sample of the Mars surface material (l). A positive metabolic response is defined by evolution of radioactive gas from a surface sample tested in an "active" cycle; and little or no evolution from a duplicate "control" cycle sample pre-heated for 3 hours at $160^\circ$C (the regimen selected to destroy or inhibit any life forms present). Extensive testing of terrestrial soils has shown these criteria to be highly reliable.

The results (2-4) obtained from such "active" and "control" cycles conducted on Mars during the Viking Mission did satisfy the life detection criteria established for the LR experiment. However, the hostile Martian environment with its improbable availability of liquid water, cold temperatures, and intense ultraviolet flux has led to a variety of speculations (2,3,5) that exotic chemical or physical reactions, instead of life, may have caused the LR positive responses on Mars. This report summarizes possible biological and chemical models of the LR flight results and presents our experimental and analytical efforts to interpret the flight data.
III. BIOLOGICAL AND CHEMICAL MODELS OF THE LR MARS RESULTS

While many chemical simulations of the LR Mars data have been attempted in our laboratory and elsewhere, we know of none that has succeeded in reproducing the active and control cycles and the additional thermal data obtained by the LR instrument on Mars. These efforts continue, but it is appropriate to consider the possible life that may exist on the surface of Mars. Biological models for Mars organisms must consider the extant knowledge about the Martian environmental conditions. Any terrestrial organisms known to survive conditions approaching those on Mars could serve as analogs.

The two Martian environmental conditions that have in the past been considered limiting for the existence of life were the absence of atmospheric nitrogen (6-8) and the extreme aridity of the planet (9). Since the Viking discovery that nitrogen is present in the atmosphere (10), water availability has become the sole critical factor. The large quantities of liquid water (11) that were apparently present at some time during the Martian geological past (11) suggest that life could have evolved on Mars. If the Martian habitat became devoid of liquid water, even transitorily (12), biological adaptations would have been required to permit utilization of either ice or water vapor as sole water sources if life were to continue. Possible adaptive mechanisms may have included the ability to withstand desiccation for sustained periods of time.
Among terrestrial life forms, several categories exist which can serve as models for putative Mars organisms and indicate that the adaptations required on Mars may have occurred through evolutionary processes. For example, terrestrial organisms are known which can enter a cryptobiotic state, a phenomenon in which certain bacteria and invertebrates (Rotifera, Nematoda, and Tardigrada) survive desiccation in a state of suspended animation (13). Other organisms that exhibit cryptobiosis during the early stages of development include spores of bacteria and fungi, seeds of higher plants, larvae of certain insects, and cysts of some crustaceans.

Cryptobiosis greatly extends the normal life-span and organisms in the cryptobiotic state exhibit resistance to environmental extremes such as those of Mars (pressure, temperature, low oxygen tension, etc.). Metabolic processes in cryptobiotic organisms are not readily detectable; however, the addition of liquid water results in revival (i.e., rapid resumption of detectable metabolic processes) of organisms stored as long as 120 years (13). The limits to the length of the storage period before irreversible changes destroy the ability to resume normal metabolic processes are not known.

Lichens, sometimes proposed as candidates for extraterrestrial life, can undergo slowing of metabolic processes and can develop a latent state (i.e., cryptobiosis) upon dehydration. Lichens are unique among organisms which undergo cryptobiosis in that
they are able to obtain water by uptake from water vapor alone (14-16). Minimum water content of lichens is generally low, ranging from 2-9% of the dry weight (14) and activity and growth have been found in lichens even without imbibition of liquid water (17). Cuthbert (18) reported measurable respiration in *Teloschistes flavicans* when only 0.4% of its maximum water content was present. Lange (15) reported that the desert lichen *Ramalina maciformis* absorbed liquid solely by uptake of water vapor while Smith (14) found indications that for some unwettable lichens, atmospheric water vapor was the only moisture source utilized. Similarly, Lange et al (16) reported that lichens in their natural habitat are able to survive without liquid water by absorbing water directly from an atmosphere containing large amounts of water vapor at night. It has been postulated (19) that this uptake of atmospheric water vapor may be facilitated by tiny crystals or the surface crust of some lichens that act as condensation nuclei.

Lichens are found in terrestrial environments ranging from polar regions to the equator, with some species living exclusively at high altitudes (20). Lichens are gray or brown in color when dry, with many species colored by incrustations of a specific lichen pigment in the cortex. Green and yellow tints occur commonly. Laudi et al (21) have speculated that these pigments may afford protection against irradiation. In addition, the prunia (a layer of crystals or dust covering the thalli and apothecia) has been interpreted as protective against insolation (22). Primitive crustose lichens form crusts firmly attached
to the substrate (usually rock) and are more resistant to unfavorable conditions than other members of the lichen family. Other lichen adaptations of interest include the ability of some lichens to exist on atmospheric nitrogen as sole nitrogen source while others exhibit resistance to temperature extremes, especially when desiccated. In addition, the resistance of phycobionts to strong illumination rapidly increases as desiccation proceeds (23).

Other terrestrial models for Martian life forms can be sought in the dry valleys of the Antarctic where the combination of extreme drought and cold temperatures constitute the most Mars-like conditions known on Earth (24). These valleys were previously believed (25) to be devoid of indigenous life until Vishniac and Mainzer (26,27) found indigenous soil microflora by in situ microbiological methods. Positive but low metabolic responses were also obtained when these in situ Antarctic samples were tested by the Labeled Release technique using Viking LR nutrient (28). More recently, several microbes, including endolithic unicellular blue-green algae, fungi, and bacteria, have been reported (24,29) to exist several millimeters beneath the surface of Antarctic rocks. It has been suggested (24) that during periods of extreme aridity, these organisms are in a cryptobiotic state and that metabolic activity is resumed when sufficient water becomes available. Although there is scant
precipitation and water generally sublimes in the Antarctic, some moisture traces resulting from infrequent snowfalls could be absorbed by porous rocks.

The recent finding of an ecological niche within rocks in an area of the Antarctic otherwise thought to be sterile provides an interesting new model for Mars. Experiments conducted in collaboration with Drs. E.I. and O.C. Friedmann have tested whether an LR signal could be obtained from such Antarctic samples. In these studies, 0.1 ml of VM1 (flight nutrient containing uniformly $^{14}$C-labeled glycine, DL alanine, DL lactate, glycolate, and formate) was added to scrapings from the endolithic band of a recently collected sample from Antarctica. The planchet-type LR experiment was run at room temperature and evolved $^{14}$CO$_2$ was monitored by gettering with Ba(OH)$_2$. The results in Figures 1 and 2 show that the radioactive gas evolved from an active sample is an order of magnitude above that obtained from a duplicate sample pretreated for 1 hour at 200°C. This difference constitutes a positive LR response. The magnitude of the response from the active sample is similar to that obtained from an active Mars sample during the Viking mission (after normalizing the data sets to the same counting efficiency) although the kinetics of gas evolution differ somewhat. When compared to activity derived from a local Maryland soil, it can be seen that the response from the Antarctic sample is relatively weak (Figure 3), possibly reflecting the relative
Endolithic rock samples (courtesy of Drs. E. I. and O. C. Friedmann) were obtained from a band of microorganisms 10 mm below the surface of Antarctic rocks. Into each of two planchets was added 0.24g of shavings from this band. One sample was then heat sterilized for 1 hour at 200°C prior to the addition of 0.1 ml VM1 (flight nutrient containing 14C-labeled organics) to each. Evolved 14CO2 was monitored by the getter technique and compared to that evolved from nutrient alone. The results are also compared to those obtained from LR Cycle 1 on Viking Lander 2 after multiplying the flight data by a factor of three to equate counting efficiency of the two systems. All data are corrected for background.
The data shown here have been replotted from Figure 1 on a linear scale.
An endolithic rock sample (courtesy of Drs. E. I. and O. C. Friedmann) was obtained from a band of microorganisms 10 mm below the surface of an Antarctic rock. Radio-respirometric activity was tested by the addition of 0.1 ml of VM1 (flight nutrient containing $^{14}$C-labeled organics) to 0.5g of shavings from the endolithic band or to local Biospherics soil. Local soil was tested with and without prior heat sterilization for one hour at 200°C. Evolved $^{14}$CO$_2$ was monitored by the getter technique and compared to that evolved from nutrient alone. All data are corrected for background.
numbers of microorganisms in each sample. These results, coupled with the discovery of microbial growth in Antarctic rock interiors, lend some credibility to a biological interpretation for the positive LR response in Mars samples by virtue of illustrating that a good terrestrial model exists that produces Mars-like LR signals. In this regard, our recent report (30) of greenish patches on some rocks near Viking Lander 1 is of considerable interest.

On the other hand, the hostile Martian environment and the failure to find organic compounds on the surface of the planet tend toward nonbiological explanations. However, to date, no chemical theory has been proposed and tested to account for all the LR experimental results obtained on Mars. A summary of the various nonbiological explanations postulated to account for the primary LR response is given below:

1. Because the LR reaction appeared to be first order and was initially thought to involve only one substrate, a reaction between formate and hydrogen peroxide was put forth as the source of the LR response (31). Hydrogen peroxide photochemically produced in the Mars atmosphere might precipitate out on the Mars surface (32) and thus be available for reaction with the LR nutrient. Alternately, Hugenin (33) has proposed that hydrogen peroxide forms on freshly weathered surface particulates in the presence of ultraviolet radiation or frost. Many
of the relative advantages and disadvantages of the hydrogen peroxide theories will be discussed later in this report.

2. Surface oxidants such as metalloperoxides, superoxides, or ozonides have also been advanced to account for both GEx and LR results. While the explanations appear plausible for GEx, difficulties with the theory for the primary LR response are that these oxidants do not possess the required thermal sensitivity (34) and that our laboratory simulations with calcium peroxide and superoxide do not replicate LR flight data.

3. The hypothesis has been advanced that the high ultraviolet flux could produce active physical states or highly oxidative compounds in the Mars surface capable of degrading one or more of the organic substrates comprising the LR nutrient. However, one flight test in which the Mars sample was obtained from under a rock gave comparable results to those obtained from a surface sample directly exposed to ambient irradiation (3). This indicates that the LR response does not depend on ultraviolet irradiation. In support, tests conducted in our laboratory (5) in which a Mars analog soil was irradiated with either ultraviolet or gamma irradiation did not replicate the LR Mars data. While gamma irradiation of silica gel did
produce LR responses comparable in magnitude to those obtained during the mission, neither the kinetics nor the thermal sensitivity matched flight data.

4. The possibility that $\gamma$-Fe$_2$O$_3$ is responsible for the oxidation of LR organics was postulated (35) following the discovery of red, magnetic surface components (36) which indicate the possible presence of $\gamma$-Fe$_2$O$_3$. Oyama demonstrated (37) the rapid evolution of CO$_2$ from a mixture of $\gamma$-Fe$_2$O$_3$, H$_2$O$_2$, and formate. The kinetics and magnitude of the reaction replicated LR flight data for the active sequence. However, the concentrations of reactants used in these experiments and the low pH of the reaction mixtures (38) suggest that this replication is fortuitous. Oyama also reported (37) a slow log-linear production of CO$_2$ when formate and $\gamma$-Fe$_2$O$_3$ were mixed in the absence of hydrogen peroxide. Such a reaction may account for the slow gas evolution observed in LR following the initial reaction.

5. It has recently been reported (39) that smectite clay minerals (such as montmorillonite and nontronite) containing iron as the exchangeable ion can catalyze CO$_2$ production both from formate and from the LR nutrient. The kinetics and magnitude of both reactions were identical to flight kinetics of the active sequence.
However, some question exists as to the sterility of the samples tested, leaving the possibility that the responses obtained in these studies were biological. Also, the fact that the iron clay catalyst did not display the temperature sensitivity of the Mars active agent tends to negate the hypothesis.

6. Rare metals known to produce CO$_2$ from formate include iridium, rhodium and rubidium (40,41). While the rare metal hypotheses have not to our knowledge been subjected to experimental testing, they appear unlikely. Iridium and rhodium were not detected on the Mars surface which is not surprising since their elemental cosmic abundances (42), based on the number of atoms per 10,000 atoms of silica, are given as 0.008 and 0.002, respectively. On Earth, these metals are generally found only alloyed with platinum. Rubidium is more universally distributed on Earth and has a cosmic abundance of 0.07 (42). This metal was found in the Mars sample at 30 ppm $\pm$ 30 ppm (37), which is at the lower end of the range generally observed on Earth. However, the principal difficulty with this hypothesis is the thermal stability of these metals.

7. Mills (43) has recently suggested that glow discharges generated by friction within dust clouds could explain many of the results observed in the Viking biology experiments. Glow discharges are effective scavengers
of even traces of organic matter from surfaces, particularly silicates, and could explain the apparent absence of carbonaceous material on the Mars surface. In support of this hypothesis, Ballou et al (44) have obtained some experimental data to account for GEx data. However, the hypothesis has not yet been tested for credibility to account for LR data.

8. Plumb (45) advanced the theory that a thermodynamically driven equilibrium reaction produced $^{14}$CO from the $^{14}$C-formate contained in the LR nutrient.

Of these nonbiological models, perhaps the leading candidate for the Mars active agent is hydrogen peroxide although metalloperoxides, superoxides, irradiation, and $\gamma$-Fe$_2$O$_3$ must also be considered further. Rare metals are not likely to be the active agent because of their thermal stability and their cosmic abundance. With regards to the gas evolved in the Mars LR reaction, both carbon dioxide and carbon monoxide must be examined for compatibility to the Mars data and for possible insight into the mechanism responsible for generation of the gaseous end product.
IV. ANALYSIS OF FLIGHT DATA

A. Kinetic Analysis of Components of the Mars LR Reaction

As demonstrated in Figure 4, several different kinetic components have been discerned (46) in the LR active reaction on Mars. Following the initial nutrient injection, one and possibly two first order reactions (#2a and #2b) occur concurrent with nutrient outgassing (Reaction #1) and with a slow linear gas evolution (Reaction #3). The contribution of Reaction #1 to the final plateau appears to be less than 500 cpm, typical of results from nutrient alone experiments routinely conducted in the LR Test Standards Module (TSM). The agent(s) responsible for Reaction(s) #2 is thermally labile whereas that responsible for Reaction #3 is thermally stable. Following the second nutrient injection, an initial spike (Reaction #4) is following by gas absorption (Reaction #5) and a subsequent slow linear gas evolution (Reaction #6). These latter three reactions are also thermally stable. The similarity of Reactions #3 and #6 suggests that a single agent is responsible for both reactions.

Our laboratory program has sought an explanation for each of these reactions. Of prime interest is the agent responsible for Reaction #2 because the thermal lability of this reaction is consistent with a biological explanation. Most of the experiments presented in the following sections are designed to test the fit of the various alternative chemical hypotheses presented in the previous section. Among these candidates, hydrogen peroxide
Figure 4
Components of Labeled Release Reaction on Mars

A) Overall LR Response

B) Component Reactions
1 - Nutrient Outgassing
2 - Thermally Sensitive First Order Reaction(s)
3 - Thermally Stable Linear Reaction
4 - Nutrient Outgassing and/or Pressure Spike
5 - Gas Adsorption
remains the most probable, although appropriate stability properties remain to be demonstrated. The other reactions, because of their thermal stability, probably reflect physico-chemical phenomenon. Experiments presented in the following sections indicate that Reaction #4 results from a pressure surge during injection whereas Reaction #5 probably results from a changed carbon dioxide water equilibrium that occurs in the LR wet mode. Reactions #3 and #6 could be caused by an inorganic catalyst, although simulation experiments presented below indicate that $\gamma$-Fe$_2$O$_3$ is not the causative agent.

B. Data from Final Two Viking LR Cycles

During this year, we have performed a detailed analysis of the data from the final two LR cycles conducted on Mars. Because of engineering constraints imposed on the lander sampling arms, these two cycles were conducted on Mars samples stored in the landers for several months at approximately 10°C. In each case, the LR response was strongly diminished. At the end of one cycle (i.e., VL-1, Cycle 4), the incubation mixture was heated to 50°C to release any radioactive gas trapped in the sample matrix. The results suggest that more than one carbon substrate is involved in the LR reaction. Detailed discussions of these data are presented below.
1. Viking Lander I, Cycle 4

The LR experiment planned for the fourth and final cycle at the Chryse landing site (VL-1) was injection of two nutrient "squirts" onto a fresh Mars sample. Previous results (3) suggested that only one of the seven $^{14}$C-labeled substrates was reacting with the Mars active agent and that this agent was inactivated or destroyed within seven sols (one Martian sol = 24 hours and forty minutes), as indicated by failure to produce a response upon addition of a second nutrient injection. The purpose of performing the double injection at the onset of Cycle 4 was to determine whether the $^{14}$C substrate or the Mars active agent was limiting the LR reaction. The time interval selected to space the two injections was three hours, sufficient to establish the magnitude of the first response, but short enough so that the second injection would occur during the vigorous portion of the initial reaction while the agent was still active.

A fresh sample of the Mars surface material was to be obtained for this experiment, but the engineers of the Surface Sampler Team expressed considerable concern that the sample arm might be damaged. Hence, the Biology Team chose to utilize the sample still stored in the hopper rather than chance not getting a fresh sample. The stored sample had been collected on Sol. 91 from the same "Sandy Flats" area that had supplied the Sol 8 and Sol 36 acquisitions which produced positive responses in Cycles
1 and 3, respectively. Between Sol 91 and the onset of Cycle 4 on Sol 230, the hopper sample had been stored in the dark, open to the Martian atmosphere, at temperatures fluctuating between 10-26°C with an average of 10-15°C. Data from previous cycles suggested that the Mars active agent was stable to such storage for at least five sols (see later). The ambient Mars surface temperature fluctuated between approximately a high of -1°C and a low of -78°C during the corresponding storage time (47).

The complete results for VL-1, Cycle 4 are presented in Figure 5. An expansion of the injection period is shown in Figure 6, and the overall response is compared to those from the other VL-1 cycles in Figure 7. As shown, the response seen in Cycle 4 following first injection was small compared to that seen in active Cycles 1 and 3. Following second injection, no stimulation in the rate of gas evolution was observed other than an initial spike typically seen upon second injection in other cycles. Gas evolution then proceeded continuously at a low rate for the duration of the cycle.

An estimate of the percent inhibition for Cycle 4 can be made by comparing the results with those obtained during comparable time intervals from VL-1, Cycle 1 and from VL-2, Cycle 1 (Table I). These active cycles were conducted on fresh samples and the magnitudes of their responses span the range of active Mars responses. At 2-½ hours following first injection, when all cycles had received only one injection, inhibition in the Cycle 4 response was between 89 and 93 percent. At 200 hours,
An active sequence was used on a sample stored at 10-26°C for 141 Sols prior to nutrient injection. A second nutrient injection was performed three hours after the first injection, as indicated. Radioactivity was measured at 16-minute intervals throughout the cycle except for the first two hours when readings were taken every 4 minutes. Radioactivity data include a background count of 730 cpm prior to the onset of the cycle. Detector and test cell temperatures were measured every 16 minutes.
Figure 6

Enlargement of Data from Double Injection in VL-1 Cycle 4

Plot of LR data from fourth sample analysis on VL-1 showing expansion of data from the first 6 hours after the initial nutrient injection. The time of the second injection is indicated. Radioactivity data have been corrected for a background count of 730 cpm prior to the onset of the cycle.
Evolved radioactivity following the first injection of radioactive nutrient to each analysis cycle of VL-1 is shown. A fresh sample was used for the active sequences of cycles 1 and 3 whereas the sample used for active cycle 4 was stored for approximately 141 Sols at 10-26°C prior to use. For cycle 2, a stored portion of the same sample used for cycle 1 was heated for 3 hours at 160°C prior to nutrient injection. All data have been corrected for background counts observed prior to nutrient injection.
### Table I

**Percent Inhibition from Stored Samples**

<table>
<thead>
<tr>
<th>Hours From 1st Injection</th>
<th>Cycle</th>
<th>No. Injections in Test Cell</th>
<th>CPM</th>
<th>Percent Inhibition</th>
</tr>
</thead>
<tbody>
<tr>
<td>2.37</td>
<td>VL-1, 1</td>
<td>1</td>
<td>2,215</td>
<td>--</td>
</tr>
<tr>
<td></td>
<td>VL-2, 1</td>
<td>1</td>
<td>3,146</td>
<td>--</td>
</tr>
<tr>
<td></td>
<td>VL-1, 4</td>
<td>1</td>
<td>234</td>
<td>89 to 93</td>
</tr>
<tr>
<td>200</td>
<td>VL-1, 1</td>
<td>2</td>
<td>7,118</td>
<td>--</td>
</tr>
<tr>
<td></td>
<td>VL-2, 1</td>
<td>2</td>
<td>11,192</td>
<td>--</td>
</tr>
<tr>
<td></td>
<td>VL-1, 4</td>
<td>2</td>
<td>1,434</td>
<td>80 to 87</td>
</tr>
<tr>
<td>1.86</td>
<td>VL-1, 1</td>
<td>1</td>
<td>2,059</td>
<td>--</td>
</tr>
<tr>
<td></td>
<td>VL-2, 1</td>
<td>1</td>
<td>2,879</td>
<td>--</td>
</tr>
<tr>
<td></td>
<td>VL-2, 5</td>
<td>1</td>
<td>252</td>
<td>88 to 91</td>
</tr>
<tr>
<td>90</td>
<td>VL-1, 1</td>
<td>1</td>
<td>9,142</td>
<td>--</td>
</tr>
<tr>
<td></td>
<td>VL-2, 1</td>
<td>1</td>
<td>12,522</td>
<td>--</td>
</tr>
<tr>
<td></td>
<td>VL-2, 5</td>
<td>1</td>
<td>356</td>
<td>96 to 97</td>
</tr>
</tbody>
</table>
when all three cycles had received two injections, the Cycle 4 inhibition was between 80 and 87 percent.

The results indicate that storage of the Mars surface sample in the hopper for 136 sols prior to nutrient injection severely dissipated the active agent. Further, additional active agent was unavailable three hours after first injection since the second injection did not produce a stimulation of gas evolution. This could be either because the amount of active agent which survived the lengthy storage was already functioning at the maximum rate (i.e., substrate saturation) or because it was largely destroyed, perhaps by water vapor, during the interim period following the first injection.

2. Viking Lander 2, Cycle 5

The fifth and final cycle planned at the Utopia landing site (VL-2) was an incubation of a fresh sample at sub-freezing temperatures, thereby better approximating ambient Martian temperatures. Since the instrument's four test cells had already been used, the fifth cycle required addition of the fresh sample on top of a sample used in an earlier cycle. Previous data acquired in the Test Standards Module (TSM), a laboratory instrument replicating the flight instruments in all essential components (1), indicated that such a "soil-on-soil" experiment detects metabolic responses, but that the changed soil/nutrient ratio could affect the kinetics of gas evolution.
This plan was interrupted when the sample arm failed to acquire the fresh sample. Nonetheless, scheduled power shutdown occurred and the biology instrument froze. When command capability was restored and the instrument warmed to 10-15°C to thaw the nutrient, the plan had to be revised to utilize a surface sample already present in a test cell. The sample selected had been acquired on Sol 145 from the same area ("Beta") which had given active responses in earlier cycles (3), stored in the hopper for 32 sols, and then placed in an LR test cell for an additional 52 sols prior to the Cycle 5 nutrient injection. This 0.5 cc sample was located above 1.2 cc of surface material from a previous "dump" of unused sample which was, in turn, on top of the 0.5 cc sample used for Cycle 1. (The Cycle 1 sample had been dried by heating 3 hours at 160°C at the end of that test.) Thus, the total sample volume was 2.2 cc contained within the 3.0 cc test cell. Nutrient budget calculations indicated that sufficient nutrient remained to assure only one nutrient injection for Cycle 5. Incubation temperatures were maintained above freezing, contrary to the original plan, because the stored sample had already been exposed to temperatures above freezing, thereby defeating the purpose of the cold incubation. Further, a second freeze might have placed undue stress on the biology instrument.
The results of Cycle 5 are given in Figure 8. Upon injection of nutrient, radioactive gas was immediately evolved, reaching a maximum of approximately 350 cpm over background after 90 hours. This level then remained essentially at a plateau until the end of the cycle at approximately 300 hours. The gas evolution in Cycle 5 was significantly reduced relative to that from the other active cycles at VL-2 as Figure 9 shows. As seen in Table I, the percent inhibition was greater than 90 percent throughout the cycle. These data support those from VL-1, Cycle 4 (see above) that long term storage of the Mars sample in the dark at approximately 10°C (see below) destroys the active agent in the Mars sample.

3. Validity of Conclusions

The conclusion from VL-1, Cycle 4 and VL-2, Cycle 5 that long term dark storage at 10°C destroys the activity of the Mars sample assumes that both cycles received a full measure of soil and nutrient. Laboratory data obtained with the TSM indicate that the rates and kinetics of gas evolution for a positive response are influenced by the soil/nutrient ratio with the total gas evolved limited by the volume of nutrient. A properly functioning instrument delivers 0.5 cc soil + 20% and 0.115 cc nutrient + 8%. A partial nutrient delivery, which could occur toward the end of the nutrient supply, would result in changed kinetics and a reduced plateau level. Excess surface
An active sequence was used on a sample stored for 34 Sols at approximately 7°C prior to nutrient injection. This sample was placed in a used test cell containing the dried sample from the first VL-2 cycle plus sample "dumps," giving a total volume of 2.2 cc. Radioactivity was measured at 16-minute intervals throughout the cycle except for the first two hours when readings were taken every 4 minutes. Data between two and four hours after injection were lost. Detector and test cell temperatures were measured every 16 minutes.
Evolved radioactivity following the first injection of radioactive nutrient to each analysis cycle of VL-2 is shown. A fresh sample was used for each cycle except cycle 5 which used a sample stored approximately 84 Sols at 7°C prior to injection. The sample used in cycle 3 was obtained from under a rock. Cycles 1, 3, and 5 were active sequences, whereas cycles 2 and 4 were control sequences in which the samples were heated for 3 hours at approximately 51.5°C and 46°C, respectively, prior to nutrient injection. Sample volumes were 0.5cc except that for cycle 5 which contained 2.2 cc. All data have been corrected for background counts observed prior to injection.
sample could diminish the rate of gas evolution, whereas failure to deliver a sample would produce a response characterized by that from nutrient alone in which a plateau of approximately 200-600 cpm is established within a few hours.

For both cycles, some question exists that a complete nutrient volume was in fact delivered because both instruments were low in the supply of nutrient. Each nutrient injection sequence requires 0.52 cc nutrient to deliver the 0.115 cc volume to the test cell. At the onset of VL-1, Cycle 4, a calculated 1.56 cc remained available, not including residual nutrient which would be left in the lines after utilizing all deliverable nutrient. This volume ensured a complete first injection and a high probability of a full second injection. However, the kinetics of gas evolution following second injection neither confirm nor deny that the second injection occurred. Typically, upon second injection, a spike is seen followed by a 30-35% drop in the total radioactive gas present such that the new level is below the plateau attained prior to second injection. In VL-1, Cycle 4, the spike occurs, followed by a 30-35% drop, but the new level is essentially the same as that seen prior to second injection. These kinetics could be explained either if there were no injection or if the injection occurred while sufficient gas was still being evolved to obliterate the drop in plateau level.
On VL-2, Cycle 5, only 0.64 cc nutrient was calculated to be available at the onset of the cycle. Further, prior to the injection, the instrument had accidentally frozen with nutrient in the lines. This may have ruptured the delivery lines or valves. Thus, the probability of a complete nutrient delivery for this cycle is considerably lower. However, at least a partial injection occurred because some radioactivity was evolved in the test cell following injection. Alternatively, this radioactivity could have resulted from residual gas present in the delivery lines which was injected into the test cell in lieu of nutrient.

The probability that sample delivery or sample size affected the results of these two cycles has also been considered. For VL-1, Cycle 4, calculations indicated that enough sample remained in the hopper to provide a full delivery. The observed kinetics are atypical of a result from nutrient alone and give evidence that a sample delivery did occur. For VL-2, Cycle 5, the large sample volume of 2.2 cc could have affected the rate of gas evolution. However, separate experiments conducted in the TSM (see later) that replicate the VL-2, Cycle 5 sequence have shown that the influence of the larger sample size on kinetics is highly soil dependent. With an iron-rich terrestrial soil, a stimulation was seen for the excess sample size, whereas with a synthetic Mars analog soil an inhibition was seen. Thus, the large sample in VL-2 cycles may have caused a reduction in gas evolution.
In summary, then, the conclusion that the low results in VL-1, Cycle 4 following first injection reflect inactivation of the stored sample appear valid. The results following second injection are probably also valid. The VL-2, Cycle 5 results tend to support the conclusion of inactivation on long-term storage although some concern exists that the low activity may reflect a low nutrient injection volume or the large sample size or both.

4. Terminal Heating of Cycle 4

A single nutrient injection into the LR test cell contains approximately 257,000 cpm with each of the 17 carbons of the seven substrates contributing approximately 15,000 cpm (corresponding to 29 nmoles of carbon). For the four cycles conducted with active surface samples (VL-1, Cycles 1 and 3; VL-2, Cycles 1 and 3), the maximum radioactivity evolved ranged from 11,000 to 16,000 cpm, possibly corresponding to total utilization of only one of the carbon substrates. This suggested (2) that a chemical reaction was occurring between formate, assumed to be the most reactive of the LR substrates, and some surface oxidant.

To test the possibility that more than one substrate may have been involved, the VL-1, Cycle 4 incubation mixture was heated to 50°C at the end of the cycle (Sol 287) to drive off any radioactive gas trapped within the sample matrix during the 55 Sol incubation period. From previous laboratory studies (1), this mild treatment was known to have no effect on the decomposition or volatilization of residual nutrient substrates.
remaining within the sample. VL-2, Cycle 4 had demonstrated that heating to 50°C severely attenuated the active agent, so that the terminal 50°C heating of the reaction mixture would likely not produce new gaseous products. When planning this experiment, it was hoped that the active sample would produce a radioactive plateau of either 16,000 cpm (from one injection) or up to 32,000 cpm (from two injections assuming active agent at the time of the second injection). If the mild heat treatment would release additional gas trapped within the sample, to exceed the 16,000 or 32,000 cpm plateau, this would establish that more than one carbon substrate was involved in the LR reaction. As discussed above, however, the stored sample used for VL-1, Cycle 4 showed a reduced response and had evolved only 3000 cpm by the time of the 50°C terminal heating.

The results of the heat treatment are shown in Figure 10. During heating, the detector heaters produce "noise" such that the gas level cannot be determined. As soon as these heaters were turned off and accurate counts obtained, the radioactivity level was 20,954 cpm, approximately 18,000 cpm above the initial value for the gas present in the headspace prior to the heating. (It should be noted that this cannot reflect simply heat-driven movement of the gas from the headspace of the test cell into the detector chamber since the detectors are simultaneously heated to 110°C, considerably
Radioactivity released from terminal 50°C heating of the VL-1, cycle 4 incubation performed 55 Sols after the onset of the reaction by injection of two nutrient "squirts". The LR heaters were turned on for a total of 3.74 hours to provide the incubation mixture with approximately 3 hours of heating at 50°C. During this time, the "noise" produced by heating the detectors to approximately 110°C prevented monitoring the level of radioactivity in the LR test cell. Readings of radioactivity resumed immediately after turning off the heaters. Radioactivity and temperature readings were taken every 16 minutes.
above the test cell 50°C.) As the test cell cooled, the radioactive gas was quickly reabsorbed and the level dropped to 6600 cpm within the 2-½ hours required for the test cell to return to 10°C. The gas level then gradually decreased at approximately a linear rate. By Sol 300, the level was 3800 cpm where it remained steady until data collection terminated on Sol 303.

Assuming the same phenomenon during previous active cycles, at least an additional 18,000 cpm would have been generated as gas and remained trapped in the sample-nutrient mixture. Thus, the 10,000-16,000 cpm level observed for active cycles may represent only a portion of the total gas evolved. Because each carbon position contributed approximately 15,000 cpm, the gas evolved may have been derived from at least two carbon positions. This could be accomplished by oxidation of one substrate composed of two carbons, or of two substrates, assuming one is formate. Alternatively, the gas could also be generated by partial oxidation of several or all substrates. However, the latter alternative is less likely in view of the apparent first order type kinetics observed (46) for the Mars LR active response.

C. Analysis of Thermal Stability of Mars Active Agent

One of the key properties of the Mars active agent is its thermal sensitivity which is consistent with a biological response and places considerable constraints on possible chemical contenders. Because of the importance of this
characteristic in delineating possible agents, we have undergone the following analysis of the thermal decomposition. The properties of the active agent forming the basis of this evaluation are summarized below:

- Destroyed by 3 hours at 160°C. (Note that sample was stored 20 Sols at approximately 10°C prior to 160°C treatment and another sol at 10°C after the 160°C treatment); VL-1, Cycle 2 (3).

- Partially destroyed (54-80%) by 3 hours heat treatment at 46°C; VL-2, Cycle 4 (4).

- Mostly destroyed (80-93%) by storage for 141 Sols at 10-26°C with an average exposure of approximately 15°C; VL-1, Cycle 4.

- Destroyed by storage for 84 Sols (or less) at -8°C to +10°C with an average exposure of 7°C. (Note that sample underwent a "freeze-thaw" cycle; conclusion also assumes full nutrient injection and no effect of sample size); VL-2, Cycle 5.

- Stable to storage for 5 Sols at 7 to 10°C. (Assumes that reduction in activity resulted from heat treatment for 3 hours at 51°C rather than from prior 5 Sol storage); VL-2, Cycle 2.

- Stable (at least partially) to storage for 2 Sols at 18°C. (Assumes stable to storage for 2 Sols at 10°C, the regime preceding active VL-1, Cycle 1.
Note, however, that since no sample was tested with a shorter storage time, stability at 10°C is an assumption); VL-1, Cycle 3.

In attempting to quantitate the thermal properties of the active agent, the actual temperature exposure of each sample must be estimated. Upon acquisition, each sample progresses through several stations, each with its own thermal regime. Samples were obtained from the top 3.5 cm of Mars surface material and transferred to the soil processor (PDA) via the collector head of the sample arm. Collector head temperatures were assumed to be those of the surrounding atmosphere. The total combined time in the collector head and PDA was generally less than two hours. The sample was sieved as it passed from the PDA into the soil hopper (SDA) where it remained until it was distributed into the LR test cell. SDA temperatures were assumed to be identical to those of the Biology Instrument mounting plate which generally experienced a diurnal temperature range between 10°C and 23°C. Temperatures in the test cell were maintained near 10°C with diurnal fluctuations ranging between 9°C and 14°C. The estimated temperature exposure of each sample at each of these stations is presented in Table II.

These time-temperature data may be used to generate an Arrhenius plot representing the temperature effect on the rate of decomposition of the Mars active agent. The overall assumptions required for this analysis are:
<table>
<thead>
<tr>
<th>Lander and Cycle</th>
<th>Soil Sample Collection</th>
<th>Surface Temp. (°C) When Collect</th>
<th>Collector Head &amp; PDA</th>
<th>SDA</th>
<th>Test Cell</th>
<th>Test Cell (Sterilization)</th>
</tr>
</thead>
<tbody>
<tr>
<td>VL-1, Cycle 1</td>
<td>8</td>
<td>-83° to -65°</td>
<td>-67° to -40°</td>
<td>1.3</td>
<td>7°</td>
<td>10° - 13°</td>
</tr>
<tr>
<td>(active)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>VL-1, Cycle 2</td>
<td>8</td>
<td>-83° to -65°</td>
<td>-67° to -34°</td>
<td>1.3</td>
<td>6° - 21° (15°)</td>
<td>399.2</td>
</tr>
<tr>
<td>(160°C control)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>VL-1, Cycle 3</td>
<td>36</td>
<td>-21°</td>
<td>-16° to +18°</td>
<td>1.8</td>
<td>17.5°</td>
<td>11° - 23° (13°)</td>
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<td>(active, long incubation)</td>
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<td></td>
<td></td>
</tr>
<tr>
<td>VL-1, Cycle 4</td>
<td>91</td>
<td>-71°</td>
<td>-80° to -60°</td>
<td>1.3</td>
<td>10° - 26° (15°)</td>
<td>3420</td>
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<td>(stored, double injection)</td>
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<tr>
<td>VL-2, Cycle 1</td>
<td>8</td>
<td>-38° to -23°</td>
<td>1° to 4°</td>
<td>1.4</td>
<td>10°</td>
<td>7° - 10°</td>
</tr>
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<td>(active)</td>
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<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>VL-2, Cycle 2</td>
<td>20</td>
<td>-38° to -23°</td>
<td>-2° to +1°</td>
<td>0.8</td>
<td>19°</td>
<td>7° - 10°</td>
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<td>(51°C control)</td>
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<tr>
<td>VL-2, Cycle 3</td>
<td>51</td>
<td>-69° to -66°</td>
<td>-51° to -21°</td>
<td>1.9</td>
<td>11°</td>
<td>6° - 9°</td>
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<tr>
<td>(active, under rock)</td>
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<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>VL-2, Cycle 4</td>
<td>145</td>
<td>-84°</td>
<td>-71° to -39°</td>
<td>1.2</td>
<td>4°</td>
<td>6° - 9°</td>
</tr>
<tr>
<td>(46°C control)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>VL-2, Cycle 5</td>
<td>145</td>
<td>-84°</td>
<td>-71° to -39°</td>
<td>1.2</td>
<td>2° - 7°</td>
<td>709.4</td>
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<tr>
<td>(soil-on-soil)</td>
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<td></td>
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</tr>
</tbody>
</table>

*T = Mean incubation from arrival to first nutrient injection but not counting sterilization which, when it occurs, occurs near the middle of this incubation.

† Numbers in parentheses = average temperature exposure
1. The thermal decomposition of the active agent follows first order kinetics such as is seen for the decomposition of hydrogen peroxide (see later).

2. The active agent at Lander Site 1 is the same as that at Lander Site 2.

3. The LR reaction is limited by the active agent and a stoichiometric relationship exists between the moles of active agent and the moles of gas evolved. Thus, the moles active agent present in the reaction mixture at the time of nutrient injection can be calculated from the radioactivity evolved, assuming a one-carbon gas. Justification for the active agent as limiting is that commandable injection did not produce additional evolved radioactivity, as would be expected if the reaction were substrate limited. Also, a higher plateau would be expected if sufficient oxidant were present and able to oxidize all LR substrates.

4. VL-1, Cycle 1; VL-1, Cycle 3; VL-2, Cycle 1; and VL-2, Cycle 3 represent reactions with fully active agent which has not undergone decomposition prior to nutrient injection. Thus, storage, at approximately 10°C for 2-3 Sols does not affect stability.
5. The initial concentration of the active agent is the same in all cycles and can be estimated from the plateau of the active cycles. (It should be noted that this necessary assumption may not be true, as indicated by the observed variation of the plateaus from active cycles. The magnitude of this error will affect the calculated percent loss in activity for each storage regime.)

6. Loss of the active agent is caused by decomposition and not vaporization.

Using these assumptions, the rate constant, \( k \), for first order decomposition can be calculated at any given temperature from the relationship:

\[
(Eq. 1) \quad k = \frac{2.303}{t} \log \frac{c_0}{c}
\]

where \( t \) is the exposure time in hours at the particular temperature, \( c_0 \) is the initial concentration of active agent, and \( c \) is the concentration remaining after the time-temperature exposure. An Arrhenius plot can then be generated from \( \log k \) versus \( 1/T \) where \( T \) is the exposure temperature in \( ^\circ \text{K} \). From the slope of this line, the activation energy, \( \Delta E_a \), for decomposition can be calculated:

\[
(Eq. 2) \quad \Delta E_a = -\text{slope} \times (2.303) \times (1.987) \text{ cal mole}^{-1}
\]
The data available for this temperature analysis are presented in Table III. For each indicated cycle, the percent loss in active agent is estimated as a function of the treatment time, in hours, at an average temperature estimated from Table II. In using these data, it is noted that $k$ cannot be calculated for cycles which show no decomposition (i.e., $c = c_0$) or total decomposition (i.e., $c = 0$). Thus, only those cycles that show intermediate decomposition (i.e., VL-2, Cycle 4; VL-1, Cycle 4; VL-2, Cycle 5) can be used to generate the Arrhenius plot. Of these three points, that from VL-2, Cycle 5 may not be valid since an injection may not have occurred and since the reaction contained excess soil which may have affected kinetics and the estimation of activity loss. Once an Arrhenius plot is generated from VL-2, Cycle 4 and VL-1, Cycle 4, $k$ at any given temperature can be graphically determined and used to test the fit of flight data from the other cycles.

The Arrhenius plot derived from data from VL-2, Cycle 4 and VL-1, Cycle 4 is shown in Figure 11. Dotted lines indicate the possible variation in slope according to variations in the percent activity loss estimated for VL-2, Cycle 4 and VL-1, Cycle 4 (54 to 80% and 80 to 93%, respectively). In estimating the validity of this plot, it should be noted that the data point derived from VL-2, Cycle 4 is perhaps the most reliable since little uncertainty exists for both the temperature and the time of exposure. The temperature estimate of VL-1, Cycle 4
## Table III

Percent Loss of LR Activity Following Various Time/Temperature Treatments

<table>
<thead>
<tr>
<th>Cycle</th>
<th>Exposure</th>
<th>Approximate % Loss of LR Response</th>
</tr>
</thead>
<tbody>
<tr>
<td>VL-1, Cycle 1</td>
<td>10°C 47 hours</td>
<td>0</td>
</tr>
<tr>
<td>VL-1, Cycle 2</td>
<td>160°C 3 hours</td>
<td>100</td>
</tr>
<tr>
<td>VL-1, Cycle 3</td>
<td>18°C 44.6 hours</td>
<td>0</td>
</tr>
<tr>
<td>VL-1, Cycle 4</td>
<td>15°C 3469 hours</td>
<td>87</td>
</tr>
<tr>
<td>VL-2, Cycle 2</td>
<td>10°C 130 hours</td>
<td>0</td>
</tr>
<tr>
<td>VL-2, Cycle 4</td>
<td>46°C 3 hours</td>
<td>60</td>
</tr>
<tr>
<td>VL-2, Cycle 5</td>
<td>7°C 2072 hours</td>
<td>97</td>
</tr>
</tbody>
</table>
The data presented in Table III for VL-1, Cycle 4 and VL-2, Cycle 4 have been used to generate an Arrhenius plot (---) of the rate of thermal decomposition (log k) of the Mars active agent as a function of reciprocal temperature in K. Broken lines represent the maximum and minimum variation in slope that could be obtained for the inhibition range observed for each cycle. For each slope, as indicated, the activation energy ($\Delta E_a$) has been calculated. The data from VL-2, Cycle 5 are also plotted for comparison although not used to generate the lines. The assumptions inherent in the data treatment are described in the text.
is subject to more error since this sample underwent diurnal fluctuations over a long period of time. However, from the line as it is plotted, the following information may be derived:

1. Decomposition of the Mars active agent has an activation energy between 35,000 and 43,000 cal mole\(^{-1}\), well within the range of 15,000 to 60,000 cal mole\(^{-1}\) cited (48) for most chemical reactions.

2. The temperature required for a 99.9% reduction within 3 hours of treatment is between 53° and 60°C. Thus, the 3 hours of treatment at 160°C that occurred on VL-1, Cycle 2 would be expected to result in 100% decomposition. This deduction is consistent with data obtained from the mission. Also, the storage for 21 Sols at 10°C in addition to the 160°C treatment prior to nutrient addition would have resulted in no more than an 8% activity loss.

3. Essentially no decomposition would be expected for the time-temperature regimes experienced prior to the onset of VL-1, Cycle 1 (2 Sols at 10°C) or VL-1, Cycle 3 (2 Sols at 18°C). Further, essentially no decomposition would be expected for the 5 sol storage of VL-2, Cycle 2 prior to cold sterilization. These predictions are also consistent with data obtained from Mars.
4. For VL-2, Cycle 5, a 10-20% decomposition is predicted. This is inconsistent with flight data which showed a 97% decomposition. Thus, with the exception of VL-2, Cycle 5, the flight data are consistent with the Arrhenius plot generated from VL-2, Cycle 4 and VL-1, Cycle 4. The inconsistency of VL-2, Cycle 5 may support the hypotheses either that injection was faulty or that the large sample affected the kinetics of gas evolution. Alternatively, the inconsistency may reflect an incorrect assumption in the highly simplified data treatment. It should be noted that the activation energy calculated from VL-2, Cycle 4 and from VL-2, Cycle 5 is between 23,000 and 27,000 cal mol$^{-1}$. However, when this value is used to test the fit of other flight data, predicted inhibitions in all cases are greater than observed on flight. This may be additional evidence that the data from VL-2, Cycle 5 may not be valid.

The data obtained from the final two cycles of the Viking LR experiment on Mars have further delineated the thermal sensitivity of the active agent responsible for the positive LR responses reported from earlier cycles. These data are consistent with a biological explanation since Mars organisms would be expected to be sensitive to temperatures significantly above that of their native habitats. The long-term storage temperatures of approximately 10°C are considerably above the -21°C to -84°C temperatures at which the samples were collected (Table II). Since few chemicals would be destroyed or dissipated by such a low temperature, these data make biology
an attractive possibility.

Of the various alternate explanations, hydrogen peroxide seems the leading candidate. The properties of hydrogen peroxide have been extensively summarized by Edwards (49) and by Schumb et al (50). At 760 mm Hg, anhydrous hydrogen peroxide melts at -1.7°C and boils at 152°C. Pressure has a significant effect and, at 30 mm Hg, the boiling point is depressed to approximately 72°C. At 6 mm Hg, the approximate pressure at which all Mars samples were stored and the pressure at which heat treatments were begun, a further depression of the boiling point to 41°C can be calculated.

Hydrogen peroxide decomposition is known to be first order (51). When hydrogen peroxide activation energies are measured under conditions such that only decomposition can account for its disappearance, values of approximately 48,000 cal mole⁻¹ (49) are obtained. Thus, were hydrogen peroxide the active agent, the decomposition activation energies expected from the Mars samples would likely have been higher than the observed 37,000 to 42,000 cal mole⁻¹. On the other hand, should some of the active agent escape through evaporation, the apparent activation energy would be lowered. In the LR experiments, both decomposition and vaporization were likely involved with vaporization playing a relatively more significant role at lower temperatures. In the long term storage experiments at
approximately 10°C, the Mars samples were open to the Mars atmosphere. The various heat treatments were performed in closed test cells, but the test cells were vented to the atmosphere for four minutes after the heating cycle. Each arrangement would probably allow a significant quantity of hydrogen peroxide vapor to escape from the reaction volume. The differences in activation energies, then, are in the correct direction and the similarity of the known hydrogen peroxide decomposition activation energy to that derived from the flight data is of considerable interest. However, another aspect of the Arrhenius plot remains to be considered. A nonstabilized 90% wt. hydrogen peroxide solution decomposes at the rate of about 0.001% per hour at 50°C (49,50). Thus, although the slope of the Arrhenius plot for hydrogen peroxide decomposition may parallel the flight data, the "y" intercepts of the two sets of data are considerably apart. In order for hydrogen peroxide to fit the flight data, its decomposition in the Mars sample would have to be catalyzed to effect a significant lowering of the temperature at which it decomposed.

The Mars surface material with its constituent metals could provide such a catalyst. To fit the necessary requirements, the particular catalyst would have to account for a postulated free radical chain length of about 10^9 in order to reconcile the frequency factor (A) calculated from the flight data with the normal frequency factor for the decomposition of
peroxides \((\log A_{\text{flight}} - \log A_{\text{normal}} = 23 - 14 = 9)\) (52). The catalyst would also have to possess the property of effecting the slow decomposition of the hydrogen peroxide under anhydrous conditions at 10°C while not precluding a continuing reaction over many hours between the peroxide and the LR labeled substrates when nutrient was added into the test cell at 10°C.

Although the data cannot at present be entirely accounted for by chemical hypotheses, they remain consistent with biology explanations. However, biological approaches to resolving the LR enigma are not possible short of another Mars mission. Hence, our laboratory efforts are directed toward chemical experiments which can reduce the area of ambiguity, ideally achieving general acceptance of biology or chemistry as the source of the LR reaction.
V. LABORATORY SIMULATION EXPERIMENTS

Simulation experiments have been conducted both at Biospherics using a getter system and at Ames Research Center using the LR Test Standards Module (TSM), a flight-like instrument closely approximating flight conditions and sequences (1). The rapid getter system has been used to screen large numbers of reaction possibilities whereas the more time-consuming TSM experiments were limited to those for which flight-like conditions were required. All getter experiments were conducted in a glove box under a nitrogen atmosphere at 760 torr and ambient temperature (approximately 23°C). Reaction mixtures were contained in liquid scintillation vials and evolved radioactive gas was trapped by Ba(OH)$_2$ soaked getter pads placed in the vial caps. All pads were dried under an infrared lamp prior to counting by gas flow technique (counting efficiency = \( \approx 10\% \)). TSM experiments were conducted at 5 torr carbon dioxide and at 10°C. Evolved radioactivity was counted at 3%, as in the flight experiments.

A. Influence of a Large Sample Size

In support of the preceding analysis of the final LR flight data, TSM experiments have been performed to determine effects of a large sample size on kinetics of gas evolution. Because the LR instrument had only four test cells on each lander, the fifth cycle in VL-2 required addition of the new
sample on top of a sample used in an earlier cycle. This 0.5 cc sample was located above 1.2 cc of the surface material from a previous "dump" which was, in turn, on top of the 0.5 cc sample used for Cycle 1. (The Cycle 1 sample had been dried by heating 3 hours at 160°C at the end of that test.) Thus, the total sample volume was 2.2 cc contained within the 3.0 cc test cell.

Previous data acquired in the TSM (53) indicated that while a "soil-on-soil" experiment detects metabolic responses, the soil/nutrient ratio can affect the kinetics of gas evolution. The possibility existed that the large sample size in VL-2, Cycle 5 could have inhibited the kinetics of gas evolution, thereby accounting for the low activity obtained from the stored sample. To test this possibility, experiments have been conducted in the TSM using an experimental sequence essentially replicating that used for VL-2, Cycle 5. Thus, an active sample (simulated equivalent of VL-2, Cycle 1) received two injections and was terminated after several days of incubation. An additional 1.7 cc sample of the same soil was then added to the test cell, equilibrated, and injected (simulated equivalent of VL-2, Cycle 5). Comparison of the gas evolved from the two sequential runs indicates the influence of the large sample size on kinetics.
The results of two such TSM experiments are shown in Figures 12 and 13. In one experiment, an iron-rich terrestrial soil (pH 7.5, 125-250 μ particle size) was selected because the high activity of this soil provided sufficient counts to establish the magnitude of any sample size effect. As shown (Figure 12), a pronounced stimulation in activity occurred in the subsequent analysis with the larger sample size. A second experiment using synthetic Mars analog soil B2 (see Appendix) prepared by the Viking Inorganic Analysis Team (pH 7.2, 10-100 μ particle size) showed the opposite effect (Figure 13); the low response of the first analysis was considerably diminished by the large sample size of the subsequent analysis. The inhibition increased with time which appeared to reflect a soil getter effect rather than a leak. However, the extent of the observed inhibition here is not sufficient to account for the magnitude of inhibition observed from VL-2, Cycle 5. Nonetheless, the effect is apparently soil dependent which prevents TSM simulations from establishing with certainty whether the low activity of VL-2, Cycle 5 resulted from long term sample storage or large sample size. As discussed in a previous section, these data must therefore be interpreted with caution.

B. Candidate Nonbiological Agents for the Active Mars Response

Laboratory experiments have examined ultraviolet irradiation, γ-Fe₂O₃, calcium peroxide and superoxide, and
Figure 12
TSM Simulation of VL-2 Cycle 5 Data Using Santa Catalina Soil

A 0.5 cc sample of Santa Catalina soil was equilibrated for approximately 25 hours at Mars atmospheric conditions in the TSM prior to injection of 0.115 ml of VM1 nutrient (- - - - ). A second nutrient injection was performed after 103 hours of incubation, as indicated. At the end of the incubation, radioactive gas was removed by purging and the reaction mixture dried by heating for 3 hours at 160°C. An additional 1.7 cc sample of the same soil was then added on top of the dried reaction mixture and equilibrated under Mars atmospheric conditions for 49 hours before injection of 0.115 ml nutrient (——-). An additional 0.5 cc aliquot of Santa Catalina soil was sterilized for 3 hours at 160°C prior to nutrient injection (-----). All experimental cycles were conducted at 10°C under Mars experimental conditions. Data have been corrected for background radioactivity observed prior to the onset of each reaction.
A 0.5 cc sample of a synthetic Mars analog soil (B2) was equilibrated for approximately 24 hours at Mars atmospheric conditions in the TSM prior to injection of 0.115 ml of VM1 nutrient (------). A second nutrient injection was performed after 69 hours of incubation, as indicated. At the end of the incubation, radioactive gas was removed by purging and the reaction mixture dried by heating for 3 hours at 150°C. An additional 1.7 cc sample of the same soil was then added on top of the dried reaction mixture and equilibrated under Mars atmospheric conditions for 27 hours before injection of 0.115 ml nutrient (-----). All experimental cycles were conducted at 10°C under Mars experimental conditions. Data have been corrected for background radioactivity observed prior to the onset of each reaction.
hydrogen peroxide as agents possibly causing the active LR response obtained following first nutrient injection to a fresh Mars surface sample. These experiments have been conducted both at Biospherics using a getter system and at Ames using the LR TSM. Pertinent results obtained with each candidate nonbiological agent are presented below:

1. Ultraviolet Irradiation

Experiments have been undertaken in cooperation with personnel at the Ames Research Center in which Mars analog soil B2 (see Appendix) was exposed to ultraviolet irradiation (25 Watt Xenon lamp) for 712 hours under simulated Mars conditions (6 torr carbon dioxide with trace amounts of oxygen, carbon monoxide, nitrogen and argon; temperatures maintained below zero at approximately -35°C). Prior to irradiation, samples were dried at 160°C for 24 hours and placed in quartz tubes under Mars atmosphere. Three samples were sealed dry and three additional samples were sealed in the presence of added water vapor. The amount of water vapor present was that in equilibrium with the Mars atmospheric pressure at the time of sealing. It should be noted that most of the vapor was probably quickly absorbed by the soil. All samples were tumbled during irradiation to ensure uniform exposure. Irradiation was terminated on August 25, 1978 and the sealed samples were stored at -15°C to -16°C until tested in the TSM in November and December 1978 using an active flight sequence at 10°C under 5 torr carbon dioxide. The results are shown in Figure 14. Upon addition of LR-nutrient, no activity was
Two samples of 0.5cc Mars analog soil B2 were each dried at 160°C for 24 hours and placed in quartz tubes under a simulated Mars atmosphere of 6 torr carbon dioxide with trace amounts of oxygen, carbon monoxide, nitrogen, and argon. One sample was sealed dry and the other was sealed in the presence of added water vapor (i.e., the amount in equilibrium with the Mars atmospheric pressure at the time of sealing). The samples were then tumbled while exposed at approximately -35°C to 712 hours ultraviolet irradiation from a 25 watt xenon lamp. At the end of the exposure, the samples were stored at approximately -15°C for 3 months prior to testing for activity in the TSM using an active flight sequence at 10°C under 5 torr carbon dioxide.
obtained with either type of sample. The results confirm previous conclusions (3,5) that ultraviolet irradiation of the soil per se cannot be directly responsible for the LR flight results.

2. Calcium Peroxide and Superoxide

Calcium peroxide and superoxide were obtained by courtesy of Dr. Ted Wydevan at the Ames Research Center and added to soil samples according to his methods. Soil samples (0.5 cc portions) were first heated at 170°C under vacuum for 18 hours and then placed in a simulated Mars atmosphere at 6 torr and -77°C for 4 hours. The samples were sealed and transferred to a glove box equilibrated under dry nitrogen at atmospheric pressure where the seals were broken and a specified amount of peroxide or superoxide added. The samples were re-sealed under nitrogen, transferred to the LR TSM, and added to the test cell under a stream of dry helium plus carbon dioxide. Samples were then tested for activity at 10°C under 5 torr carbon dioxide using an active flight sequence.

Results with iron-rich (≈ 13%) Santa Catalina soil (sieved to <125 μ particle size) are shown in Figure 15. In the absence of added peroxide or superoxide, the soil shows a response typical of sterile soils. Addition of small amounts of calcium peroxide (0.7 mg) or calcium superoxide (0.41 mg) stimulates the evolved gas approximately two-fold. Upon second injection of nutrient, gas evolution again increases, in contrast to the flight response where gas absorption follows the second nutrient addition. As the amount of oxidant added to the soil increases, the reaction following first nutrient injection
Aliquots of 0.5 cc Santa Catalina soil were heated under vacuum at 170°C for 18 hours and then placed at 6 torr carbon dioxide and -77°C for 4 hours. The samples were sealed and transferred to a glove box equilibrated under dry nitrogen at 760 mm Hg where the indicated amount of calcium peroxide (CaO₂) or calcium superoxide ((CaO₂)₂) was added. The samples were re-sealed under nitrogen and then transferred to the TSM where they were added to the test cell under a stream of dry helium plus carbon dioxide. After equilibrating at 10°C under 5 torr carbon dioxide, the VM1 nutrient was injected, as indicated, according to the flight sequence. At the end of the run, the sample was removed from the TSM test cell for determination of the pH of the mixture.
is not further enhanced. Rather, a pronounced gettering effect is observed (Figure 15), perhaps caused by the increased soil pH that accompanies the peroxide or superoxide addition.

With Mars analog soil #1, the addition of 1.75 mg calcium superoxide initially stimulates the reaction following first nutrient injection (Figure 16) although the data again do not resemble flight data either in magnitude or kinetics (compare Figures 14 and 16). However, upon second nutrient injection, a spike is seen followed by a dramatic gas absorption, a response which is similar to the flight response following second injection. Increased amounts of oxidant in the soil enhance the gettering effect following each nutrient addition but do not produce a flight-like response following first nutrient injection. Essentially identical results have been obtained with Mars analog soil B2.

Further experiments with Mars analog soils containing these oxidants have been conducted in which $^{14}$CO$_2$ or $^{14}$CO was first equilibrated with soil. Water was then added to the TSM test cell in place of nutrient using the flight injection sequence. The results with $^{14}$CO$_2$ and Mars analog soil #1 are shown in Figure 17. When 2.09 mg calcium superoxide or 1.97 mg calcium peroxide are present in the soil, a sharp spike with subsequent gas adsorption immediately follows initial water injection. Again, the results cannot account for flight kinetics following the first liquid injection. That the adsorption phenomenon observed in Figure 17 may be related to the water-carbon dioxide equilibrium is suggested by the fact that no adsorption occurred when $^{14}$CO was substituted for $^{14}$CO$_2$. 
Figure 16

EFFECT OF CALCIUM PEROXIDE AND SUPEROXIDE ON REACTION BETWEEN VM1 AND MARS ANALOG SOIL #1

Aliquots of 0.5 cc Mars analog soil #1 were heated under vacuum at 170°C for 18 hours and then placed at 6 torr carbon dioxide and -77°C for 4 hours. The samples were sealed and transferred to a glove box equilibrated under dry nitrogen at 760 mm Hg where the indicated amount of calcium peroxide (CaO₂) or calcium superoxide ((CaO₂)₂) was added. The samples were re-sealed under nitrogen and then transferred to the TSM where they were added to the test cell under a stream of dry helium plus carbon dioxide. After equilibrating at 10°C under 5 torr carbon dioxide, the VM1 nutrient was injected, as indicated, according to the flight sequence. At the end of the run, the sample was removed from the TSM test cell for determination of the pH of the mixture.
Mars analog soil, with or without calcium peroxide or superoxide, as indicated, was prepared by desiccating for six days, sealing under unlabeled carbon dioxide, and adding to the TSM test cell. Radioactive carbon dioxide (specific radioactivity = 0.041mCi/m mole) was then injected into the TSM test cell to provide approximately 2700 nanomoles and 9500 cpm. The resulting test cell pressure was approximately 7 torr. After equilibration, 0.115 cc of sterile water was injected into the test cell by the flight injection sequence. Injections \( (I_x) \) are indicated for each run where \( x \) indicates the xth injection into the test cell during the run.
It is concluded that although these metallo-oxidants can produce a flight-like getter response following second nutrient injection, they cannot account for the initial active LR response on Mars. Coupled with the thermal stability (34) of the metalloperoxides and superoxides, the results indicate that these agents cannot be responsible for the Viking LR response.

3. \textit{\textit{Gamma} \textit{Fe}_2\textit{O}_3}

\textit{Gamma Fe}_2\textit{O}_3 (Cobaloy X4107, Lot #2848114) was obtained by courtesy of Vance Oyama and tested in the LR TSM for activity with LR nutrient (VM1) using an active flight sequence at 10°C under 5 torr carbon dioxide. However, as shown in Figure 18, essentially no activity was seen upon addition of the LR flight nutrient (VM1) to 0.5 cc \textit{\gamma-Fe}_2\textit{O}_3 contained in the TSM test cell. In addition, kinetics following second nutrient injection do not resemble flight data. These results indicate that \textit{\gamma-Fe}_2\textit{O}_3 alone cannot be responsible for any portion of the active flight response, not even the slow, linear, heat stable gas evolution (see Figure 4, Reactions #3 and #6) observed during the mission superimposed on the heat-labile active response.

4. Hydrogen Peroxide

The results of an experiment whereby VM1 is added to the TSM test cell containing 0.5 cc hydrogen peroxide at 10°C under 5 torr carbon dioxide are shown in Figure 19. As shown, both the kinetics and magnitude of the subsequent \textsuperscript{14}C-labeled gas evolution are similar (but not identical) to those observed during the Viking mission. Upon injection of additional nutrient, a brief spike precedes a faster rate of
A 0.5 cc sample of gamma Fe$_2$O$_3$ was equilibrated at 6 torr carbon dioxide in the TSM prior to the injection of 0.115 ml of VM1 nutrient. After 63 hours of incubation at 10°C, a second nutrient injection was performed. Evolved radioactivity is compared to that obtained from the first LR cycle on each of the Viking landers.
A 0.5 cc aliquot of $1.2 \times 10^{-1}$ M hydrogen peroxide was adjusted to pH 6.4 and added to the TSM test cell. After equilibration in the sealed test cell at 10°C under 6 torr carbon dioxide, 0.115 ml of VM1 was injected according to the flight sequence. A second nutrient injection was added after approximately 113 hours of incubation. Evolved radioactivity (---) is compared to that obtained from the first LR cycle on each of the Viking landers.
gas evolution. This latter response does not resemble flight data and indicates that nutrient limited the TSM response following first nutrient injection. This conclusion is in contrast to the flight data where the active Mars agent appears to be absent at the time of the second injection.

The getter technique has been used to explore the large number of variables required to delineate the nature of the VM1-hydrogen peroxide reaction observed in the TSM. Each of the substrates comprising VM1 has been prepared separately at the same concentration as in VM1. Uniformly-labeled glycine, formate, and glycolate were prepared at $2.5 \times 10^{-4}$ M whereas DL-alanine and L-lactate were prepared at $5 \times 10^{-4}$ M. (Note that lactate was racemic in VM1; however, for purposes of this study, the easily obtainable and less expensive L isomer was used instead of the DL mixture.)

The results obtained when each substrate was mixed with $10^{-1}$ M hydrogen peroxide are compared in Figure 20 to those obtained with VM1 and $10^{-1}$ M hydrogen peroxide. As shown, all substrates react with hydrogen peroxide, although at different rates. The fact that more gas is evolved from glycine than from VM1 is probably related to the fact that the flight VM1 has been stored for approximately four years since its preparation in 1974 and may have partially decomposed. To determine the most reactive substrates, the percent evolved $^{14}$CO$_2$ relative
Uniformly labeled glycine, formate, and glycolate solutions were separately prepared at $2.5 \times 10^{-4}$ M whereas DL-alanine and L-lactate solutions were separately prepared at $5 \times 10^{-4}$ M. Each separate substrate is comparable in concentration and in radioactivity to that present in VM1. Experiments were initiated by adding 0.22 ml of $1.5 \times 10^{-4}$ M hydrogen peroxide (adjusted to pH 6.4) to a liquid scintillation vial and equilibrating in a glove box under nitrogen at 760 mm Hg and room temperature. After equilibration, 0.11 ml of VM1 or individual substrate was added. The vial was immediately sealed and evolved $^{14}CO_2$ trapped with a Ba(OH)$_2$ soaked getter pad. Pads were dried and counted by the gas flow technique at approximately 10% efficiency. The number given in parentheses indicates the percent evolved $^{14}CO_2$ relative to the radioactivity available in each substrate.
to the available radioactivity in each uniformly labeled substrate (2 μCi/carbon) has been calculated (Figure 20). As shown, glycine is the most reactive compound with formate the second most reactive. The magnitude of these reactions is highly dependent on the hydrogen peroxide concentration (Figure 21). As shown, evolved $^{14}\text{CO}_2$ approximately doubles for each ten-fold increase in hydrogen peroxide concentration.

The possibility exists that the order of reactivity of the VM1 constituent substrates with hydrogen peroxide may reflect the presence of trace amounts of a metal catalyst despite the fact that unstabilized solutions of hydrogen peroxide (i.e., no stannous ions present) were used for these experiments. To test this possibility, the chelator ethylenediamine-tetra-acetate (EDTA) was added to each reaction mixture. Inhibition of activity between hydrogen peroxide and all substrates was found at EDTA concentrations as low as $10^{-6}$M EDTA, indicating the presence of a trace metal catalyst. At EDTA concentrations sufficiently high ($10^{-2}$M) to totally prevent participation of any endogenous metal catalyst, only lactate and formate showed a reaction with the hydrogen peroxide (Figure 22), albeit at a greatly inhibited rate for both substrates. Although more gas was evolved from lactate, formate was the most reactive compound from the viewpoint of percent decomposition. This is in agreement with statements by various investigators (31,52) that hydrogen peroxide is expected to be more reactive with these two substrates than with the other VM1 constituents.
Aliquots of 0.22 ml of an appropriate concentration of hydrogen peroxide (adjusted to pH 6.4) were added to liquid scintillation vials and equilibrated in a glove box under nitrogen at 760 mm Hg and room temperature. After equilibration, 0.11 ml of VM1 or its constituent substrates (see legend to Figure 20) was added such that the final concentration of hydrogen peroxide was 10^{-3} M, 10^{-2} M, or 10^{-1} M. The vials were immediately sealed and evolved \(^{14}\text{CO}_2\) trapped with a Ba (OH)_2 soaked getter pad. Pads were dried and counted by the gas flow technique at approximately 10% efficiency.
EFFECT OF $10^{-2}$M EDTA ON REACTION BETWEEN $H_2O_2$ AND VM1 CONSTITUENTS

<table>
<thead>
<tr>
<th>Substrate</th>
<th>$-H_2O_2$</th>
<th>$-H_2O_2 + EDTA$</th>
<th>% inhibition</th>
</tr>
</thead>
<tbody>
<tr>
<td>Glycine</td>
<td>24,096</td>
<td>176</td>
<td>99.5</td>
</tr>
<tr>
<td>VM1</td>
<td>19,208</td>
<td>1322</td>
<td>33.1</td>
</tr>
<tr>
<td>Formate</td>
<td>5,222</td>
<td>472</td>
<td>91.0</td>
</tr>
<tr>
<td>Lactate</td>
<td>2,304</td>
<td>1804</td>
<td>22.7</td>
</tr>
<tr>
<td>Glycolate</td>
<td>1,392</td>
<td>974</td>
<td>30.0</td>
</tr>
<tr>
<td>Alanine</td>
<td>1,096</td>
<td>304</td>
<td>74.6</td>
</tr>
</tbody>
</table>

Aliquots of 0.22 ml of $1.5 \times 10^{-1}$M hydrogen peroxide and $1.5 \times 10^{-2}$M EDTA (adjusted to pH 6.4) were added to liquid scintillation vials and equilibrated in a glove box under nitrogen at 760 mm Hg and room temperature. After equilibration, 0.11 ml of VM1 or its constituent substrates (see legend to Figure 20) was added such that the final concentration of hydrogen peroxide was $10^{-1}$M and of EDTA was $10^{-2}$M. Evolved $^{14}C$O$_2$ was trapped with a Ba(OH)$_2$ soaked getter pad. Pads were dried and counted by the gas flow technique at approximately 10% efficiency. Data plotted have been corrected by subtracting radioactivity evolved from each substrate alone. The percent inhibition by EDTA is shown in the accompanying table.
It may be concluded that hydrogen peroxide at relatively high concentrations ($10^{-1}$M) may react with VML to produce a flight-like positive response and that the reaction is apparently catalyzed by the presence of trace amounts of an unknown metal catalyst. Further, if hydrogen peroxide is the active Mars agent, more than one of the VML substrates is involved in the Viking LR reaction. However, before the hydrogen peroxide hypothesis can be accepted as accounting for the Mars response, its thermal sensitivity must be compared to that of the Mars active agent.

5. **Hydrogen Peroxide Plus Gamma Fe$_2$O$_3$**

A study was next undertaken to determine the effect of pure gamma iron on the reaction of $\text{H}_2\text{O}_2$ with VML and some of its components. Getter-type reactions were conducted in which $^{14}$C-labeled substrate was added to hydrogen peroxide (final concentration = $10^{-1}$M) contained on 0.5 cc pure $\gamma$-Fe$_2$O$_3$ as "soil". The results in Figure 23 compare the gas evolutions achieved with VML, formate, or glycine as substrates. As shown, $\gamma$-Fe$_2$O$_3$ in the absence of hydrogen peroxide produced no activity from any of the organic substrates tested. However, in combination with hydrogen peroxide, it produces strong effects: activity with formate is enhanced whereas activity with glycine is inhibited. The demonstrated net effect of $\gamma$-Fe$_2$O$_3$ on VML reactivity with hydrogen peroxide is an overall stimulation. Similar results have been obtained when the amount of added pure $\gamma$-Fe$_2$O$_3$ is reduced to a few grains ($\sim$ 1-2 mg) per reaction.
EFFECT OF 0.5cc $\gamma$Fe$_2$O$_3$ ON REACTION BETWEEN H$_2$O$_2$ AND VM1 OR CONSTITUENTS*

Liquid scintillation vials containing 0.5 cc gamma Fe$_2$O$_3$ ($\Delta - \Delta$), 0.22 ml of $1.5 \times 10^{-1}$ M hydrogen peroxide ($\Delta - \Delta$), or both 0.5 cc gamma Fe$_2$O$_3$ and 0.22 ml of $1.5 \times 10^{-1}$ M hydrogen peroxide ($\Delta - \Delta$) were placed in a glove box and equilibrated under nitrogen at 1,600 mm Hg and room temperature. After equilibration, 0.11 ml of VM1, $^{14}$C-formate or $^{14}$C-glycine, as indicated, was added to each vial such that the final hydrogen peroxide concentration (when present) was $10^{-1}$ M. The vials were immediately sealed and evolved $^{14}$CO$_2$, trapped with a Ba(OH)$_2$ soaked getter pad. Pads were dried and counted by the gas flow technique at approximately 10% efficiency.

* Order of Addition: $\gamma$Fe$_2$O$_3$, H$_2$O$_2$. Followed immediately by VM1 or Constituents.
Some preliminary experiments have also been conducted in which other metals have been added to the reaction between $^{14}$C-glycine and $10^{-1}$M hydrogen peroxide. The results indicate that gas evolution is stimulated by the presence of $10^{-3}$M cupric sulfate and inhibited by $10^{-3}$M ferric sulfate to an extent comparable to the inhibition seen with gamma iron. Thus, the effects observed with gamma iron are not unique to this metal catalyst.

Figure 19 shows that a concentration of $10^{-1}$M hydrogen peroxide was required to produce a response with VM1 that simulated flight results. However, the participation of a metal catalyst derived from the Mars surface sample might further lower the amount of hydrogen peroxide necessary to produce a response of flight magnitude. However, such a catalyst must not catalyze the anhydrous degradation of hydrogen peroxide over a two sol period at $10^0$C nor cause a rapid decomposition in the presence of water (otherwise the production of radioactive gas would occur only briefly).

C. Stability of Hydrogen Peroxide

Our laboratory program has demonstrated that hydrogen peroxide, with and without added $\gamma$-$Fe_2O_3$, can interact with VM1 to produce a flight-like active cycle response. However, in order to qualify as the Mars active agent, hydrogen peroxide must meet two additional criteria. First, it must display
the thermal instability (3,4,54) observed in the Viking LR reactions (i.e., completely destroyed by 3 hours heating at 160°C, partially destroyed by 3 hours at 50°C, and mostly destroyed by several months storage at approximately 10°C). Second, it must be stable on the fine particulate material such as is present on Mars. During the Viking mission, any hydrogen peroxide present would have had to be stable for two to three sols in the test cell prior to the addition of VM1.

According to Dr. John Edwards at Brown University (52), the high surface area of the fine Mars material should catalyze the rapid decomposition of hydrogen peroxide. Laboratory simulation experiments delineating the stability of hydrogen peroxide to temperature and on various analog soils are presented below.

1. Thermal Stability

Experiments testing the thermal sensitivity of hydrogen peroxide were conducted in the TSM by placing 0.5 ml of 1.2 \times 10^{-1} M hydrogen peroxide in the test cell, evacuating to 5 torr carbon dioxide, and heating in the closed cell at the desired temperature for 3 hours. After then cooling to 10°C, the test cell was vented for 4 minutes through S/52 and 0.115 ml of VM1 added according to the flight sequence (final hydrogen peroxide concentration = 10^{-1} M). Subsequent
14C-labeled gas evolution was monitored by the TSM detectors at a counting efficiency of 3%. The results of several experiments (Figure 24) show that pre-heating at 160°C, 50°C, and even 40°C, essentially destroys or renders hydrogen peroxide unavailable for reaction with VM1 in the early stages of the experiment. Some unexplained residual gas evolution occurs later in the time course following nutrient injection onto a 160°C-treated sample.

Hydrogen peroxide is known to be essentially stable to three hours treatment at 50°C (49,50). Because the TSM results conflict with the predicted results, additional experiments were conducted in our laboratory to study the thermal sensitivity of hydrogen peroxide under a variety of conditions. Getter-type experiments were conducted by placing 0.22 ml of 1.5 x 10^{-1} M hydrogen peroxide (adjusted to pH 6.4) in the bottom of a sterile liquid scintillation vial contained in a glove box under nitrogen at 760 mm Hg and placing the vial in a Mars Simulation Chamber. This chamber, described fully elsewhere (55) and pictured in Figure 25, has been constructed at Biospherics from a 2000 ml Pyrex dessicator with a top fitted with a ground glass seal. The top contains four ground glass openings which allow atmospheric exchange and monitoring, vacuum application and measurement, and a means for manipulating vials contained within. After adding the vials, they are opened and the chamber sealed, evacuated, flushed five times with nitrogen, and finally evacuated to the desired pressure.
A 0.5 cc aliquot of $1.2 \times 10^{-1}$ M hydrogen peroxide was adjusted to pH 6.4 and added to the TSM test cell. After equilibration in the sealed test cell at $10^\circ C$ under 6 torr carbon dioxide, the sample was heated for 3 hours at $160^\circ C$ (---), $50^\circ C$ (-----), or $40^\circ C$ (-----) as indicated. After cooling and venting for 4 minutes through S/52, 0.115 ml of VM1 was added to the test cell according to the flight sequence. Evolved radioactivity is compared to that obtained from an unheated sample (-----) and from nutrient alone (-----).
Assembled Mars Simulation Chamber
The vials inside the chamber are then sealed via the manipulative system. The chamber is then brought to atmospheric pressure and the vials removed and placed in an appropriate incubator (23°, 50°, or 160°C) for three hours. After cooling for 45 minutes in the glove box equilibrated at 760 mm Hg under nitrogen, the vial caps are opened for pressure equilibration with the glove box atmosphere. Hydrogen peroxide decomposition resulting from heat treatment is monitored by subsequent reactivity with 0.11 ml VM1 (pH 6.5) added to each vial (final concentration of hydrogen peroxide is 10⁻¹ M).

Preliminary experiments using this technique established that a 10⁻¹ M hydrogen peroxide solution was stable to 3 hours storage at room temperature (app. 23°C) under anaerobic conditions. Results of a typical experiment showing the sensitivity of hydrogen peroxide to preheating at 50°C or at 160°C are shown in Figure 26. Three hours treatment at 160°C under 10 torr nitrogen destroys all reactivity of hydrogen peroxide whereas no decomposition is obtained following treatment at 50°C. Several additional experiments also established that higher pressures (80 torr) did not influence these results. It is concluded that hydrogen peroxide alone cannot account for the sensitivity of the Mars active agent at 50°C.

The reason for the discrepancy in thermal sensitivity of hydrogen peroxide in a glass container versus in the TSM
Aliquots of 0.22 ml of $1.5 \times 10^{-1}$M hydrogen peroxide were added to liquid scintillation vials which were equilibrated in the Mars Simulation Chamber under 10 torr nitrogen. The vials were sealed, removed from the chamber, and heated for 3 hours at 23°C (●●●), 50°C (○○○), or 160°C (△△△). After cooling for 45 minutes in a glove box under nitrogen at 760 mm Hg and room temperature, the vials were opened and 0.11 ml VM1 added such that the final hydrogen peroxide concentration was calculated to be $10^{-1}$ M (assuming no concentration effects by heating). The vials were immediately sealed and evolved $^{14}$CO$_2$ trapped by Ba(OH)$_2$ getter pads. The pads were dried and counted by the gas flow technique at approximately 10% efficiency.
test cell has been the subject of several additional experiments. Getter-type experiments have shown that the stainless steel composition of the TSM test cell does not catalyze decomposition at 50°C nor does the carbon dioxide atmosphere impact the results. It is tentatively concluded that the experimental sequence somehow influences the outcome of the results, perhaps through volatilization rather than decomposition. The results also suggest that hydrogen peroxide in the TSM (and in the flight instrument) may be too temperature sensitive to account for the Mars active agent.

The possibility that a metal such as $\gamma$-$\text{Fe}_2\text{O}_3$ could lower the thermal sensitivity of hydrogen peroxide has also been examined in getter-type experiments. Figure 27 shows the results of an experiment conducted under 80 torr nitrogen in which hydrogen peroxide was preheated at 23°, 50°, or 160°C in the presence of $\gamma$-$\text{Fe}_2\text{O}_3$ only added either in trace amounts (1-2 mg) or as a 0.5 cc volume. As shown, $\gamma$-$\text{Fe}_2\text{O}_3$ considerably enhances the reactivity of hydrogen peroxide with VM1 at 23°C, in agreement with results reported in a previous section. Separate experiments also established that hydrogen peroxide in the presence of $\gamma$-$\text{Fe}_2\text{O}_3$ only was stable to three hours of incubation at 23°C. Preheating hydrogen peroxide in the presence of iron significantly lowers the reactivity (Figure 27). Separate experiments have shown that the residual gas evolution following heating at 160°C results from nutrient outgassing rather
Aliquots of 0.22 ml of $1.5 \times 10^{-1}$M hydrogen peroxide were added to liquid scintillation vials with or without 0.5 cc gamma Fe$_2$O$_3$, as indicated. To vials lacking 0.5 cc gamma Fe$_2$O$_3$, a few grains (1-2 mg) of gamma Fe$_2$O$_3$ was then added to the hydrogen peroxide. After equilibration in the Mars Simulation Chamber under 80 torr nitrogen at room temperature, the vials were sealed, removed from the chamber, and heated for 3 hours at 23°C (•••), 50°C (○○○), or 160°C (△△△). After cooling for 45 minutes in a glove box under nitrogen at 760 mm Hg and room temperature, the vials were opened and 0.11 ml VM1 added such that the final hydrogen peroxide concentration was calculated to be $10^{-5}$ M (assuming no concentration effects by heating). The vials were immediately sealed and evolved $^{14}$CO$_2$, trapped by Ba(OH)$_2$ soaked getter pads. The pads were dried and counted by the gas flow technique at approximately 10% efficiency.
than from interaction with VM1. (The Batch 1 VM1 used for this experiment was prepared in 1972 and has a high nonbiological background). Control experiments in which $\gamma$-$Fe_2O_3$ alone was heated to 160°C also established that 160°C had no effect on $\gamma$-$Fe_2O_3$. Thus, the 160°C treatment destroyed hydrogen peroxide. When pre-heated at 50°C, hydrogen peroxide in the presence of $\gamma$-$Fe_2O_3$ is also destroyed or partially destroyed.

In summary of these experiments, hydrogen peroxide is stable to pre-heating at 50°C except in the presence of a metal catalyst when it may be partially or totally decomposed. However, in the TSM, hydrogen peroxide in the absence of a metal is totally dissipated by 50°C. Thus, only if a complex between hydrogen peroxide and soil somehow stabilized hydrogen peroxide to accommodate the thermal characteristics observed during the mission could hydrogen peroxide be responsible for the active LR response obtained on Mars.

2. **Stability on Soils**

Preliminary experiments have been conducted to assess the survival of hydrogen peroxide on various analogs of the Mars surface material. In these getter-type experiments, 0.5 cc of the analog soil is added to a sterile liquid scintillation vial and equilibrated in a glove box under a nitrogen atmosphere at 760 mm Hg. To each soil is then added 0.22 ml of a $1.5 \times 10^{-1}$M solution of hydrogen peroxide (adjusted to pH 6.4) followed, either immediately or after 3 hours, by 0.11 ml of VM1 (pH 6.4). After the VM1 addition, the vials are sealed and
evolved $^{14}$CO$_2$ is trapped with a Ba(OH)$_2$ soaked getter pad. At
intervals, the pads are replaced with fresh pads and the exposed
pads are dried and counted by the gas flow technique (approximately
10% efficiency).

Results with Mars analog soil B2 and with a fine
silica powder are shown in Figure 28. The particle sizes of these
soils range from 10-100 μ for the B2 soil and 50-200 μ for
the silica powder. With both soils, hydrogen peroxide is
destroyed within three hours of contact. The fine particle
size of each analog soil supports the suggestion of Edwards
(52) that hydrogen peroxide may not be stable on the fine surface
material of Mars. An alternative hypothesis for hydrogen
peroxide destruction on the analogs is that hydrogen peroxide
may have been destroyed by the trace organic matter present in
each soil. However, TOC analyses indicate that the organic
content of the B2 soil and the silica powder are 800 and less
than 30 ppm, respectively. The 30 ppm is probably insufficient
to account for destruction of all available hydrogen peroxide.
Further, results (not shown) obtained when hydrogen peroxide
is added to each analog followed immediately by VM1 show gas
evolution with B2 but not with silica powder. Thus, the
organic hypothesis is not consistent with the data. Against the
particulate theory, however, is the finding (Figure 27) that
Aliquots of 0.22 ml of 1.5 x 10^-1 M hydrogen peroxide were added under nitrogen at 760 mm Hg to empty liquid scintillation vials (●●●) or to vials containing either 0.5 cc Mars analog soil B2 (○○○) or 0.5 cc silica powder (△△△). The particle size of the B2 soil ranged from 10-100 μ whereas that for the silica powder ranged from 50-200 μ. After incubating for 3 hours under nitrogen at 760 mm Hg and room temperature, the vials were opened and 0.11 ml of VM1 added. Evolved 14CO2 was trapped with a Ba(OH)2 soaked getter pad. After drying, the pads were counted by the gas flow technique at approximately 10% efficiency.
hydrogen peroxide is stable to incubation at 23°C for 3 hours on 0.5 cc of γ-Fe₂O₃. The particle size of γ-Fe₂O₃ is also in the 10-100 μ range. Thus, although our preliminary results suggest that hydrogen peroxide may not survive on Mars, certain inconsistencies exist in the data which prevent a premature conclusion and necessitate further work with other analog soils and with hydrogen peroxide - soil complexes that might represent those present on Mars.

Even assuming that conditions could be defined whereby hydrogen peroxide is stable on fine Martian particulate material, other conditions on Mars may preclude the survival of hydrogen peroxide. In a hypothetical model of the aeronomy of the lower atmosphere of Mars, Parkinson and Hunten (56) and McElroy et al (32) have postulated that hydrogen peroxide is produced in the Martian atmosphere and freezes out on the surface of the planet. Hydrogen peroxide is highly susceptible to photolysis by ultraviolet light which penetrates to the Mars surface virtually unattenuated. The rate coefficients cited (32, 56), for the formation and destruction of hydrogen peroxide on Mars favor its destruction by a factor exceeding 10⁷. Freezing probably would not protect the compound from photolysis (52). However, the active agent on Mars is apparently stable to ultraviolet radiation as evidenced by its presence on or just
below the exposed Mars surface. Thus, in order for hydrogen peroxide to be the active Mars agent, some form of protection or complexing would be necessary to preserve the compound and form a UV stable, but thermolabile, complex or precursor complex. On the other hand, it should be noted that the stability of the active Mars agent apparently does not depend on direct ultraviolet radiation since activity observed with a sample taken from under a rock (VL-2, Cycle 3) was essentially identical with that obtained from an exposed surface sample (3).

D. TSM Studies with Injected Radioactive Gases

When a second injection of nutrient was added to an LR reaction on Mars, 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. We report here a series of experiments supporting a physico-chemical explanation, rather than a biological explanation, for that portion of the flight kinetics immediately following second nutrient injection. For these studies, the LR TSM 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 effects of wetting on the gas equilibrium between soil and headspace can be studied independent of biology. To experimentally distinguish between carbon monoxide and carbon dioxide as possible end products of the LR reaction on Mars, TSM studies have been performed with both $^{14}$C-labeled carbon monoxide and $^{14}$C-labeled carbon dioxide. The responses of each gas to liquid injections and to temperature cycles mimicking those observed in the test cell during the mission have been examined for possible matches to flight data.

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 test and detector 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.

All experiments were conducted at 10°C 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 10°C and 5 torr carbon dioxide for 2.5 hours, if desired, the soil was then sterilized according to the flight sequence by raising the temperature in the test cell to 160°C for 3 hours. After cooling for 9 hours at room temperature, the test cell was vented for four minutes and the soil then incubated at 10°C, the temperature of the Mars experiments, for an additional 1.5 hours.
Experiments were initiated by the injection of 100 μl radioactive gas into the test cell which provided a total cell pressure of approximately 7.5 torr. Subsequent injections of sterile distilled water were performed using the flight nutrient injection sequence (1) whereby the test cell was pressurized by two sequential additions of 18 torr helium each prior to injection of 0.115 cc water and 18 torr helium. At the end of the injection, the pressure was approximately 69 torr, including 10 torr from the vapor pressure of water. At this point, the test cell liquid and gas contents become 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.

Typical results obtained upon injection of 14C-labeled carbon dioxide into the empty test cell are shown in Figure 29. 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 an hour. A later injection of water into the test cell resulted in a spike of radioactivity followed by a decrease to a new plateau level.
Mars analog soil was prepared by desiccating for five 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 hours at 160°C according to the flight sequence. Radioactive carbon dioxide (specific radioactivity = 0.041mCi/m mole) 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.
somewhat lower than that observed prior to the injection. A second water injection also caused a brief spike and then 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 #1 present in the test cell, injected $^{14}$C-labeled carbon dioxide immediately began to disappear, reaching equilibrium with the soil at approximately 50 hours (Figure 29). 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 hours 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 runs. If corrections were made for the estimated leak rate, the results of both runs would be similar.

For soil pre-equilibrated with carbon dioxide, sterilization in the test cell for three hours at 160°C did not affect the results.

Upon injection of water into the test cell containing $^{14}$CO$_2$ in equilibrium with Mars analog soil #1, all soil samples released carbon dioxide such that the gas level in the headspace attained a new plateau level (Figure 29). In the case of soil pre-equilibrated with 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, $^{14}\text{CO}_2$ evolution following first water injection is not equivalent to $^{14}\text{CO}_2$ 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 $^{14}\text{CO}_2$ in equilibrium with Mars analog soil B2. The results of duplicate runs are compared in Figure 30 to results obtained in the presence and absence of Mars analog soil #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 $\gamma-\text{Fe}_2\text{O}_3$ amplifies changes in $^{14}\text{CO}_2$ levels, the changes differ from those obtained with the Mars analog soil #1.
Mars analog soils were prepared by desiccating for six days, sealing under unlabeled carbon dioxide, and adding to the TSM test cell. Radioactive carbon dioxide (specific radioactivity = 0.021mCi/m mole) was then injected into the TSM test cell in the presence and absence of 0.5 cc Mars Analog Soil No. 1 or B2, as indicated, to provide approximately 2700 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.
A similar series of TSM experiments was next conducted with $^{14}\text{C}$-labeled carbon monoxide (Figure 31). After injection of water into the TSM test cell containing $^{14}\text{CO}$ (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 #1 has no impact on these results. The failure of $^{14}\text{CO}$ to show changes in plateau level in response to water injections suggests that carbon monoxide was not the gas evolved on Mars.

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 (Figure 32). To determine the influence of temperature on levels of $^{14}\text{CO}_2$ or $^{14}\text{CO}$ present at the end of the experiments shown in Figures 29-31, the test cell temperature was cycled between $9^\circ\text{C}$ and $16^\circ\text{C}$ in a pattern approximating that on flight. With $^{14}\text{CO}$ present, no changes in gas level subsequently occurred either in the presence or absence of Mars analog soil. With $^{14}\text{CO}_2$ 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
Mars analog soil was prepared by desiccating for five days, sealing under either nitrogen or unlabeled carbon dioxide, and adding to the TSM test cell. Radioactive carbon monoxide (specific radioactivity = 0.21mCi/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 3500 nanomoles and 5000 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 injection sequence. Injections (I<sub>x</sub>) are indicated for each run where x indicates the xth injection into the test cell during the run. For the run receiving three injections, the third injection was with helium (the normal amount accompanying a water injection) only, without water.
Temperature data is obtained from VL-1, Cycle 1 approximately 93 lows after first nutrient injection.
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 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.

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 Figure 33 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.

A summary of percent increase observed for each of the spikes in Figure 33 is given in Table IV along with similar data derived from other TSM experiments in which Mars analog
Radioactive carbon dioxide (specific radioactivity = 0.041 mCi/mmole) or carbon monoxide (specific radioactivity = 0.021 mCi/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.
### TABLE IV

Effect of Injection Into LR Test Cell on Level of $^{14}$C-Gas

<table>
<thead>
<tr>
<th>TEST CELL CONTENTS</th>
<th>INSTRUMENT</th>
<th>TYPE INJECTION</th>
<th>% INCREASE</th>
</tr>
</thead>
<tbody>
<tr>
<td>$^{14}$CO$_2$</td>
<td>TSM</td>
<td>1 - Helium</td>
<td>44</td>
</tr>
<tr>
<td></td>
<td></td>
<td>2 - H$_2$O</td>
<td>20</td>
</tr>
<tr>
<td></td>
<td></td>
<td>3 - He helium</td>
<td>17</td>
</tr>
<tr>
<td>$^{14}$CO$_2$</td>
<td>TSM</td>
<td>1 - H$_2$O</td>
<td>32</td>
</tr>
<tr>
<td></td>
<td></td>
<td>2 - H$_2$O</td>
<td>14</td>
</tr>
<tr>
<td>$^{14}$CO</td>
<td>TSM</td>
<td>1 - H$_2$O</td>
<td>26</td>
</tr>
<tr>
<td></td>
<td></td>
<td>2 - H$_2$O</td>
<td>17</td>
</tr>
<tr>
<td>$^{14}$CO$_2$, Mars Analog Soil No. 1</td>
<td>TSM</td>
<td>1 - H$_2$O</td>
<td>83</td>
</tr>
<tr>
<td></td>
<td></td>
<td>2 - H$_2$O</td>
<td>14</td>
</tr>
<tr>
<td>$^{14}$CO$_2$, Mars Analog Soil *B2</td>
<td>TSM</td>
<td>1 - H$_2$O</td>
<td>49</td>
</tr>
<tr>
<td></td>
<td></td>
<td>2 - H$_2$O</td>
<td>16</td>
</tr>
<tr>
<td>$^{14}$CO, Mars Analog Soil No. 1</td>
<td>TSM</td>
<td>1 - H$_2$O</td>
<td>33</td>
</tr>
<tr>
<td></td>
<td></td>
<td>2 - H$_2$O</td>
<td>12</td>
</tr>
<tr>
<td>Mars Soil, $^{14}$C-gas</td>
<td>Lander 1, Cycle 1</td>
<td>2 - VML</td>
<td>15</td>
</tr>
<tr>
<td></td>
<td>Lander 1, Cycle 3</td>
<td>2 - VML</td>
<td>8</td>
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<tr>
<td></td>
<td>Lander 1, Cycle 3</td>
<td>3 - VML</td>
<td>5</td>
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<tr>
<td></td>
<td>Lander 2, Cycle 1</td>
<td>2 - VML</td>
<td>15</td>
</tr>
<tr>
<td></td>
<td>Lander 2, Cycle 3</td>
<td>2 - VML</td>
<td>14</td>
</tr>
</tbody>
</table>

Injections of either water, helium or LR nutrient (VML) 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.
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.

In conclusion, that part of the LR flight data observed following second nutrient injection can be accounted for by a physico-chemical explanation. 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. It is also of interest to note that the reaction with Mars analog soil #1 more closely simulate 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.
VI. STATUS OF BIOLOGICAL AND CHEMICAL INTERPRETATIONS OF LR FLIGHT DATA AND RECOMMENDATIONS FOR FUTURE WORK

The LR response on Mars following first nutrient injection is consistent with a biological interpretation and our results to date cannot eliminate living Martian microorganisms as the causative agents. Of the various nonbiological models examined, none are entirely consistent with the flight data, although physico-chemical explanations readily account for that portion of the data obtained following second nutrient injection. Nonbiological agents eliminated as candidate active agents for the LR response are ultraviolet irradiation, $\gamma$-Fe$_2$O$_3$, and metalloperoxides and superoxides. On the other hand, hydrogen peroxide could conceivably account for the initial active response except for certain inconsistencies between our laboratory results and the flight data which pose serious questions to this hypothesis. These may be summarized as follows:

1. TSM simulation experiments indicate that hydrogen peroxide alone as an active agent is still reactive with VM1 upon a second nutrient injection after 4 sols. On flight, the active agent was dissipated when nutrient was injected a second time after 7 sols.

2. Hydrogen peroxide can fulfill the thermal sensitivity required by the Mars active agent, provided
a suitable metal is present to catalyze partial decomposition at 50°C. On the other hand, in the TSM and therefore presumably on flight, hydrogen peroxide shows enhanced thermal sensitivity such that it is completely inactivated by 3 hours pre-heating at 50°C, perhaps due to volatilization rather than decomposition. Thus, hydrogen peroxide does not appear to account for flight data.

3. Hydrogen peroxide may not survive on the surface of Mars. This may reflect surface sample particle size as well as instability to the intense UV flux present on the surface of Mars. On the other hand, the active Mars agent is stable to UV but does not depend on direct UV irradiation for its existence. Nonetheless, the similarity of kinetic and thermal data obtained with hydrogen peroxide to flight data are tantalizing. It is possible that the cited discrepancies might be resolved if hydrogen peroxide could be complexed with soil in a manner that would reduce susceptibility to surface UV and, perhaps, prevent volatilization in the flight instrument at 50°C. Further, if a metal such as γ-Fe₂O₃ were a constituent of the complex, partial decomposition of hydrogen peroxide would be expected by heating for three hours at 50°C. Because hydrogen peroxide appears to be the only serious remaining contender for a non-
biological interpretation, it is important to determine whether or not the putative complex of hydrogen peroxide seems feasible. Thus, it is recommended that future laboratory efforts center on attempts to construct complexes between hydrogen peroxide and various Mars analog soils. Any such complexes should be examined for stability to thermal decomposition at 50°C and to ambient Martian environmental conditions.

Respectfully submitted,

Patricia Ann Straat, Ph.D.
Director of Research Services

Approved by:

Gilbert V. Levin, Ph.D.
Principal Investigator
VII. ACKNOWLEDGEMENTS

We gratefully acknowledge the excellent technical assistance of Cynthia Ann Waldman in reduction of flight data, Jon Calomiris in performing TSM experiments, and Kim Schroader in performing getter experiments. We also wish to thank Donald G. Shaheen and Dr. John Edwards for suggestions and helpful discussions related to the thermal analysis reported herein.


46. Levin, G.V. and Straat, P.A., unpublished data.


Appendix I:

Composition of Mars Analog Soils
Composition of Mars Analog Soils

Two Mars analog soils were prepared for the Viking Biology Team by the Viking Inorganic Analysis Team to match, as closely as possible, the inorganic X-ray fluorescence analysis obtained from Mars. The first soil, called Mars analog #1, was prepared following tentative analysis of the data from Chryse, the first landing site on Mars. As the Mars data became more refined, the second analog, called B2, was prepared. Although the soils have several minor differences, the most significant differences are that the iron in B2 is approximately 11% gamma Fe$_2$O$_3$ whereas that in #1 consists mainly of alpha Fe$_2$O$_3$ and the sulfate content in B2 is about two-fold higher than in #1. Both soils have a pH of approximately 7.2 and a particle size range of 10 – 100 µ.

According to A.K. Baird (Viking Inorganic Analysis Team, personal communication), the weight percent mineral composition of the two soils is as follows:

<table>
<thead>
<tr>
<th>Mineral</th>
<th>#1</th>
<th>B2</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nontronite</td>
<td>51.1</td>
<td>17.5</td>
</tr>
<tr>
<td>Bentonite</td>
<td>25.5</td>
<td>29.8</td>
</tr>
<tr>
<td>Kieserite (MgSO$_4$)</td>
<td>9.4</td>
<td>18.5</td>
</tr>
<tr>
<td>Quartz (SiO$_2$)</td>
<td>--</td>
<td>14.4</td>
</tr>
<tr>
<td>Calcite (CaCO$_3$)</td>
<td>6.0</td>
<td>7.0</td>
</tr>
<tr>
<td>Maghemite (γ-Fe$_2$O$_3$)</td>
<td>--</td>
<td>11.1</td>
</tr>
<tr>
<td>Hematite (γ-Fe$_2$O$_3$)</td>
<td>5.0</td>
<td>--</td>
</tr>
<tr>
<td>Magnetite (Fe$_3$O$_4$)</td>
<td>3.0</td>
<td>--</td>
</tr>
<tr>
<td>Rutile (TiO$_2$)</td>
<td>--</td>
<td>1.0</td>
</tr>
<tr>
<td>Halite (NaCl)</td>
<td>--</td>
<td>0.8</td>
</tr>
</tbody>
</table>
Nontronite, a naturally occurring clay mineral from a quarry at Riverside, California, was collected in separate lots for each soil. Bentonite is a commercial grade material obtained from Wyoming, and maghemite was obtained from Memorex Corporation who certify it as pure gamma iron oxide. All other minerals are reagent grade chemicals. Analog soils were prepared by mixing these minerals in the proportions shown above for 24 hours in homogenizing roller mills. The resulting mixtures yield x-ray spectrographic results in flight-like Viking instruments that closely approximate spectra obtained from Mars.

The elemental analyses for the two Mars analog soils are given below along with that of bentonite and nontronite. By convention, chemical analyses are reported as weight percent oxides, although the elements are not necessarily present as oxides. Total iron is reported as Fe$_2$O$_3$ but includes Fe$_3$O$_4$.

<table>
<thead>
<tr>
<th>Oxide</th>
<th>#1</th>
<th>B2</th>
<th>Bentonite</th>
<th>Nontronite</th>
</tr>
</thead>
<tbody>
<tr>
<td>SiO$_2$</td>
<td>39.5</td>
<td>42.2</td>
<td>63.5</td>
<td>50.5</td>
</tr>
<tr>
<td>Al$_2$O$_3$</td>
<td>7.0</td>
<td>5.9</td>
<td>17.3</td>
<td>4.0</td>
</tr>
<tr>
<td>Fe$_2$O$_3$</td>
<td>21.7</td>
<td>17.0</td>
<td>2.7</td>
<td>28.9</td>
</tr>
<tr>
<td>MgO</td>
<td>7.3</td>
<td>7.0</td>
<td>1.5</td>
<td>2.2</td>
</tr>
<tr>
<td>CaO</td>
<td>7.0</td>
<td>5.7</td>
<td>1.4</td>
<td>7.7</td>
</tr>
<tr>
<td>Na$_2$O</td>
<td>0.5</td>
<td>0.6</td>
<td>1.7</td>
<td>0.5</td>
</tr>
<tr>
<td>K$_2$O</td>
<td>0.2</td>
<td>0.2</td>
<td>0.6</td>
<td>0.1</td>
</tr>
<tr>
<td>TiO$_2$</td>
<td>0.3</td>
<td>1.1</td>
<td>0.2</td>
<td>0.5</td>
</tr>
<tr>
<td>P$_2$O$_5$</td>
<td>0.02</td>
<td>0.1</td>
<td>0.1</td>
<td>0.2</td>
</tr>
<tr>
<td>SO$_3$</td>
<td>5.5</td>
<td>12.5</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Cl</td>
<td>--</td>
<td>0.5</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>CO$_2$</td>
<td>2.6</td>
<td>3.0</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>

Values for bentonite and nontronite represent chemical analytical data whereas values for Mars analog soils #1 and B2 have been calculated from the composition of the constituent minerals.
assuming stoichiometry of the constituents. For each sample, most of the remaining weight is water.