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FINAL REPORT
DEVELOPMENT OF BIOLOGICAL AND NONBIOLOGICAL EXPLANATIONS FOR THE VIKING LABELED RELEASE DATA

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I. **Summary**

This report covers the final year of an intensive three-year post-Viking effort to develop nonbiological explanations of the positive responses obtained on Mars by the Labeled Release life detection experiment. During the study, a variety of hypotheses were explored. All were contraindicated by experimental evidence with the exception of one which postulates hydrogen peroxide as the active agent. During this past year, an intensive investigation was continued to examine the plausibility that hydrogen peroxide, widely distributed within the Mars surface material, was responsible for the evocative responses obtained by the Viking Labeled Release (LR) experiment on Mars.

Our results have shown that hydrogen peroxide in aqueous solution at a concentration of $10^{-1}$ M can react with the LR nutrient in a flight-like instrument to produce a positive response similar to that obtained during the mission. The hydrogen peroxide concentration required to produce the positive response can be lowered by an order of magnitude or more, if a metal catalyst is introduced to stimulate the reaction. Also, it is dissipated by three hours heat treatment at 160°C to fulfill the "control requirement". However, to make hydrogen peroxide in a flight-like instrument fulfill the thermal criteria of the Viking LR is more difficult. When heated for three hours at 50°C, it is volatilized out of the test cell so that, on flight, it should not have been available for subsequent reaction with the LR nutrient. Experiments conducted by placing
hydrogen peroxide on an appropriate soil sample partially stabilized it to volatilization at 50°C. Thus, if an appropriate complex exists on Mars between hydrogen peroxide and the surface material, it would be possible to obtain partial reduction of activity with the LR nutrient following the 50°C heating regime.

With regards to stability, hydrogen peroxide at room temperature is stable on low pH soils but not on neutral or alkaline soils. Lowering the temperature to -70°C preserves hydrogen peroxide on neutral or high pH soils, suggesting that if a mechanism exists for formation of hydrogen peroxide on Mars, then the low ambient surface temperatures would contribute to its stability and accumulation. After low temperature storage on select soil, hydrogen peroxide can partially survive the subsequent temperature rise to 10°C for the two sols prior to nutrient addition. However, we estimate that this would require concentrations of hydrogen peroxide on the surface of Mars in the order of 1.0M. Further, the destructive effect on hydrogen peroxide of the ultraviolet flux incident to the Mars surface would have to be mitigated. No solution to this problem has been found.

Two major problems stand in the way of the hydrogen peroxide theory: (1) demonstration that it is formed or deposited on the Mars surface in quantities sufficient to account for the LR flight data (no experimental evidence has yet accounted for largescale hydrogen peroxide formation) and (2) since hydrogen peroxide is highly susceptible to ultraviolet radiation, some
yet unknown method for preserving it on the surface in considerable excess over that required for the LR experiment must be postulated. In order for the hydrogen peroxide theory to be compatible with laboratory and flight data, the Mars surface material must catalyze the reaction between hydrogen peroxide and the LR nutrient, and must also form a complex with hydrogen peroxide that will stabilize it to ultraviolet decomposition, volatilization at 50°C, and to storage for two sols at 10°C. Although we report on a mixture of gamma Fe₂O₃ and silica sand that stimulated the LR nutrient reaction with hydrogen peroxide and reduced the rate of hydrogen decomposition under various storage conditions, the Mars analog soil prepared by the Viking Inorganic Analysis Team to match the Mars analytical data does not cause such effects. Nor is adequate resistance to UV irradiation shown.

On the basis of the results and consideration presented herein, while the hydrogen peroxide theory remains the most, if not only, attractive chemical explanation of the LR data, it remains unconvincing on critical points. Until problems concerning the formation and stabilization of hydrogen peroxide on the surface of Mars can be overcome, adherence to the scientific evidence requires serious consideration of the biological theory.
II. Introduction

The Viking Labeled Release (LR) life detection experiment on the surface of Mars produced data consistent with a biological interpretation (1-3). In considering the plausibility of this interpretation, terrestrial life forms have been identified which could serve as models for Martian microbial life (4) and analysis of the landscape at the Mars landing site has indicated color and feature changes that may be attributable to biological activity (5). 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, several nonbiological explanations have been advanced as alternate explanations of the Viking LR data. Most of these nonbiological theories, such as reactions caused by or with ultraviolet radiation, gamma Fe₂O₃, metalloperoxides or superoxides, have been largely discounted on the basis of our earlier investigations (4,6). Reaction of the LR nutrient with putative surface hydrogen peroxide remains the leading nonbiological candidate. Hydrogen peroxide has been shown to react with the LR nutrient to produce responses in some respects similar to those observed on Mars (4).

Unlike the biological possibility, the hydrogen peroxide theory can be intensively examined on Earth under controlled experimental conditions to determine its probability. In order for hydrogen peroxide to account for the flight data, it must fulfill the following four criteria:
It must produce an active flight-like response with the LR nutrient under simulated flight conditions.

It must display thermal sensitivity to 160°C, 50°C and to long-term storage at 10°C similar to that observed (1-3) on flight.

A mechanism must exist for its formation and accumulation in sufficient quantities to account for the magnitude of the flight response.

A mechanism must exist for its stability on the Mars surface material even in the presence of intense ultraviolet radiation.

During this contract period, we have experimentally examined each of these issues in an attempt to develop the hydrogen peroxide explanation as far as possible. These data, their interpretations, and their adequacy in accounting for flight data from the Viking Labeled Release experiment are presented in detail herein, evaluated, and briefly compared to the adequacy of a biological explanation for the LR data.
III. Reactivity of Hydrogen Peroxide with Labeled Release Nutrient

Experiments have been conducted in the LR Test Standards Module (TSM), an instrument replicating flight configuration in all essential components (7), to determine whether hydrogen peroxide can mimic the Mars active agent by producing a positive LR response upon addition of the LR nutrient. In these experiments, 0.5 cc hydrogen peroxide were added to the LR TSM test cell and equilibrated for several hours at 10°C under 5 torr carbon dioxide; the hydrogen peroxide concentration was such that the final concentration would be $10^{-1}$M after the addition of 0.1 ml nutrient. The results following the addition of VM1 are reproduced from an earlier report (4) in Figure 1. As shown, both the kinetics and magnitude of the subsequent $^{14}$C-labeled gas evolution are similar (but not identical) to those observed during the Viking mission. Upon injection of additional nutrient, after approximately 120 hours of incubation, a brief spike precedes a faster rate of gas evolution. Although the spike resembles flight data, the subsequent increased response contrasts sharply with the absorption of headspace gas observed in flight data. In the TSM, nutrient appeared to limit the TSM response following first nutrient injection. This is in contrast to the flight data in which the active Mars agent appears to be absent at the time of the second injection.
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.
Although hydrogen peroxide at a concentration of $10^{-1}$M can react with the LR nutrient in a flight-like instrument to produce a flight-like active response, it is not likely that hydrogen peroxide could be present on the surface of Mars at such high concentrations. However, it is known from previous work (4) that the hydrogen peroxide reaction with LR nutrient is stimulated by metal catalysts. Thus, in the presence of Mars metal catalysts, it may be possible to produce a response of the magnitude observed in Figure 1 with lesser amounts of hydrogen peroxide.

Figure 2 presents the results of experiments designed to determine how far the hydrogen peroxide concentration can be lowered in the presence of metal catalyst and still produce a response of flight magnitude. Because time constraints preclude use of the TSM, these experiments were performed by the getter technique. Thus, hydrogen peroxide was added to a 0.5 cc sample of a Mars analog soil contained in a sterile screw cap vial. The soil selected was a mixture of gamma Fe$_2$O$_3$ and silica sand (15/85%) on which hydrogen peroxide was found to be stable (see later). After equilibration in a glove box at room temperature under 760 mm nitrogen, 0.1 ml of LR nutrient was added to each test vial and the vials immediately capped with a Ba(OH)$_2$-soaked getter pad. Getter pads were changed at intervals, dried under an infrared lamp, and counted for evolved radioactivity in a Nuclear Chicago gas flow counter. Counting
Screw cap vials containing 0.5 cc of a mixture of gamma Fe$_2$O$_3$/silica sand (15/85%) were placed in a glove box equilibrated under one atmosphere of nitrogen at room temperature and 0.22 ml of a hydrogen peroxide solution was added to each vial. Immediately thereafter, each vial received 0.11 ml of the LR nutrient. The concentration of hydrogen peroxide in each vial was such that after the nutrient addition the final concentration would be $10^{-1}$ M (●—●), $10^{-2}$ M (▲—▲), $10^{-3}$ M (■—■), or $10^{-4}$ M (▼—▼), as indicated. Evolved radioactive gas was collected on Ba(OH)$_2$-soaked getter pads which were changed at various intervals and counted by the gas flow technique at approximately 10% counting efficiency. Results are compared to those obtained from LR nutrient and $10^{-1}$ M hydrogen peroxide in the absence of soil (○—○) and from nutrient alone (x—x). All reactions were conducted in duplicate. The scale on the left gives raw data obtained whereas the scale on the right gives normalized counts had the experiment been conducted either in the TSM or in a flight instrument.
efficiency was estimated at 10% versus the 3% counting efficiency of the solid state beta detectors in the TSM and flight instruments.

Results obtained by mixing LR nutrient, gamma Fe$_2$O$_3$, and varying concentrations of hydrogen peroxide are compared in Figure 2 to a control in which LR nutrient was added to 10$^{-1}$M (final concentration) hydrogen peroxide in the absence of a metal-containing soil. Because the conditions of this control are similar to the same control used in Figure 1, this control permits approximate normalization of Figure 2 data to the data presented in Figure 1 which was obtained with the TSM and with the flight instruments. (It should be noted that differences in kinetics between Figures 1 and 2 are in part attributed to differences in test cell geometry between the two systems and in part to the fact that evolved radioactive gas in the TSM remains in equilibrium with the soil whereas it is removed from the headspace in a getter experiment.)

The results in Figure 2 show that the metal catalyst (gamma Fe$_2$O$_3$) significantly stimulates the reaction between hydrogen peroxide and the LR nutrient. Although difficult to quantitate the relationship between results in Figures 1 and 2, it nonetheless appears that the hydrogen peroxide concentration can be lowered to the 10$^{-3}$ to 10$^{-2}$M range in the presence of a metal catalyst and still produce a positive response of flight magnitude with LR nutrient. Further, for all concentrations of
hydrogen peroxide, the kinetics of gas evolution in the presence of the metal catalyst are initially faster and then slower than those observed with $10^{-1}$M hydrogen peroxide in the absence of a metal catalyst. This shape was also observed with flight data (see Figure 1). It is concluded that, in the presence of a suitable metal catalyst, hydrogen peroxide can fulfill the first criteria of the Mars active agent, namely, that of mimicking the LR active response in the presence of a metal catalyst.
IV. Thermal Sensitivity of Hydrogen Peroxide in the TSM

Labeled Release data returned from Mars showed that the active response with the LR nutrient was obliterated by pretreatment of the Mars surface material at 160°C for three hours. The active agent was also partially destroyed by heat treatment for three hours at 50°C, a finding that gave strong support to the biological interpretation. In order for hydrogen peroxide to be the active agent on Mars, it must show similar patterns of thermal sensitivity.

A. Liquid Hydrogen Peroxide

Hydrogen peroxide is known (8,9) to be decomposed at 160°C although it would be stable to the thermal regime at 50°C. This has been verified by our previous experimental results (4) in which hydrogen peroxide stability to various pretreatments was measured by its subsequent ability to react with the LR nutrient in a getter-type experiment. Under 10 torr nitrogen, hydrogen peroxide was destroyed by three hours heating at 160°C but not by three hours heating at 50°C. However, in the presence of a metal catalyst such as gamma Fe₂O₃, hydrogen peroxide could be either partially or totally decomposed at 50°C, depending on the amount of iron present with the hydrogen peroxide during the heat treatment. From these results, it was initially concluded that hydrogen peroxide could meet the thermal criteria established for the active agent in the Mars surface material.
Similar studies have been conducted this year in the LR TSM in order to take into account any peculiarities of the flight instrument. In these experiments, liquid hydrogen peroxide was added to the LR TSM test cell and equilibrated at 5 torr carbon dioxide. The test cell was then sealed and, as appropriate, the hydrogen peroxide subjected to a thermal pretreatment essentially identical to that conducted on flight. After then cooling to 10°C and equilibrating at that temperature for several hours, LR nutrient was injected into the test cell via the flight injection sequence (7) and the hydrogen peroxide activity present was monitored by the amount of radioactive carbon dioxide subsequently evolved. As in the flight instrument, radioactive gas was counted at 3% efficiency.

The results of the hydrogen peroxide thermal sensitivity studies in the TSM are presented in Figure 3. As shown, pre-treatment for three hours at 160°C, 50°C, and even 40°C, essentially destroys or renders hydrogen peroxide unavailable for reaction with the LR nutrient. Some unexplained residual gas evolution occurred later in the time course following nutrient injection onto a 160°C treated sample.

This unexpected sensitivity of hydrogen peroxide to heat treatment in the TSM, reported previously (4), has been the subject of considerable investigation during this contract period.
A 0.5 cc aliquot of $1.2 \times 10^{-1}$ M hydrogen peroxide was adjusted to pH 6.4 (same pH as LR nutrient pH) and added to the TSM test cell. After equilibration in the sealed test cell at 10°C under 6 torr carbon dioxide, the sample was heated for 3 hours at 160°C (---), 50°C (---), 40°C (---), or 23°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 (-----).
Thus, the results have been extended (Figure 3) to show partial reduction (approximately 50%) of subsequent hydrogen peroxide activity with LR nutrient following three hours exposure of hydrogen peroxide to temperatures as low as 23°C. This extreme temperature sensitivity in the TSM is inconsistent with known properties of hydrogen peroxide (8,9) and with our previous results (4) and suggests that hydrogen peroxide in the flight instrument would be too temperature sensitive to be the active agent which produced the LR results on Mars.

In pursuing an explanation for the enhanced thermal sensitivity of hydrogen peroxide in the TSM, the hypothesis that the test cell material (stainless steel) may catalyze the thermal decomposition has been considered. However, in a separate experiment, hydrogen peroxide was added to each of four test cells. One was the TSM stainless steel test cell and the other three were of identical size and configuration but composed of glass (made for special-purpose TSM studies). The test cells were sealed with Parafilm under one atmosphere air; glass cells were maintained for three hours at 10°C, 23°C, or 40°C, whereas the stainless steel cell was maintained for three hours at 40°C. After the cells equilibrated at room temperature, the Parafilm was removed and LR nutrient added to the pretreated hydrogen peroxide. Evolved radioactive gas was collected with barium hydroxide-soaked getter pads which were then dried and counted by liquid scintillation. The results showed that hydrogen
peroxide activity was unimpaired in each test cell, demonstrating that the material comprising the TSM test cell was not responsible for the enhanced thermal sensitivity observed during the flight sequences for heat treatment. In confirmation of this conclusion, a large volume of hydrogen peroxide was heated in the TSM test cell for three hours at 50°C according to the flight sequence. After cooling to 10°C, the test cell was brought to one atmosphere, opened, and the hydrogen peroxide removed by pipette. A portion was then added to a glass cell and tested for activity by adding LR nutrient and collecting evolved gas by the getter technique. A second portion was added back into the TSM test cell, equilibrated under 5 torr carbon dioxide, and tested for activity with VM1 added by the normal nutrient injection sequence. Both portions were fully active. The results show that heat treatment per se at 50°C in the TSM does not decompose hydrogen peroxide. This is in accordance with published data (8,9) on the thermal sensitivity of hydrogen peroxide.

A possible explanation for these observations is that during the heat treatment, hydrogen peroxide was volatilized out of the test cell to a colder part of the LR TSM. Candidate areas include valve seats (e.g., S/52) in the head-end of the test cell and the long swanneck tube leading to the solid state detectors. (Detailed descriptions of the TSM instrument have been presented earlier (7).) By the time nutrient was injected,
hydrogen peroxide was no longer present in the test cell and the resulting gas evolution pattern was typical of a nutrient-alone response. However, this explanation appeared tentative in that, at the end of each TSM experiment, the test cell had been opened and the volume contained therein was measured. Approximately 70 - 80% of the calculated total volume (0.5 ml hydrogen peroxide plus 0.1 ml nutrient) was routinely recovered and shown to be radioactive, thereby confirming that injection had occurred.

It was next theorized that, perhaps, at the end of the TSM experiment, the sequence of operations for opening the test cell may have driven volatilized hydrogen peroxide back into the test cell where it then recondensed.

A series of experiments was conducted in the TSM to test this hypothesis. In these experiments, 0.5 ml hydrogen peroxide was added to the test cell and heated to 50°C under Mars-like conditions according to the flight sequence. After performing various operations in the sequence, the test cell could be opened for observations of liquid present. This was accomplished under Mars conditions by dropping the test cell and observing it through the glass bell jar, or by opening the test cell after bringing it and the bell jar to atmospheric pressure, removing the bell jar, and measuring the liquid present by pipette. The significant findings from many experiments are summarized below (refer to Figure 4 for valve location):
Figure 4

SCHEMATIC OF THE Labeled RELEASE EXPERIMENT

![Diagram of the labeled release experiment]

Legend:
- Valve
- Heater
1. If after cooling to 10°C, the test cell is vented out through S/52 to Mars atmosphere, and then both bell jar and test cell (via S/52) are simultaneously raised to one atmosphere, almost full volume is recovered upon opening the test cell.

2. If after cooling to 10°C, the test cell venting to Mars pressure through S/52 is omitted, and both bell jar and test cell pressures are raised to one atmosphere together through S/52 prior to opening the cell, no liquid is recovered.

3. If the test cell is lowered while heating at 50°C, no liquid is observed in the test cell. If the test cell is then closed and cooled to 10°C, and the bell jar pressure lowered, reopening the test cell "pulls" liquid out of the swanneck and the S/52 valve seat back into the test cell.

4. If foil is placed across the head-end, thereby preventing volatilization into other TSM regions during heating, all of the liquid is recovered after the sequence. Without the foil, the best recovery is about 70-80%.

5. Lowering the test cell after the S/52 vent to Mars pressure results in only partial recovery at this stage. Some liquid is found in the test cell and some is apparent around the openings into the test cell.
These results confirm the hypothesis that hydrogen peroxide is volatilized out of the test cell during 50°C heat sterilization and returns to the test cell only after the cell is vented through S/52 and opened to atmospheric pressure. However, it should be noted that, because these experiments deviate from the flight sequence whenever the test cell is opened or the pressure changed, some extrapolation is necessary. Thus, it has not been definitely established that any liquid, actually drops into the test cell after venting through S/52. Study of similar phenomena following heating at 160°C is complicated by the fact that the test cell cannot be lowered without briefly heating to prevent damage to the seal. However, that no such re-entry of liquid hydrogen peroxide occurs is indicated by the kinetic data following nutrient injection (Figure 3), although the late residual gas evolution after the 160°C sterilizations could reflect small re-entries into the test cell.

In considering the implications of these findings to flight data, it should be noted that in the flight sequence, heating at either 160°C or 50°C is followed by cooling to 10°C and a four minute vent to Mars atmosphere through S/52 prior to nutrient injection. If the results obtained in the TSM are applicable to the flight instrument, then hydrogen peroxide would have been totally removed from the flight test cell in the heat sterilization portion of the process. Venting through S/52 may have "pulled" and condensed hydrogen peroxide onto the head end area rather than into the test cell bottom where it could have reacted with the LR nutrient.
Thus, by this analysis, hydrogen peroxide cannot account for the Mars active agent unless it is complexed with the Mars soil in such a manner as to stabilize it to volatilization.

The applicability of these conclusions to the LR flight data is dependent upon the degree of similarity of the TSM configuration to that of the flight instrument. On the LR flight instrument, the main test cell heater (H-21) is contained in the head end assembly and a "trim" heater is located in the valve block. Both heaters must be used to achieve 160°C sterilization temperatures although the trim heater alone was used on flight to obtain the 50°C sterilization. The flight instrument also contains a detector heater (H-25) which is heated to approximately 110°C during sterilization. The 13 inch swan-neck line between the test cell and the detector is unheated but is probably at the temperature of the LR module (approximately 10°C) although it could increase somewhat during sterilization due to conduction from the head end and detector heaters.

The major difference between the flight instrument and the TSM is in the heaters. In all other respects, the TSM is essentially identical to the flight instrument. The TSM has both a detector heater and a head end heater, similar to those on flight. In addition, there is also an auxiliary heater strapped around the test cell. To obtain sterilization temperatures of 160°C, both the auxiliary heater and the head end heater are used.
along with the detector heater. For the 50°C heating on the TSM, only the head end heater was used (along with the detector heater). The line between the test cell and the detector is at room temperature (approximately 23°C) although it is subject to heating by conduction during sterilization. The information regarding the differences between the flight instrument and the TSM thermal patterns during the 50°C heating is summarized in Table I. The major difference is that on the flight instrument, the valve block temperature during the heating cycle is higher than the head end whereas the reverse is true in the TSM experiments. In considering the importance of this difference, we located an obsolete TSM trim heater on the back of the underside of the TSM valve block. Consultation with TSM engineers (10) established that the location, although not identical with that of the flight instrument, closely approximated it. After connecting this heater, an experiment was performed in which only the TSM valve block heater was used to obtain temperatures of 50°C. These experiments confirm that the hydrogen peroxide originally contained in the test cell had disappeared or lost all activity by the time VM1 was subsequently injected in the flight injection sequence. It is concluded that the experiments conducted in the TSM with the auxiliary heater showing hydrogen peroxide volatilization are directly applicable to the flight instrument. This analysis would predict that no
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hydrogen peroxide could have remained in the LR test cells on Mars after the 50°C heatings to explain the responses obtained.

B. Effect of Various Mars Analog Soils on Hydrogen Peroxide Stability to 50°C

The complete volatilization of hydrogen peroxide out of the TSM test cell during the 50°C heating regime (see preceding section) would be expected to occur in the Viking instrument on Mars. However, to press the matter further, it was theorized that some mechanism exists on Mars to stabilize hydrogen peroxide to volatilization at 50°C while partially decomposing it through metal catalysis. The only apparent mechanism would be formation of a complex between hydrogen peroxide and the Mars surface material (which contains approximately 18% iron oxide).

Experiments conducted this quarter have examined the ability of gamma Fe$_2$O$_3$ and silica sand (analytically determined to be present on Mars, reference 11), to stabilize hydrogen peroxide. In each experiment, 0.5 cc of soil sample were placed in the TSM test cell and 0.25 ml of hydrogen peroxide added (final concentration after nutrient addition = 10^{-1}M). The test cell was sealed and the mixture equilibrated at 10°C under 5 torr carbon dioxide. As appropriate, the test cell was then heated for three hours at 50°C and then re-cooled to 10°C prior to nutrient addition; control samples were incubated for an equal period of time at 10°C prior to nutrient addition. LR nutrient (0.115 ml) was then injected onto the mixture via the normal flight injection sequence and the evolved $^{14}$C-labeled gas was
followed as a monitor of hydrogen peroxide activity. Counting efficiency in the TSM is approximately 3%.

Initial experiments were conducted with samples of pure silica sand (pH 6.9) or with pure gamma Fe₂O₃ (pH 3.0). (Separate experiments reported in later sections had already established that hydrogen peroxide was stable at room temperature for three hours on both samples and that neither sample showed biological activity with LR nutrient alone.) As shown in Figure 5, hydrogen peroxide was stable on both samples for the nine hour incubation period at 10°C; silica sand had essentially no effect on subsequent reactivity with the LR nutrient whereas the iron sample significantly stimulated the reaction, as reported earlier (4). However, the 50°C heat treatment of hydrogen peroxide in contact with either sample resulted in total loss of all activity upon subsequent addition of the LR nutrient, indicating that neither sample had stabilized hydrogen peroxide to volatilization during heating.

The ambient Mars temperature suggested yet a further attempt to generate a heat-stable complex between hydrogen peroxide and a Mars analog soil. Accordingly, experiments were conducted in which hydrogen peroxide was added onto the soil sample frozen at -30°C. A liquid nitrogen bath was placed around the TSM test cell to accomplish the cooling. Hydrogen peroxide added to a soil sample contained in the cooled TSM test cell froze instantly upon contact. The test cell was then held at -30°C
Samples of 0.5cc of either γFe₂O₃ or of silica sand were added to the TSM test cell under an atmosphere of carbon dioxide. After sample equilibration at either 10°C or at -30°C, as indicated in parentheses, 0.3 ml of a 1.4 x 10⁻³ M hydrogen peroxide solution (pH 6.5) was added. The test cell was quickly sealed under 5 torr carbon dioxide and incubation continued for an additional three hours at either 10°C or -30°C. All samples designated for "active" sequences were then maintained at 10°C for an additional six hours whereas samples designated for "sterile" sequences were heated to 50°C for three hours and then cooled to 10°C for an additional three hours. After the pre-incubation regime, 0.115 ml of LR nutrient was injected via the flight sequence and subsequently evolved radioactivity monitored. Assuming complete mixing of nutrient and hydrogen peroxide, the final hydrogen peroxide concentration was 10⁻³ M. The results with γFe₂O₃ or with silica sand are compared to those obtained with 10⁻³ M hydrogen peroxide alone (no soil) after a nine hour pre-incubation at 10°C. All data have been corrected for background counts.
for an additional three hours after which the temperature was raised to 10°C with the test cell sealed under 5 torr carbon dioxide. The sample was then heated for three hours at 50°C according to the "cold sterilization" flight regime. After again cooling the sample to 10°C, LR nutrient was added via the flight injection sequence. A control similarly treated at -30°C was maintained at 10°C for a time period comparable to that used during the 50°C regime. The results obtained with pure gamma Fe₂O₃ (Figure 5) indicate that freezing had no appreciable effect on hydrogen peroxide activity with the LR nutrient; further, no complex formation occurred to stabilize hydrogen peroxide to volatilization at the 50°C treatment.

The possibility that complex formation with hydrogen peroxide could require an analog soil that contains both silica and a metal component was then examined using a mixture of 15% gamma Fe₂O₃ and 85% silica sand. Hydrogen peroxide was added to 0.5 cc samples of this mixture contained in the TSM test cell and pre-equilibrated at either 10°C or at -30°C. For each pre-equilibration temperature, samples were also examined with and without subsequent heat treatment for three hours at 50°C. The results of these experiments are shown in Figure 6. As shown, for samples not heated at 50°C, hydrogen peroxide pre-equilibrated at either 10°C or at -30°C with the iron/silica sand mixture reacted when LR nutrient was added. (The lower
Experimental conditions and procedures are identical to those described for Figure 1 except that the 0.5cc sample consisted of a mixture of 15% Fe₂O₃ and 85% silica sand. One run, as indicated, had a test cell leak although the magnitude of the leak could not be determined. The results obtained are thus lower than would have been observed in the absence of this leak.
reactivity of the sample pre-equilibrated at 100°C reflects an observed leak in the test cell.) For samples subjected to the 50°C heat regime, a differential effect on hydrogen peroxide is observed depending on the pre-equilibration temperature. Following 100°C pre-equilibration, no reactivity occurred with the LR nutrient. However, for the -30°C pre-equilibration, a moderate level of reactivity was observed after the 50°C heating. This apparent inhibition of the active agent is reminiscent of that observed on Mars. This result could only be obtained if the sought-for complex between hydrogen peroxide and the iron/silica sand mixture had, indeed, formed at -30°C such that the resulting complex was stabilized against subsequent volatilization at 50°C. The partial reactivity observed could reflect either partial stabilization or total stabilization coupled with partial decomposition by metal catalysis. Thus, if soil composition and conditions on Mars permit this mechanism to operate, and if sufficient quantities of hydrogen peroxide accumulate on Mars, hydrogen peroxide could be responsible for the 50°C heating response as well as for the active and the 160°C sterilization response obtained on Mars.

In an effort to gain further support for this concept, similar experiments have been conducted in the TSM with the Mars analog soil B2. This soil was prepared by the Viking Inorganic Analysis Team to match the analytical spectra of
the Mars surface material (11) containing approximately 42% SiO$_2$ and 17% iron oxide, approximately 11% of which is gamma Fe$_2$O$_3$ (12). The B2 analog soil was shown to be biologically sterile by adding LR nutrient to a 0.5 cc sample contained in the TSM test cell. Subsequent experiments were conducted with hydrogen peroxide added to the B2 soil and pre-equilibrated at -30°C. However, as had been found earlier (4) in experiments in which hydrogen peroxide was added to the B2 soil at room temperature, contact with the frozen sample resulted in rapid decomposition of hydrogen peroxide. Thus, if this model of the Mars soil is correct, any hydrogen peroxide present on Mars could not have been stabilized by the low temperature.
V. Stability of Hydrogen Peroxide on Various Mars Analog Soils

A. Room Temperature Studies

Preliminary experiments reported earlier (4) indicated that hydrogen peroxide may not be stable on the surface of Mars. It was shown that three hours of contact with Mars Analog B2 or with a fine glass powder destroyed hydrogen peroxide. The fine particle size of each analog soil (from 10-100 μ for the B2 soil and from 50-200 μ for the glass powder) supports the suggestion of Edwards (13) that hydrogen peroxide may not be stable on the fine surface material of Mars. An alternate hypothesis was that hydrogen peroxide may have been destroyed by the trace organic matter present in the analog soils. However, TOC analyses indicated that the organic content of the B2 soil and the glass powder are 800 ppm and less than 30 ppm, respectively; the 30 ppm is probably insufficient to account for destruction of all hydrogen peroxide present. Thus, the organic contaminant hypothesis does not appear consistent with the data. Attempts to remove organics from the Mars Analog Soil B2 did not improve subsequent hydrogen peroxide stability on those soils. Casting doubt on size theory, however, is the finding (4) that hydrogen peroxide is stable to incubation for three hours on gamma Fe₂O₃ despite its particle size range of 10-100 μ.
Although the preliminary results suggested that hydrogen peroxide may not survive on Mars, the perplexing flight data prompted a further investigation into the stability of hydrogen peroxide on candidate Mars analog soils. In getter-type experiments, 0.5 cc of the analog soil was added to a sterile glass vial and equilibrated in a room temperature glove box under a nitrogen atmosphere at 760 mm Hg. To each soil were added 0.22 ml of a $1.5 \times 10^{-1}$M aqueous solution of hydrogen peroxide (adjusted to approximately pH 6.5) followed, either immediately or after three hours, by 0.11 ml of LR nutrient (pH 6.4). After the nutrient addition, the vials were sealed and evolved $^{14}\text{CO}_2$ was trapped with a Ba(OH)$_2$-soaked getter pad placed inside the caps. At intervals, the pads were replaced with fresh pads and the exposed pads dried and counted by the gas flow technique (approximately 10% efficiency).

Six Mars analog soils were selected for stability studies with hydrogen peroxide. Two of these samples were prepared by the Viking Inorganic Analysis Team to match the Mars inorganic spectra (11,12) whereas the other samples consisted of pure gamma Fe$_2$O$_3$, pure silica, and a mixture of gamma Fe$_2$O$_3$ and glass powder. The samples covered a wide range of particle sizes, organic content, and pH values as follows:
Results with each of these soils are shown in Figures 7 and 8. As shown in Figure 7, hydrogen peroxide is stable for at least three hours at room temperature on silica sand and on gamma $\text{Fe}_2\text{O}_3$. Results with silica sand resemble those obtained in the absence of any soil whereas hydrogen peroxide activity with the LR nutrient is significantly stimulated in the presence of gamma $\text{Fe}_2\text{O}_3$. On the other hand, hydrogen peroxide is highly unstable on glass powder and shows intermediate stability with the iron/glass powder mixture. With Mars Analog Soil B2 (Figure 8), a stimulatory effect is evident without pre-incubation although all hydrogen peroxide activity is essentially destroyed following three hours of incubation on the soil. In considering all the data in Figures 7 and 8, the stability of hydrogen peroxide did not appear to correlate with either organic content or with particle size although some correlation between stability and low pH became apparent.
Sterile screw cap vials were prepared either (a) without a soil sample, or with a 0.4 g sample of (b) pure gamma Fe₂O₃, (c) silica sand, (d) glass powder, or (e) a 15/85% mixture of gamma Fe₂O₃ and glass powder. After placing the vials in a glove box equilibrated under one atmosphere of nitrogen at room temperature, 0.22 ml of a solution of 1.5 x 10⁻¹⁴ M hydrogen peroxide (pH 6.5) was added to each vial. Either immediately (---) or three hours after (o---o) the hydrogen peroxide addition, each vial then received 0.11 ml of the LR nutrient. For each sample, a control was also conducted in which water was substituted for hydrogen peroxide as a monitor of biological or nonbiological activity of the LR nutrient (□—□). Evolved radioactive gas was collected on Ba(OH)₂ soaked getter pads which were changed at various intervals and counted by the gas flow technique at approximately 10% counting efficiency. All reaction vials were conducted in duplicate.
Sterile glass screw cap vials containing 0.4 g of Mars Analog B2 soil were placed in a glove box equilibrated under one atmosphere of nitrogen at room temperature and 0.22 ml of a solution of 1.5 x 10⁻³ M hydrogen peroxide (pH 6.5) was added to each vial. Either immediately (●—●) or three hours after (○—○) the hydrogen peroxide addition, each vial received 0.11 ml of the LR nutrient. Evolved radioactive gas was collected on Ba(OH)₂ soaked getter pads which were changed at various intervals and counted by the gas flow technique at approximately 10% counting efficiency. Results are compared to those obtained from LR nutrient and hydrogen peroxide in the absence of soil (△—△). A control was also conducted in which LR nutrient was added to soil in the absence of hydrogen peroxide (□—□) to monitor for biological or nonbiological activity of nutrient with soil. All reactions were conducted in duplicate.
In pursuing the possible correlation between hydrogen peroxide stability and soil pH, we obtained five additional iron samples for comparison to the original gamma Fe$_2$O$_3$ sample. The six samples, listed in Table II, were found to span a broad range of pH values and include samples of either gamma or alpha iron. The additional five samples were obtained by courtesy of Dr. Robert Hargraves of Princeton University who commented that they "behave" differently and differ in pH depending upon their source (preparation) (14).

Results of stability studies in which hydrogen peroxide was incubated for zero or three hours with these samples prior to the addition of the LR nutrient are shown in Figure 9. All experiments were conducted at room temperature under nitrogen at 760 mm Hg. As shown, without pre-incubation, hydrogen peroxide is stable on all soils except that at the highest pH. On the (Mapico Brown 422 Maghemite, pH 8.7 sample, hydrogen peroxide loses activity immediately. For the other alkaline sample (Lepidocrocite, pH 8.0), hydrogen peroxide activity is retained without pre-incubation, but is totally lost after three hours of pre-incubation with the soil. All other samples show considerable activity upon addition of the LR nutrient after three hours of pre-incubation of hydrogen peroxide with the sample, although activity losses range from minor to major.

These results support the hypothesis that hydrogen peroxide stability or soil is favored by low pH. That more than
<table>
<thead>
<tr>
<th>Sample</th>
<th>Texture</th>
<th>pH</th>
</tr>
</thead>
<tbody>
<tr>
<td>YFe₂O₃</td>
<td>MO2230 Maghemite</td>
<td>2.5</td>
</tr>
<tr>
<td>YFe₂O₃</td>
<td>MO4228 Maghemite</td>
<td>2.5</td>
</tr>
<tr>
<td>YFe₂O₃</td>
<td>Cobaloy x4107</td>
<td>3.0</td>
</tr>
<tr>
<td>αFeO·OH</td>
<td>Goethite</td>
<td>3.8</td>
</tr>
<tr>
<td>γFeO·OH</td>
<td>Lepidocrocite</td>
<td>8.0</td>
</tr>
<tr>
<td>YFe₂O₃</td>
<td>Mapico Brown 422 Maghemite</td>
<td>8.7</td>
</tr>
</tbody>
</table>

* Gamma iron obtained from Vance Oyama and used for all previous work.
Sterile screw cap vials were prepared containing 0.4 g of each of six gamma or alpha iron samples, as indicated. After placing the vials in a glove box equilibrated under one atmosphere of nitrogen at room temperature, 0.22 ml of a solution of 1.5 x 10^{-1} M hydrogen peroxide (pH 6.5) was added to each vial. Either immediately (---o) or three hours after (o-o-o) the hydrogen peroxide addition, 0.11 ml of the LR nutrient was added to each vial. For each sample, a control was also conducted in which water was substituted for hydrogen peroxide as a monitor of biological or nonbiological activity of the LR nutrient (0--0). Evolved radioactive gas was collected on Ba(OH)_{2} soaked getter pads which were changed at various intervals and counted by the gas flow technique at approximately 10% counting efficiency. All reaction vials were conducted in duplicate. Five of these soils were obtained from Dr. Hargraves and one was obtained from Mr. Oyama (as indicated). Soil pH was determined separately and is shown for each sample.
pH alone is involved in stability is indicated by separate studies in which aqueous solutions of hydrogen peroxide without the soils were adjusted to varying pH values from pH 3 to 10 and LR nutrient added before and after three hours standing at the indicated pH. The hydrogen peroxide solutions were found to be stable and essentially no difference in stability was found among solutions at different pH values. These findings are consistent with published literature (8,9). This suggests that some additional factor in the soil-metal composition is present to catalyze hydrogen peroxide instability in high pH soils or that the soil particulates will catalyze hydrogen peroxide instability except when this effect is prevented by low pH.

B. Effect of Low Temperature on Hydrogen Peroxide Stability with Soil

Studies were next undertaken to determine whether low temperatures, in the presence and absence of ultraviolet light, could enhance hydrogen peroxide stability on neutral or high pH soils. (The effects of UV will be discussed in a later section). The soils selected for these studies are Mars analog B2 soil (pH 7.9) and the 15/85% mixture of gamma iron/silica powder (pH 9.1). (Note that this is a different mixture than the gamma iron/silica sand mixture used for Figure 2 and 6 data in previous sections. Hydrogen peroxide is stable at room temperature on the low pH silica sand mixture but not on the high pH silica powder mixture.) All experiments were conducted by the getter technique in which 0.5 cc of the analog soil was added to a
sterile glass screw cap vial and the vial placed on dry ice 
(<-70°C) contained within a glove box under nitrogen atmosphere 
at 760 mm Hg. After temperature equilibration of the soil for 
15 minutes, 0.22 ml of a 1.5 x 10^-3M solution of hydrogen 
peroxide was added which instantly froze on the sample. The 
vials were sealed and both vial and dry ice removed from the 
glove box; after further incubation of the sample at <-70°C for 
zero or three hours, the vial was returned to the glove box, 
the temperature raised to 10°C or 23°C, as appropriate, for 
approximately 10 minutes and 0.11 ml of LR nutrient (pH 6.4) 
added to the sample. The vials were quickly sealed and evolved 
$^{14}$CO$_2$ was trapped by a Ba(OH)$_2$ getter pad placed in the vial cap. 
At appropriate intervals, the pads were replaced with fresh pads 
and the exposed pads dried and counted by the gas flow technique 
(approximately 10% counting efficiency).

Results obtained with the gamma iron/silica powder 
mixture are shown in Figure 10. In agreement with previous 
data (Figure 6) this soil stimulates the reaction between hydrogen 
peroxide and LR nutrient when both are added together onto the 
soil sample at room temperature. Pre-incubation of hydrogen 
peroxide on the soil for three hours at room temperature destroys 
all subsequent activity with the LR nutrient. However, pre- 
incubation on the soil at <-70°C for either three or six hours 
preserves most of the hydrogen peroxide activity as monitored 
by subsequent nutrient addition. If, after incubation at <-70°C
A mixture of 15% $\gamma$Fe$_2$O$_3$ and 85% silica powder was prepared and 0.5cc samples added to liquid scintillation vials. The vials were placed in a glove box under nitrogen at 760 mm Hg and equilibrated at either room temperature (A) or on dry ice at approximately -70°C (B). After temperature equilibration for 15 minutes, 0.22 ml of a 1.5 x 10^-3 M hydrogen peroxide solution (pH 6.5) was added. For samples maintained at room temperature (A), 0.11 ml of LR nutrient was added with (o---o) and without (e---e) a three hour pre-incubation at room temperature. Reduced temperature pre-incubation regimes (B) were 3 hours at -70°C (■—■), 6 hours at -70°C (□—□), 3 hours at -70°C followed by 3 hours at 10°C (△—△), and 6 hours at -70°C with UV present (1620 µW/cm²; 254 nm) during the last three hours (▲—▲). Samples pre-incubated at reduced temperatures were raised to room temperature for approximately 10 minutes prior to nutrient addition. Radioactivity evolved following the nutrient addition was monitored by the getter technique using a Ba(OH)$_2$ soaked filter pad placed in the vial cap. Getter pads were changed at various intervals, dried, and counted in a gas flow counter at approximately 10% efficiency.
for three hours, hydrogen peroxide is maintained at 10°C for an additional three hours prior to the nutrient addition, about half of the hydrogen peroxide activity remains. With respect to flight data, this suggests that little, if any, would be expected to survive the two sol incubation at 10°C prior to nutrient addition.

Similar tests have been conducted with the Mars analog B2 soil. As shown in Figure 11, pre-incubation of hydrogen peroxide on B2 for three hours at room temperature destroys all subsequent activity with the LR nutrient whereas pre-incubation for three or six hours at <-70°C preserves most activity. In contrast to the results obtained with the gamma iron/silica powder mixture, however, three hours incubation at <-70°C followed by an additional three hours at 10°C essentially destroys all activity.

These results suggest that, if a mechanism exists for the formation of hydrogen peroxide on the surface of Mars, the low ambient temperatures would contribute significantly to its preservation on the surface of Mars. However, if the ambient Mars temperature approaches 10°C, even briefly (i.e., three hours), as might be expected at some latitudes during the Martian summer, the peroxide would disappear from the surface. Further, the minimum two day incubation period at 10°C prior to nutrient injection in the Mars LR experiment would have
Figure 11

Effect of Freezing ± Ultraviolet Radiation on Stability of \( \text{H}_2\text{O}_2 \) on Mars Analog B2

Experimental conditions and procedures are identical to those described for Figure 3 except that the 0.5cc sample consisted of Mars Analog Soil B2. One additional run was also conducted in which the sample was pre-incubated with hydrogen peroxide for 3 hours and 20 minutes at \(-70^\circ\text{C}\) with UV present during the last 20 minutes (△- △).
eliminated reactivity of hydrogen peroxide if Mars soil is composed as reported by the Inorganic Analysis experiment.
C. Effect of Flight Pre-Incubation Thermal Regime on Hydrogen Peroxide Stability

In the flight experiments, the Mars surface temperature at the time of the various sample acquisitions ranged from -21°C to -84°C (3). After acquisition, Mars samples that later gave positive LR responses were placed into the R test cell and maintained at an average temperature of about 10°C (diurnal range of approximately 9°C to 14°C) for two sols prior to nutrient addition. The question next arises as to whether any hydrogen peroxide contained on the surface material could have remained stable under these storage conditions, particularly since the results in Figures 10 and 11 indicate that most activity is lost after three hours from the gamma iron/silica powder mixture and all is lost from the Mars analog B2 soil.

This question has been further examined using soil samples on which hydrogen peroxide has been shown (Figure 9) to be differentially stable for three hours at room temperature. In these experiments, hydrogen peroxide was added to the various samples and incubated for three hours at <-70°C under a nitrogen atmosphere at 760 mm Hg. The temperature was then raised to 10°C and, after 48 hours at this temperature, nutrient was added to monitor the activity of any remaining hydrogen peroxide. The results were compared to control samples in which hydrogen peroxide was added to the sample at 23°C followed by nutrient added either immediately or after 48 hours incubation at 23°C.
The results of experiments conducted with several iron oxide samples are shown in Table III. For reactions conducted at room temperature, pre-incubation for 48 hours significantly reduces hydrogen peroxide activity with LR nutrient. All activity is destroyed by incubation with alkaline pH samples whereas, in agreement with previous results (Figure 8), some activity remains with the low pH samples. Pre-incubation for three hours at <-70°C followed by 48 hours at 10°C preserves up to 50% activity with low pH (<3.8) samples but does not prevent complete destruction of hydrogen peroxide by high pH samples.

The mixture of 15% gamma iron and 85% silica sand was also tested in these experiments. This sample is of particular interest because TSM results (Figure 2) suggest that hydrogen peroxide may form a complex with this mixture at <-70°C that stabilizes it to volatilization at 50°C. The results are also shown in Table III. As shown, hydrogen peroxide on this sample at 23°C has lower reactivity with LR nutrient than observed when placed on the other iron samples, probably because of the lower amount of stimulatory iron present in the mixture. However, hydrogen peroxide on this sample is considerably more stable than on the pure iron samples; 45% activity remains after 48 hours storage at room temperature and 70% remains after three hours at <-70°C followed by 48 hours at 10°C. These results provide further support that hydrogen peroxide could account
# TABLE III

## Stability of Hydrogen Peroxide on Various Iron Samples After Various Pre-Incubation Temperature Regimes

<table>
<thead>
<tr>
<th>Iron Sample</th>
<th>pH</th>
<th>Soil Pre-Incubation Temperature</th>
<th>H₂O₂ Pre-Incubation Temperature</th>
<th>CPM Evolved from LR Nutrient in 25 Hours After 0 or 48 Hours</th>
<th>% H₂O₂ Remaining Inhibition After 48 Hours</th>
</tr>
</thead>
<tbody>
<tr>
<td>YFe₂O₃-MG02230</td>
<td>2.5</td>
<td>-70°C, 10°C</td>
<td>23</td>
<td>80,000</td>
<td>11</td>
</tr>
<tr>
<td></td>
<td>2.5</td>
<td>-70°C, 10°C</td>
<td>10</td>
<td>9,000</td>
<td>33</td>
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<tr>
<td>YFe₂O₃-MG04228</td>
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<td>-70°C, 10°C</td>
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<td>10</td>
<td>2,000</td>
<td>17</td>
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<tr>
<td>YFe₂O₃-COBALOY*</td>
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<td></td>
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<td>FeO·OH-Goethite</td>
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<td>-70°C, 10°C</td>
<td>23</td>
<td>72,000</td>
<td>25</td>
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<tr>
<td></td>
<td>3.8</td>
<td>-70°C, 10°C</td>
<td>10</td>
<td>18,000</td>
<td>50</td>
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<tr>
<td>YFeO·OH-Lepidociocite</td>
<td>8.0</td>
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<td>23</td>
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<tr>
<td></td>
<td>8.0</td>
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<tr>
<td>YFe₂O₃-MAPICO</td>
<td>8.7</td>
<td>-70°C, 10°C</td>
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<td>&lt;1,000</td>
<td>0</td>
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<tr>
<td></td>
<td>8.7</td>
<td>-70°C, 10°C</td>
<td>10</td>
<td>&lt;1,000</td>
<td>0</td>
</tr>
<tr>
<td>YFe₂O₃/Silica Sand (15/85)</td>
<td>3.2</td>
<td>-70°C, 10°C</td>
<td>23</td>
<td>40,000</td>
<td>45</td>
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<tr>
<td></td>
<td>3.2</td>
<td>-70°C, 10°C</td>
<td>10</td>
<td>8,000</td>
<td>70</td>
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</tbody>
</table>

All aliquots (0.5cc) of the indicated samples were placed in sterile glass screw cap vials and the vials placed in a glove box under nitrogen at 760 mm Hg. After temperature equilibration at either room temperature (app. 23°C) or at -70°C for 15 minutes, 0.22 ml of a 1.5 × 10⁻⁴ M hydrogen peroxide solution (pH 6.5) was added. LR nutrient (0.11 ml) was then added either immediately or after approximately 48 hours pre-incubation at either room temperature or reduced temperature. The reduced temperature pre-incubation regime consisted of three hours at -70°C, 48 hours at 10°C, and 10 minutes at room temperature prior to nutrient addition. Radioactivity evolved following the nutrient addition was monitored by the \( ^{3} \)H and the \( ^{14} \)C activity. CPM evolved from pre-incubated samples to that evolved from samples without pre-incubation and at room temperature.

* This sample was used for experiments reported in Figures 2, 4, 5, 6 and 9 and for the mixture examined in Tables 1 and 2.
for the active agent in the flight experiments, provided the pH and composition of the Mars surface material were highly acidic to stabilize hydrogen peroxide during the 10°C storage. However, because of the alkaline pH of the Mars sample suggested by other experimenters (11,15), the inference must be interpreted with caution.

In a final attempt to ascertain hydrogen peroxide stability under conditions simulating those prevailing during the Viking mission, an additional experiment was conducted in which hydrogen peroxide was added onto either the B2 Mars analog soil or the gamma Fe₂O₃/silica sand mixture (15/85%) contained in 0.5 cc portions in liquid scintillation vials. Both soils were pre-equilibrated on dry ice at <-70°C under 760 mm nitrogen at the time of addition such that the hydrogen peroxide solution froze instantaneously upon addition. (Sufficient hydrogen peroxide was added such that the final concentration would be 10⁻¹M following the later addition of LR nutrient.) Immediately after adding the hydrogen peroxide, the vials and dry ice were placed in a plastic glove box under nitrogen and transferred to a walk-in freezer at -30°C. All vials were maintained for 19 days in the presence and absence of ultraviolet irradiation (see next section for discussions of UV exposure and effects) with the temperature of the vials gradually rising from -70°C to -30°C as the dry ice evaporated. At the end of the 19 day incubation, vials containing each soil (with and without UV) were
separated into four groups to receive one of the following treatments prior to addition of LR nutrient:

- Warm for 10 minutes at 10°C, then for 10 minutes at room temperature.
- Warm for 10 minutes at 10°C, then heat 3 hours at 50°C, then cool for 10 minutes at room temperature (simulates VL2-4).
- Warm at 10°C for 24 hours, then for 10 minutes at room temperature (simulates all active runs although on flight the storage time was about 48 hours).
- Warm for 36 days at 10°C, then for 10 minutes at room temperature (simulates long term storage in VL1-4 and VL2-5).

As a control for this experiment, an additional set of vials was conducted in which hydrogen peroxide was added to soil at room temperature and LR nutrient added immediately. This control was conducted just prior to the 19 day storage using the same hydrogen peroxide solution used in the storage portion of the experiment. The entire experiment was conducted by the getter technique with all getter pads counted in a Nuclear Chicago gas flow counter at approximately 10% counting efficiency.

The results of this experiment are shown in Table IV. For the gamma Fe₂O₃/silica sand mixture in the absence of UV, approximately 20 percent activity remains after 19 days of cold incubation followed by 20 minutes of warming prior to addition of nutrient. However, only 12 percent remains if this warming period at 10°C is extended to 24 hours. Thus, in order for sufficient hydrogen peroxide to be present after 48 hours of warming at 10°C to give a response of flight magnitude, its initial concentration
### Table IV

<table>
<thead>
<tr>
<th>Soil</th>
<th>Pre-Incubation Treatment of H$_2$O$_2$ on Soil</th>
<th>Subsequent Treatment</th>
<th>-CPM Evolved* 20 Hours After Add LR Nutrient</th>
<th>-% Activity Remaining</th>
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<tr>
<td>γFe$_2$O$_3$/Silica Sand</td>
<td>NONE</td>
<td>NONE</td>
<td>80,000</td>
<td>100</td>
</tr>
<tr>
<td></td>
<td>19 days at -30°C, no UV</td>
<td>20 min at 10°C</td>
<td>16,000</td>
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<tr>
<td></td>
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<td>24 hours at 10°C</td>
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<td>36 days at 10°C</td>
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<tr>
<td></td>
<td>19 days at -30°C, +UV</td>
<td>20 min at 10°C</td>
<td>4,000</td>
<td>5</td>
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<td></td>
<td></td>
<td>36 days at 10°C</td>
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</tr>
<tr>
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<td>100</td>
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</table>

* Data corrected for CPM evolved from nutrient alone. Counting efficiency =10% or approximately threefold higher than on flight.

Aliquots (0.5 cc) of the indicated samples were placed in screw cap vials and the vials placed on dry ice in a glove box under nitrogen at 760 mm Hg. After temperature equilibration for 15 minutes, 0.22 ml of a 1.5 x 10$^{-4}$ M hydrogen peroxide solution (pH 6.5) was added. The vials were capped, moved into a plastic portable glove box under nitrogen at 760 mm Hg, and moved into a cold room at -30°C for 19 days. Vials were stored either capped or uncapped and exposed to an ultraviolet source at 254 μm placed 4 inches above the soil surface to give a calculated exposure of 1620 μW/cm$^2$. After the 19 day incubation, vials of each type (i.e., each soil with and without UV) were divided into four groups which were then stored at 10°C for 10 minutes, 24 hours, or 36 days or heat treated for 3 hours at 50°C. After each of these treatments, vials stored at room temperature under nitrogen for 10 minutes prior to the addition of 0.11 ml LR nutrient. Radioactivity evolved following the nutrient addition was monitored by the getter technique. Percent hydrogen peroxide remaining after the various treatments was calculated by comparing to radioactivity evolved from samples in which the LR nutrient was added to vials at room temperature immediately after the hydrogen peroxide addition to the soil. All reactions were conducted in duplicate.
would have had to be considerably greater than $10^{-1} \text{M}$. (Note that counting efficiency in the flight instruments was 3%; since active flight responses ranged from 10,000 to 16,000 cpm, these numbers must be multiplied by about 3 to relate them to the data presented in Table IV). The remaining data with this unexposed soil is in agreement with flight data. Thus, about half of the activity seen after the 24 hour storage is lost by three hours heat treatment at 50°C whereas almost all is lost by long term storage (i.e., 36 days) at 10°C. (See next section for discussion of UV effects.)

The results with the B2 Mars analog soil shown in Table IV are essentially the same as those presented in Figure 11. Thus, hydrogen peroxide is not stable when placed on this soil. Even prior to the freezing regime, hydrogen peroxide reactivity with the LR nutrient on this soil is considerably less than on the gamma Fe$_2$O$_3$/silica sand mixture (see Table IV) despite the fact that both soil samples contain approximately 15% gamma Fe$_2$O$_3$.

In summary of the Table IV results, it is apparent that the extent of hydrogen peroxide stability on a soil sample is highly dependent upon the composition and pH of that soil sample. The results with the gamma Fe$_2$O$_3$/silica sand sample suggest that, if a mechanism exists for formation and accumulation of hydrogen peroxide on the surface of Mars, it may have been possible for hydrogen peroxide to be present in the LR experiment. However, in order to obtain a response with the LR nutrient of flight magnitude, extremely high concentrations (i.e., $>10^{-1} \text{M}$ and approaching 1.0M) must have been present on the Mars surface.
if the gamma Fe$_2$O$_3$/silica sand mixture is a reasonable representation of the Mars surface material. Alternatively, either the Mars soil composition imparts a greater stability to hydrogen peroxide or a metal catalyst is present which is more effective than gamma Fe$_2$O$_3$. It should be noted, however, that because the Mars analog B2 soil was prepared by the Viking Inorganic Analysis Team to match the Mars Spectra (11), the results obtained with this soil cast serious doubt on the ability of hydrogen peroxide to survive on the surface of Mars. Finally, perhaps the most difficult obstacle to be overcome is the provision of some mechanism to protect the hydrogen peroxide from destruction by UV - even when the type of otherwise stabilizing complex formed in the laboratory is present.

D. Effect of Ultraviolet Radiation on Hydrogen Peroxide Stability

Hydrogen peroxide is highly susceptible to photolysis by ultraviolet light which, on Mars, penetrates to the surface virtually unattenuated by an ozone layer. In fact, it has been reported that the rate of destruction on Mars favors the rate of formation by a factor exceeding $10^7$ (16,17).

The possibility that freezing or complexing could protect hydrogen peroxide to ultraviolet irradiation was explored this year. In a preliminary experiment, hydrogen peroxide placed on either the gamma Fe$_2$O$_3$/silica powder mixture or on the Mars analog B2 soil at <-70°C was exposed to ultraviolet radiation
during subsequent maintenance at $\leq 70^\circ C$. This was accomplished by removing the cap from the reaction vial containing the soil plus hydrogen peroxide and placing a 254 mÅ source approximately 4 inches above the soil surface to give a calculated dosage to the soil surface of $1620 \mu W/cm^2$. This exposure is approximately one order of magnitude greater than that estimated (18,19) for the Mars surface. Total incubation time at $\leq 70^\circ C$ was six hours with UV present during the last three hours. As shown in Figure 10, with the gamma Fe$_2$O$_3$/silica powder mixture, this treatment enhanced subsequent reactivity with the LR nutrient. With the B2 soil (Figure 11), a small stimulation was also obtained; however, this stimulation was not observed when the ultraviolet exposure was reduced from three hours to 20 minutes (i.e., total pre-incubation at $\leq 70^\circ C$ was three hours and 20 minutes).

Because the short UV exposure time did not produce a deleterious effect on hydrogen peroxide and because this result is in contrast to the known (8,9) sensitivity of hydrogen peroxide to UV photolysis, a further experiment was conducted in which exposure times were extended to 19 days (Table IV). As in the experiments cited above (Figures 10,11), the UV source was a 254 mÅ lamp placed approximately 4 inches above the soil surface to give a calculated surface exposure of $1620 \mu W/cm^2$. Exposure was initiated at $\leq 70^\circ C$ under nitrogen although temperatures gradually rose during the 19 day incubation to $-30^\circ C$ (see previous section for experimental details). Soil samples selected
were the gamma Fe$_2$O$_3$/silica sand mixture (15/85%) on which hydrogen peroxide is known (Figure 6) to be more stable than on the gamma Fe$_2$O$_3$/silica powder mixture, and the Mars analog B2 soil. Following the 19 day radiation exposure, samples were warmed and assayed with LR nutrient after storage for 10 minutes, 24 hours, or 36 days at 10$^\circ$C or after heat treatment for 3 hours at 50$^\circ$C. As shown in Table IV, in the two cases where any activity remained, namely for storage of 10 minutes or 24 hours at 10$^\circ$C on the gamma Fe$_2$O$_3$/silica sand mixture, the ultraviolet exposure diminished hydrogen peroxide activity with LR nutrient more than in samples incubated 19 days in the absence of ultraviolet radiation. In all other cases, insufficient activity remained even in the absence of ultraviolet exposure to ascertain any further deleterious effect of UV. However, because some activity remained in the UV exposed sample that simulated the flight regime for an active response, the results do not preclude the possibility that some hydrogen peroxide could have been present with the Mars sample in the Viking LR experiment. In this case, however, even higher concentrations would have had to have been on the surface than indicated in the previous section. Thus, the results seriously challenge the possibility that the Mars sample composition could stabilize sufficient quantities of hydrogen peroxide on the Mars surface to account for the LR active responses.
VI. Hydrogen Peroxide Formation on Mars Analog Soil

A necessary prerequisite to considering that hydrogen peroxide might be the active agent in the LR experiment is evidence for hydrogen peroxide formation under Mars environmental conditions. Mechanisms postulated for its formation include production in the Mars atmosphere coupled with freezing out on the planet surface (16,17) and ultraviolet induced formation by a reaction between water molecules and the Mars surface material (20).

Experiments reported earlier (4), in which Mars analog soil B2 was exposed to ultraviolet radiation for 712 hours under 5 torr carbon dioxide in the presence and absence of water vapor, failed to produce an agent that would react with the LR nutrient in the TSM. However, because these experiments could have been water limited, we have conducted additional getter-type experiments in a further attempt to generate hydrogen peroxide from water in contact with Mars analog soil. Variable amounts of water (0, 50, 100, 200 μl) were added to the gamma Fe₂O₃/silica sand mixture at <70°C and incubated for three hours at that temperature. Duplicate samples were incubated in the presence and absence of ultraviolet radiation from a 254 μm source at an estimated exposure of 1620 μW/cm² (approximately two orders of magnitude greater than on Mars). In no case was any evidence obtained for formation of an active agent.
A similar experiment conducted in the TSM in which 100 µl water was added to this soil at -30°C under 5 torr carbon dioxide in the presence and absence of water vapor failed to produce an agent that would react with the LR nutrient.

In additional experiments conducted this quarter in a further attempt to generate hydrogen peroxide from water in contact with Mars analog soil, variable amounts of water (0, 50, 100, 200 µl) were added to the gamma iron/silica sand mixture at <-70°C and incubated for three hours at that temperature. Duplicate samples were incubated in the presence and absence of ultraviolet radiation from a 254 nm source at an estimated exposure of 1620 µW/cm² (approximately two orders of magnitude greater than on Mars). The temperature was then raised to 10°C and LR nutrient added. In no case was any evidence obtained for formation of an active agent.

A similar experiment conducted in the TSM in which 100 µl water was added to this soil at -30°C under 5 torr carbon dioxide in the absence of UV also failed to provide evidence for hydrogen peroxide formation.
VII. Status of Biological and Chemical Interpretations of LR Flight Data

Several different kinetic components have been discerned (4) in the active LR response obtained from the Mars surface samples (1-3). These are reviewed diagramatically in Figure 12. 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 range of 10,000 to 16,000 cpm appears to be less than 500 cpm, typical of results from nutrient alone experiments routinely conducted in the LR 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 followed 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 and all except Reaction #2 can now be accounted for by physico-chemical phenomena (4,12). On the other hand, the thermal lability of the Mars agent responsible for Reaction #2 remains consistent with a biological explanation.
Figure 12
Components of Labeled Release Reaction on Mars

A) Overall LR Response

B) Component Reactions
1 – Nutrient Outgassing
2 – Thermally Sensitive First Order Reactions(s)
3 – Thermally Stable Linear Reaction
4 – Nutrient Outgassing and/or Pressure Spike
5 – Gas Adsorption
6 – Thermally Stable Linear Reaction
Because the biological interpretation is not subject to direct experimentation but can only gain strength through elimination of candidate nonbiological explanations, we have undertaken an intensive systematic examination of the plausibility of the various chemical alternatives. Our previous efforts (4) have shown that ultraviolet irradiation, gamma Fe_2O_3, and metalloperoxides and superoxides can be eliminated as candidate active agents for Reaction #2. Under select conditions, however, hydrogen peroxide can fulfill several, but not all, of the necessary criteria for the Mars active agent.

Major problems remain with the hypothesis that hydrogen peroxide is the active agent in the Mars surface material. Most significant among these is demonstration that hydrogen peroxide can be formed and survive on the Mars surface in quantities sufficient to produce an LR response comparable in magnitude to the flight response. Our experiments to date have not demonstrated hydrogen peroxide formation under Mars-like conditions. If the gamma Fe_2O_3/silica sand mixture can be considered a reasonable Mars model soil, then our experiments permit a rough calculation of the hydrogen peroxide concentration in the Mars soil that would produce a LR positive response:

As seen in Table IV, when 0.5cc of gamma Fe_2O_3/silica sand, which had been pretreated by holding at -30°C under UV flux equivalent to 190 days on Mars, and which had then been
held at $10^0\text{C}$ for 24 hours, was injected with 0.22 ml of $1.5 \times 10^{-1}\text{M}$ hydrogen peroxide, a response totalling 5,000 cpm was produced. Adjusting for the higher counting efficiency of the getter technique, this equates to a response in the viking instrument of approximately 1,600 cpm, about one-tenth that obtained on Mars. This indicates that, to produce a Mars-type response, a hydrogen peroxide concentration of 1.5M would be necessary.

A 1.5M solution of $\text{H}_2\text{O}_2$ contains $1.5 \times 34 = 51 \text{ g/l} = 51 \text{ mg/ml}$.

$0.22 \text{ ml} \times 51 \text{ mg/ml} = 11.22 \text{ mg H}_2\text{O}_2$.

This amount in the 0.5cc sample would produce a Mars magnitude response.

Assuming a specific gravity of 1.0 for the soil, the concentration of $\text{H}_2\text{O}_2$ would be $\frac{11.22 \times 100}{500} = 2.24\%$.

Thus, to have been responsible for the LR Mars response, hydrogen peroxide would have to constitute more than two percent of the Mars soil. However, since the LR nutrient may only partially wet the soil, at least initially, the hydrogen peroxide concentration in the soil may have to be higher than 2%. Because hydrogen peroxide on the surface is susceptible to destruction by ultraviolet radiation, and to Mars temperatures approaching $10^0\text{C}$,
a mechanism must also exist for formation of surface hydrogen peroxide at even higher concentrations and for frequent replenishment in the course of a year.
The only possible means of lowering the required concentration while still producing a response of flight magnitude would be to have a more effective metal catalyst in the Mars soil and/or to form a more stable complex in the Mars surface material. Even then, the problem of survival of hydrogen peroxide under the Martian UV flux remains.

Another major difficulty with the hydrogen peroxide interpretation is the number of constraints imposed on the composition of the Mars soil. Thus, although our laboratory experiments have demonstrated that complex formation with analog soil can stabilize hydrogen peroxide to volatilization at 50°C, can partially stabilize it to decomposition on Mars soils even in the presence of UV by storage at low temperatures (i.e., -30°C or less), and can stabilize it to the two sol storage at 10°C, these stabilizations are highly soil dependent. To meet all criteria, the soil must be highly acid and contain both silica and iron (gamma iron, according to our experiments). However, it should be noted that the Mars surface material is believed to be of neutral (11) or high pH (15) and that the Mars analog soil B2 that matches the Mars inorganic spectra does not convey these stability properties to hydrogen peroxide. Thus, while such complexing can exist under certain specific conditions, it remains to be demonstrated that it is in fact possible with the Mars surface material.
There is yet another constraint to be imposed on any complex between hydrogen peroxide and the Mars surface material. During the mission, the Mars active agent had been dissipated before the second nutrient addition, presumably either because it was limiting or because the experimental conditions had destroyed any remaining active agent. In our TSM experiments, excess hydrogen peroxide was not destroyed by the assay conditions but was available for interaction with nutrient added five days after the first nutrient injection. This implies either that if hydrogen peroxide were the active agent on flight, it was either limiting or complexed with surface material in such a manner as to render it susceptible to destruction by assay conditions. However, to produce a response comparable in magnitude to the flight response, hydrogen peroxide in our experiments has been approximately three orders of magnitude in excess of the amount of carbon oxidized on flight. Thus, in order for hydrogen peroxide to be limiting and account for the flight data, concentrations must be significantly lowered and a metal catalyst must strongly stimulate the reaction to replicate the magnitude of the flight response. Our experiments with pure gamma iron have not demonstrated stimulatory effects of this magnitude.

Thus, while the hydrogen peroxide theory remains an attractive chemical explanation of the LR flight data, several problems remain with this interpretation that probably cannot be resolved short of another mission. Most serious is whether
hydrogen peroxide can form and persist in sufficient amounts on the Mars surface material under Mars environmental conditions.

On the other hand, a biological explanation can also account for the observed flight data and we have previously discussed (4) various terrestrial life forms that can serve as models for putative Mars organisms. These include cryptobiotic bacteria and invertebrates, lichens, and endolithic algae, fungi, and bacteria that exist beneath rock surfaces in Antarctica's dry valleys. Such models lend some plausibility to a biological interpretation for the positive LR response found on Mars. In this regard, our study of the Viking lander pictures reporting (5) greenish patches on some rocks near Viking Lander 1 and some changes in color pattern with time is of considerable interest.

One of the major obstacles for acceptance of a biological interpretation of the Viking LR results has been the failure of the Viking Organic Analysis experiment to detect organic compounds in the Mars surface material (21,22). However, the possibility exists that the sensitivity of the mass spectroscopy experiment may not have been sufficiently low. Thus, on theoretical grounds, we have estimated that a soil sample must contain approximately $5 \times 10^8$ microorganisms per gram in order to detect specific compounds or pyrolysis products. This estimate is based on 1) a dry weight of approximately $5 \times 10^{-4}$ g carbon/organism which would require $5 \times 10^5$ organisms per gram soil to provide 1 ppb total carbon assuming 50% of cell weight is carbon, 2) the assumption that two orders of magnitude higher would be required to obtain 1 ppb of a specific compound,
and detection limits in the Viking Organic Analysis experiment of 5-10 ppb for benzene and toluene. This estimate can be contrasted with the fact that positive LR responses have been obtained from a terrestrial sample containing $10^2$ microorganisms/gram and that responses of flight magnitude are estimated to require $10^4$ cells/gram terrestrial soil. Thus, all aspects of the Viking results are compatible with the biological hypothesis while considerable problems remain to be solved to raise the chemical hypothesis to that status. However, the paramount importance of making a correct conclusion with respect to the LR data requires further investigation, preferably by means of a new mission to Mars.
Respectfully submitted,

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Co-Investigator
VIII. Acknowledgements

We gratefully acknowledge the dedication and excellent technical assistance of Jon Calomiris in performing all TSM experiments cited herein and of Jed Fahey and Susan Olson in performing all getter experiments.
IX. References


IX. References (continued)


