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A Search for a Nonbiological Explanation of the Viking Labeled Release Life Detection Experiment

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The Viking Labeled Release (LR) data obtained on Mars satisfy the criteria established for a biological response. The importance of the issue, especially when viewed against the harsh environment on Mars, requires careful consideration of possible nonbiological reactions that may have produced false positive results. A 3½-year laboratory effort to investigate possible chemical, physical, and physicochemical agents or mechanisms has been concluded. Among nonbiological possibilities, hydrogen peroxide, putatively on Mars, emerged as the principal candidate. When placed on analog Mars soils prepared to match the Viking inorganic analysis of the Mars surface material, hydrogen peroxide did not duplicate the LR Mars data. When other materials were used as substrate, hydrogen peroxide could be made to evoke the type of responses obtained by the LR Mars experiment. However, essential criteria concerning the formation, accumulation, and preservation of hydrogen peroxide to qualify it as the active agent on Mars have not been met and new data show it to be essentially absent from the Mars atmosphere. The presence of a biological agent on Mars must still be considered. This interpretation of the LR results is strengthened by a recent report that the Viking organic analysis instrument (GCMS) failed to detect organics in an Antarctic soil in which the LR instrument had demonstrated the presence of microorganisms.

INTRODUCTION

The Viking Labeled Release (LR) life detection experiment, designed to detect heterotrophic microbial life, produced a positive response on the surface of Mars (Levin and Straat, 1976b, 1977b, 1979b). Terrestrial life forms have been identified which survive simulated Martian environments and which could serve as models for Martian microbial life (Levin and Straat, 1977a). Analysis of the landscape at the Viking 1 landing site has indicated color and feature changes that may be attributable to biological activity (Levin *et al.,* 1978). However, because of the failure to find organics on the surface of Mars (Biemann *et al.,* 1976, 1977) and the hostility of the Martian environment to most terrestrial life forms, nonbiological explanations of the LR results must be considered. Most of the nonbiological theories advanced, such as reactions resulting from ultraviolet radiation and the possible presence of theta-Fe_2O_3, metalloperoxides, or superoxides, have previously been investigated and found wanting (Levin and Straat, 1977a,c, 1977a). Reaction of the LR nutrient with putative surface hydrogen peroxide remained as the leading nonbiological candidate.

In order for hydrogen peroxide to constitute a tenable explanation for the Mars LR data: (1) It must produce an active, Marslike response with the LR nutrient under simulated Mars experimental conditions; (2) It must display thermal sensitivities to 160 and 50°C similar to those observed (Levin and Straat, 1976b, 1977b) during the LR experiments on Mars; (3) Its stability on the Mars sample stored in the dark at 10°C must account for an active response after 2-sol (a "sol," 1 day on Mars, equals 24.65 Earth hours) storage but, also, loss of activity after 2 or 3 months of storage (Levin and Straat, 1979b); (4) a mechanism must exist for the formation of hydrogen peroxide on Mars in sufficient quantities to account for the magnitude of the flight response; and (5) A mechanism must exist to stabilize and accumulate hydrogen peroxide on the Mars surface in the presence of intense ultraviolet radiation and other destructive forces. This report describes attempts to assess hydrogen peroxide against these criteria. The biological interpretation of the LR results is also briefly explored.

EXPERIMENTAL

Mars analog soil B2 was prepared by the Viking Inorganic Analysis Team to match, as closely as possible, the analysis of Mars samples obtained by the Viking X-ray fluorescence instrument (Clark and Baird, 1977). As described previously (Levin and Straat, 1979c), this synthetic soil contains approximately 18% ferric oxide and 42% silica oxide, has a pH of 7.2, and ranges in particle size from 10 to 100 µm. The iron is in the form of theta-Fe_2O_3, a compound believed to be present on Mars (Hargraves et al., 1977) and hypothesized (Oyama, 1976; Oyama *et al.,* 1978) to be involved in the LR reaction. Other samples used as analog soils consisted of silica sand (Fisher Scientific), silica glass powder (Fisher Scientific), and pure theta-Fe_2O_3 (Cobaloy X4107, courtesy of V. Oyama). Mixtures of this theta-Fe_2O_3 and either silica sand or glass powder were prepared in a ratio of 15:85 w/w, respectively. Five additional soil samples (goethite, M02230 maghemite, M04228 maghemite, Mapico Brown 422 maghemite, lepidocrocite) containing primarily alpha and theta iron were obtained by courtesy of R. Hargraves. All Mars analog soils were determined to be biologically sterile by conducting TSM and getter experiments in which LR nutrient was added to 0.5 cm^3 samples of each analog at room temperature. In each case, no significant evolution Of $^{14}CO_2$ occurred. Antarctic soil No. 726 was provided from the NASA Ames Antarctic soil bank courtesy of E. Merek.

The LR nutrient contains seven organic substrates (formate, glycolate, glycine, DL-alanine, DL-lactate) uniformly labeled with ^{14}C and adjusted approximately to pH 6.2 (Levin and Straat, 1976a). The nutrient sent to Mars and used in the 1976 Viking experiments had been prepared during the summer of 1973. A portion of this nutrient had been retained and was available for the LR Test Standards Module (TSM) experiments reported herein. Getter experiments, however, were conducted with freshly prepared nutrient.

The LR TSM replicates the Viking flight instrument configuration in all essential components (Levin and Straat, 1976a). Basically, the instrument consists of a 3.5-cm^3 capacity stainless steel test cell which is connected to a detector cell containing two solid-state beta detectors via a 33 cm long, 0.2-cm i.d. diameter "swan neck" tube. The total volume of the test cell and detector assembly is approximately 8.5 cm^3, and any radioactive carbon gas contained in the total volume is counted with an efficiency of approximately 3%. Liquid LR nutrient is stored in a reservoir adjacent to the test cell and connected by 0. 1-cm i.d. diameter tubing. Solenoid valves control the introduction of 0.115 ml of nutrient into the test cell. Flight experimental conditions are simulated by evacuating the entire apparatus to 5 Torr carbon dioxide and by maintaining the test cell temperature at 10°C, the nominal temperature maintained in the lander instrument on Mars.

In conducting TSM experiments, an aqueous solution of hydrogen peroxide (pH 6.5) was manually added to the LR TSM test cell at a concentration such that the final concentration would be 10^{-1} *M* after the addition of 0.115 ml of nutrient. The hydrogen peroxide volume was either 0.5 ml when used alone, or 0.1 ml when a 0.5-cm^3 Mars analog soil sample was also present in the test cell. After addition of hydrogen peroxide in the presence or absence of soil, the TSM test cell was equilibrated for several hours at 10°C under 5 Torr carbon dioxide before the addition of LR nutrient. For select experiments, the test cell temperature was reduced to approximately -30°C during the equilibration period by surrounding the outside of the test cell with liquid nitrogen; in these cases, the test cell was warmed until it attained 10°C just prior to the addition of nutrient.

Because the TSM can accommodate only one reaction at a time, getter-type experiments were conducted when it was desirable to compare several reactions simultaneously. In getter experiments, 0.5 cm^3 of an analog soil was added to a sterile glass vial and equilibrated in a glovebox under a nitrogen atmosphere at 760 mm Hg. Equilibration was either at room temperature or on dry ice (-78.5°C). To each soil was added 0.22 ml of a 1.5×10^{-1} *M* solution of hydrogen peroxide (adjusted to pH 6.5) which froze immediately at the lower temperature. The volume and concentration of hydrogen peroxide were selected as convenient to supply the absolute amount required in a manner to mix readily with the LR nutrient. This was also deemed appropriate to Mars because hydrogen peroxide, infinitely soluble in water, would, even if formed anhydrously, take up water vapor which diurnally approaches saturation of the atmosphere near the Mars surface. The longer the survival time of the hydrogen peroxide, the more dilute the solution would become. Exposure to above-freezing temperature which is maintained inside the LR instrument for 2 or more days prior to addition of the nutrient would assure complete solution. As given later in this paper, the quantity of water vapor on Mars greatly exceeds the upper limit possible for hydrogen peroxide which also argues for its substantial dilution. As explained in the following paragraph, the effect of ultraviolet radiation on a given amount of hydrogen peroxide is essentially independent of the concentration. Either immediately following addition of the hydrogen peroxide, or after several hours, 0.11 ml of LR nutrient was added. After nutrient addition, the calculated concentration of hydrogen peroxide in the vial was 10^{-1} *M*. Where preincubation with hydrogen peroxide had been at -78.5°C, the soil was allowed to warm to room temperature just prior to nutrient addition. The vials were then quickly sealed and evolved $^{14}CO_2$ 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 at approximately 10% efficiency.

Getter experiments were also conducted in which soils were preincubated with hydrogen peroxide solution, at room temperature or at -78.5°C, in the presence of ultraviolet irradiation prior to addition of LR nutrient. The solar flux strikes the surface of Mars relatively unattentuated in the ultraviolet region ranging from 200 to 300 nm (Averner and MacElroy, 1976, Table 16 and p. 34). Hydrogen peroxide primarily absorbs in this range, resulting in dissociation (Schumb *et al.,* 1955, p. 288). Previous work (Holt *et al.,* 1948) had established 254 nm as a conventional wavelength to be used for absorption and decomposition studies with dilute solutions and vapors of hydrogen peroxide. A review of a number of studies (Schumb *et al.,* 1955, p. 289) concluded that the absorption coefficient for both liquid and vapor phases of hydrogen peroxide is the same. Relatively little differences in absorption coefficients exist over the wide range of solution and vapor concentrations reported (Schumb *et al.,* 1955, Fig. 19, p. 287), 6.5 to 99.3% (Schumb *et al.* extend the range to include anhydrous) hydrogen peroxide. Thus, the rate of hydrogen peroxide decomposition by ultraviolet radiation is essentially directly proportional to hydrogen peroxide concentration over the range of dilute to concentrated solutions (Schumb *et al.,* 1955, p. 459). Although some departures from Beer's Law at higher concentrations are reported (Taylor and Cross, 1949), the corresponding differences in absorption are of the order of only several percent.

The 254-nm wavelength commonly used for the study of hydrogen peroxide decomposition is near the center of the ultraviolet band, is moderately absorbed (Schumb *et al.,* 1955, Fig. 19, p. 287), and is thus fairly representative of the frequencies absorbed. A commercially available lamp (General Electric G8T5) of this frequency was, therefore, selected for this study of hydrogen peroxide survivability. Exposure was accomplished by removing the cap from the reaction vial containing the soil plus hydrogen peroxide solution and placing the 254-nm source approximately 10 cm above the soil surface to give a calculated dosage to the soil surface of 1620 µW/cm^2 (based upon the lamp manufacturer's calibration). This flux is between one and two orders of magnitude greater than that estimated (Glasstone, 1968; Shorthill, 1977; Zill *et al.,* 1979) for the surface of Mars.

While the foregoing experiment examined the destruction of hydrogen peroxide by ultraviolet light, another experiment was conducted to investigate the ultraviolet-induced production of that compound. Since the 254-nm lamp used in the previous experiment does not photolyze water, a prime potential source for hydrogen peroxide formation, this experiment was conducted using the Mars Ultraviolet Simulation Facility at the NASA Ames Research Center. Its source was a 2500-W xenon lamp (Spectrosun Model X-25 Solar Radiation Simulator, Spectrolab, Inc.) with the beam passed through a heat-dissipating water filter. The spectral irradiance of the beam closely followed that of the average solar flux from 215 to 240 nm at the Viking sites and thereafter exceeded the maximum average solar flux by up to one order of magnitude (Zill *et al.,* 1979). Two 0.5-cm^3 samples of Mars analog soil B2 were dried at 160°C for 24 hr 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 water vapor in equilibrium with the simulated Mars atmosphere at the time of sealing. The samples were tumbled in a sample-holding rotor in the Mars Ultraviolet Simulation Facility for 712 hr while at -35°C. At the end of the exposure, the samples were stored at approximately -15°C for 3 months prior to testing for activity when injected with LR nutrient in the TSM using an active flight sequence at 10°C under 5 Torr carbon dioxide.

Additional experiments attempted to generate hydrogen peroxide through ultraviolet-induced physical disjunctions in each of several Mars analog soil compositions. Various amounts of water (0 to 200 µl) were added to 0.5-cm^3 portions of the soils at -78.5°C. The water froze instantly and the samples were incubated for 3 hr at -78.5°C. Duplicate samples were incubated for 3 hr in the presence and absence of ultraviolet radiation from the 254-nm source at a calculated flux of 1620 µW/cm^2. The temperature was then raised to 10°C and LR nutrient added in getter-type experiments.

RESULTS

Reactivity of Hydrogen Peroxide with Labeled Release Nutrient

The results following the addition of LR nutrient onto 10^{-1} *M* hydrogen peroxide contained in the TSM test cell under simulated flight conditions are shown in Fig. 1. 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 hr 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 and with the previously reported (Levin and Straat, 1979c) simulation of this effect. In the TSM, nutrient appeared to limit the TSM response following the 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.

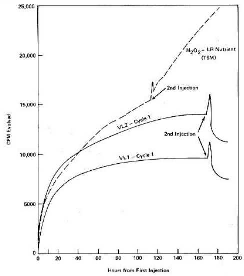
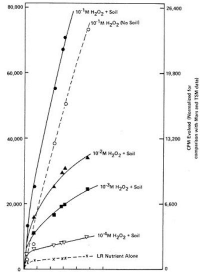


FIG. 1. Reaction between hydrogen peroxide and LR nutrient in TSM. A 0.5-ml aliquot of 1.2×10^{-1} *M* hydrogen peroxide (pH 6.4) was added to the TSM test cell and equilibrated at 10°C under 5 Torr carbon dioxide. After subsequent injection of 0.115 ml of LR nutrient according to flight injection sequence, the hydrogen peroxide concentration was assumed to be $10^{-1}M$. A second nutrient injection was added after approximately 113 hr of incubation. Evolved radioactivity (---) is compared to that obtained from the first LR cycle on each Viking lander, as indicated.

Hydrogen peroxide, presumed on the surface of Mars, has been estimated (Clark, 1979) at 30 ppm, significantly less than required to produce a 10^{-1} *M* solution in the injected nutrient. However, catalysts may be present in the Mars surface material such that lesser amounts of hydrogen peroxide could produce a response of flight magnitude. Figure 2 presents the results of getter experiments conducted with various concentrations of hydrogen peroxide added to a metal catalyst (theta-Fe_2O_3) mixed with silica sand (15:85 w/w). The results show significant stimulation of the reaction.



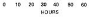


FIG. 2. Effect of hydrogen peroxide concentration on reaction with LR nutrient in the presence of an iron/silica mixture. Screw cap vials containing 0.5 cm^3 of the γ-Fe_2O_3/silica sand mixture (15 : 85 w/w) were equilibrated under 1 atm nitrogen at room temperature before adding 0.22 ml of a hydrogen peroxide solution to each vial. The final concentration in each vial was 10^{-1} *M* (●), 10^{-2} *M* (▲), 10^{-3} *M* (■), or 10^{-4} *M* (▽), as indicated. Evolved radiactive gas was collected by the getter technique (app. 10% counting efficiency). Results are compared to those obtained in the absence of soil (○) and from nutrient alone (×). All reactions were conducted in duplicate. The scale on the left gives raw data, whereas the scale on the right gives normalized counts had the experiment been conducted either in the TSM or in a flight instrument (adjusting for counting efficiency).

Although kinetics of the getter and TSM reactions differ, conditions of the Fig. 2. control, in which hydrogen peroxide and LR nutrient react in the absence of metal catalyst, are similar to those of the TSM experiment reported in Fig. 1. This permits an approximately normalization of the Fig. 2 data to those presented in Fig. 1. Comparison of the two sets of data suggests that the hydrogen peroxide concentration in the nutrient 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, at least for the initial part of the reaction. Further, for all concentrations of hydrogen peroxide, the kinetics of gas evolution in the presence of metal are initially faster and then slower than those observed in the absence of the metal catalyst. Similar kinetics were observed in flight data (see Fig. 1). It is concluded that hydrogen peroxide, if present in the Mars soil in amount sufficient to produce a 10^{-3}-10^{-2} *M* solution in the LR nutrient at the time of reaction, and in the presence of an appropriate catalyst, can fulfill the first criterion of the Mars agent. Although the second injection response differs from the sharp spike and immediate absorption of $^{14}CO_2$ observed on Mars, this is deemed of lesser significance since a simulation of this effect has been reported (Levin and Straat, 1979c).

It had been hypothesized (Oro, 1976) that the LR response on Mars may have resulted from a reaction between hydrogen peroxide and only one of the substrates of the LR nutrient, namely, formate. This hypothesis was based on the facts that the LR Mars reaction appeared to be first order and that the magnitude of the flight response suggested that only one carbon substrate was involved (Levin and Straat, 1976b). Late Viking Lander data (Levin and Straat, 1979a) indicated that at least two carbon atoms had reacted, making it unlikely that formate was the only reacting substrate. To confirm that hydrogen peroxide could react with more than one of the LR substrates, separate getter-type experiments were conducted in which hydrogen peroxide was tested with each of the nutrient constituent substrates individually. The results (Fig. 3) show that, if hydrogen peroxide were present in the Mars sample, all LR substrates in the nutrient would have reacted, albeit at different rates.

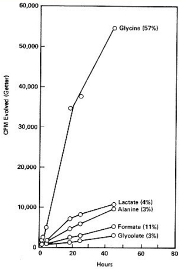


FIG. 3. Effect of hydrogen peroxide on LR nutrient and its constituents. Uniformly labeled glycine, formate, and glycolate solutions were separately prepared at 2.5×10^{-4} *M*, whereas DL-alanine and L-lactate solutions were separately prepared at 5×10^{-4} *M*. Each separate substrate is comparable in concentration and in radioactivity to that present in the LR nutrient. Experiments were initiated by adding 0.22 ml of 1.5×10^{-1} *M* hydrogen peroxide (pH 6.4) to a sterile screw cap vial and equilibrating under nitrogen at 760 mm Hg and at room temperature. After equilibrating, 0.11 ml of LR nutrient or individual substrate, as indicated, were added giving a final hydrogen peroxide concentration of 10^{-1} *M*. Evolved $^{14}CO_2$ was monitored by the getter technique. The number given in parentheses indicates the percentage evolved $^{14}CO_2$ relative to the radioactivity available in each substrate.

Thermal Sensitivity of Hydrogen Peroxide in the TSM

The active reponse with the LR nutrient was obliterated by pretreatment of the Mars surface material at 160°C for 3 hr. The active Mars agent was also partially destroyed by heat treatment for 3 hr 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.

Hydrogen peroxide, known (Edwards, 1962; Schumb *et al.,* 1955) to decompose completely in 3 hr at 160°C, is essentially unaffected for that period at 50°C. Experiments in which heating was conducted in glass vials verified these facts. However, subjecting hydrogen peroxide in the TSM test cell to flight-like thermal regimes produced different results. As shown in Fig. 4, pretreatment for 3 hr at 160, 50, and even 40°C, essentially destroys or renders hydrogen peroxide unavailable for subsequent reaction with the LR nutrient. Even exposure to room temperature (23°C) for 3 hr prior to nutrient addition resulted in partial reduction (approximately 50%) of subsequent hydrogen peroxide activity with LR nutrient. The reason for the peculiar increase beginning at approximately 12 hr for the 160°C curve is unexplained, but this anomoly never occurred when the hydrogen peroxide was placed on soils in the TSM.

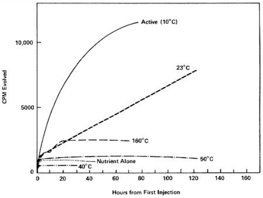


FIG. 4. Effect of preheating hydrogen peroxide on subsequent reaction with LR nutrient in TSM. A 0.5-ml aliquot of 1.2×10^{-1} *M* hydrogen peroxide (pH 6.4) was equilibrated in the sealed TSM test cell at 10°C under 5 Torr carbon dioxide. The sample was heated for 3 hr at 160°C (———), 50°C (– · –), 40°C (– · –), or 23°C (– – –), as indicated. After cooling to 10°C and venting the test cell for 4 min, 0.115 ml of LR nutrient was added to the test cell according to flight sequence (final hydrogen peroxide concentration is 10^{-1} *M*). Evolved radioactivity is compared to that obtained from an unheated sample(———) and from nutrient alone (· · · · ·).

In a series of studies designed to determine the impact of the TSM configuration on the thermal properties of hydrogen peroxide, it was demonstrated that this extreme thermal sensitivity results from volatilization of hydrogen peroxide out of the test cell to unheated parts of the instrument, where it condenses and remains unavailable for reaction when the LR nutrient is injected. Escape of hydrogen peroxide from the test cell is an artifact of the instrument design and would occur in the instruments landed on Mars as well as in the TSM. Thus, hydrogen peroxide could not be the Mars active agent unless it were complexed with the Mars soil in such a manner as to be significantly stabilized against volatilization at 50°C.

To this end, the influence of theta-Fe_2O_3/silica sand mixture (15:85 w/w) on the thermal characteristics of hydrogen peroxide at 50°C was explored. In these experiments, hydrogen peroxide was added to 0.5-cm^3 samples of this mixture contained in the TSM test cell and preeqilibrated at either 10 or -30°C. For each preequilibration temperature, samples were also examined with and without subsequent heat treatment for 3 hr at 50°C. As shown in Fig. 5, for samples not heated at 50°C, hydrogen peroxide, preequilibrated at either 10 or -30°C with the iron/silica sand mixture, reacted when LR nutrient was added. (The lower reactivity of the sample preequilibrated at 10°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 preequilibration temperature. Following 10°C preequilibration, no reactivity occurred with the LR nutrient. However, for the -30°C preequilibration, a moderate level of reactivity was observed after the 50°C heating. Such inhibition of the hydrogen peroxide activity is reminiscent of that observed on Mars. This result is evidence that the sought-for complex between hydrogen peroxide and the iron/silica sand mixture had, indeed, formed at -30°C and 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 metal catalyzed partial decomposition. Other complexes could exist on Mars that might effect better stabilization of hydrogen peroxide.

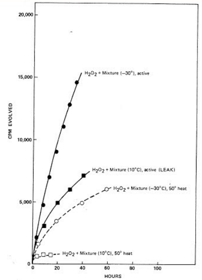


FIG. 5. Effect of iron/silica sand mixture on volatilization of hydrogen peroxide at 50°C in LR TSM. A 0.5-cm^3 sample of the γ-Fe_2O_3/silica sand mixture (15 : 85 w/w) was equilibrated in the TSM test cell at 5 Torr carbon dioxide at either 10 or −30°C, as indicated. A 0.3-ml aliquot of 1.4×10^{-1} *M* hydrogen peroxide solution (pH 6.4) was then added and incubated at the indicated temperature for an additional 3 hr. All samples designated for "active" sequences were then maintained at 10°C for an additional 6 hr, whereas samples designated for "sterile" sequences were heated to 50°C for 3 hr and then cooled to 10°C for an additional 3 hr. After the preincubation regime, 0.115 ml of LR nutrient was injected via the flight sequence (final hydrogen peroxide concentration = 10^{-1} *M*) and subsequently evolved radioactivity was monitored. 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 a leak.

On the other hand, similar series of experiments have been conducted in the TSM with silica sand alone, with theta-Fe_2O_3 alone, and with the Mars analog soil B2. Even at -30°C, hydrogen peroxide was not stabilized by any of these soils to subsequent volatilization at 50°C. These results emphasize the high dependency of the phenomenon on the composition of the soil and suggest that only under yet undefined specific conditions could hydrogen peroxide fulfill the thermal characteristics of the 50' flight data required for satisfaction of the second criterion.

Stability of Hydrogen Peroxide on Various Mars Analog Soils

The third criterion, stability on the Mars surface material during storage in the hopper at 10°C shielded from light was then examined. Edwards (1978) has suggested that hydrogen peroxide may not be stable on the fine surface material of Mars even in the absence of ultraviolet radiation.

To test the stability of hydrogen peroxide on soil, five Mars analog soils were selected for getter-type experiments. The analogs covered a wide range of particle sizes, organic content, and pH values. Their properties are listed in Table I along with those of the theta-Fe_2O_3/silica sand mixture reported in Figs 2 and 5. Hydrogen peroxide was added to each soil according to the protocol described under "Experimental," followed by LR nutrient. Resultant $^{14}CO_2$ evolution, compared to a control in the absence of soil, reflects the stability of hydrogen peroxide on the particular analog.

TABLE I

CHARACTERISTICS OF ANALOG SOILS TESTED

Soil	**Organics (ppm)**	**Particle size (µm)**	**pH**
theta-Fe_2O_3 (Cobaloy X4107)	800	10-100	3.0
Silica sand	170	420-840	6.8
Silica glass powder	30	50-210	9.9
theta-Fe_2O_3/Silica glass powder mix (15:85 w/w)	145[a]	10-210	9.1
Mars analog B2	600	10-100	7.2
theta-Fe_2O_3/silica sand	265[a]	10-840	app. 6.0

[a] Calculated (all other values determined by TOC).

The results are shown in Figs. 6 and 7. As shown in Fig. 6, hydrogen peroxide is stable for at least 3 hr at room temperature on silica sand and loses partial activity after 3 hr contact with theta-Fe_2O_3. Results with silica sand resemble those obtained in the absence of soil, whereas theta-Fe_2O_3 significantly stimulates the rate of the hydrogen peroxide reaction when immediately mixed with the LR nutrient. Hydrogen peroxide is highly unstable on glass powder under either test condition and shows severe loss of activity after 3 hr on the iron/silica powder mixture. Without preincubation on this mixture, hydrogen peroxide shows the enhanced initial reaction rate characteristic of the theta-Fe_2O_3 stimulation, but a reduced plateau. With Mars analog soil B2 (Fig. 7), an initial stimulatory effect is evident without preincubation, but hydrogen peroxide activity is largely destroyed following three hours of preincubation on the soil. Considering all the data in Table I and Figs. 6 and 7, the stability of hydrogen peroxide does not appear to correlate with either organic content or with particle size. However, some correlation between stability and pH became apparent.

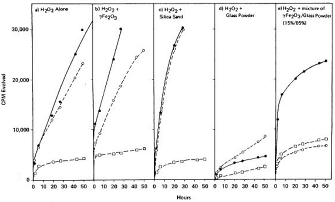


FIG. 6. Effects of γ-Fe_2O_3, silica sand, and glass powder on hydrogen peroxide stability. Sterile screw cap vials were prepared either (a) without a soil sample, or with a 0.4-g sample of (b) pure γ-Fe_2O_3, (c) silica sand, (d) glass powder, or (e) a γ-Fe_2O_3/silica powder mixture (15:85 w/w). After equilibrating the vials under 1 atm nitrogen at room temperature, 0.22 ml of 1.5×10^{-1} *M* hydrogen peroxide (pH 6.5) was added to each vial. Either immediately (●) or 3 hr after (○) the hydrogen peroxide addition, each vial 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 for biological or nonbiological activity of the LR nutrient (□). Evolved radioactive gas was collected by the getter technique. All reaction vials were conducted in duplicate.

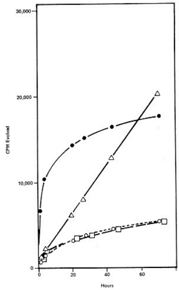


FIG. 7. Effect of Mars analog soil B2 on hydrogen peroxide stability. Sterile glass screw cap vials containing 0.4 g of Mars analog soil B2 were equilibrated under 1 atm nitrogen at room temperature with 0.22 ml of 1.5×10^{-1} *M* hydrogen peroxide (pH 6.5) added to each vial. Either immediately (●) or 3 hr after (○) the hydrogen peroxide addition, each vial received 0.11 ml of the LR nutrient. Evolved radioactive gas was collected by the getter technique. Results are compared to those obtained from LR nutrient and hydrogen peroxide in the absence of soil (△) and from LR nutrient and soil in the absence of hydrogen peroxide (□). All reactions were conducted in duplicate.

To examine the relationship between hydrogen peroxide stability and soil pH, five additional iron samples were obtained and tested. These samples (Table II) were selected to span a broad pH range and included samples of both theta- and alpha-iron. Their properties are given in Table II along with those of the theta-Fe_2O_3 (Cobaloy X4107) listed in Table I. Results of getter-type stability studies, in which hydrogen peroxide was incubated for 0 or 3 hr with these six samples prior to the addition of the LR nutrient, are shown in Fig. 8. Without preincubation, hydrogen peroxide reacts with LR nutrient on all soils except Mapico Brown 422 maghemite, pH 8.7, on which it loses activity immediately. For the other alkaline sample (lepidocrocite, pH 8.0), hydrogen peroxide activity is found when the reaction is performed immediately, but is totally lost within 3 hr contact with the soil. All other samples show activity losses, ranging from moderate to major, when LR nutrient is added after 3 hr of preincubation of hydrogen peroxide with the sample. All samples retaining activity after 3 hr incubation are at low pH.

TABLE II

pH OF IRON (theta AND alpha) SAMPLES TESTED

Sample		pH
theta-Fe_2O_3	MO2230 Maghemite	2.5
theta-Fe_2O_3	MO4228 Maghemite	2.5
theta-Fe_2O_3	Cobaloy X4107	3.0
alpha-FeO · OH	Goethite	3.8
theta-FeO · OH	Lepidocrocite	8.0
theta-Fe_2O_3	Mapico Brown 422 maghemite	8.7

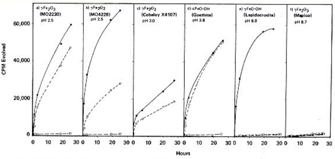


FIG. 8. Effect of various iron samples on hydrogen peroxide stability. Sterile screw cap vials containing 0.4 g of various γ- or α-iron samples, as indicated, were equilibrated under 1 atm nitrogen at room temperature with 0.22 ml of $1.5 \times 10^{-1} M$ hydrogen peroxide (pH 6.5). Either immediately (●) or 3 hr after (○) the hydrogen peroxide addition, 0.11 ml of the LR nutrient was added to each vial. For each mineral sample, a control was also conducted in which water was substituted for hydrogen peroxide as a monitor for biological or nonbiological activity of the LR nutrient (□). Evolved radioactive gas was collected by the getter technique. All reaction vials were conducted in duplicate.

These results support the hypothesis that hydrogen peroxide survival on soil requires low pH. However, that more than pH is involved in stability is indicated by separate studies in which aqueous solutions of hydrogen peroxide without soils were adjusted to various pH values from pH 3 to 10 and LR nutrient added before and after 3 hr standing at the indicated pH. In agreement with published literature (Edwards, 1962; Schumb *et al.,* 1955), the hydrogen peroxide solutions were found to be stable and essentially no difference in stability was found among solutions at different pH values. This demonstrates that some additional factor in the soil-metal compositions also affects stability.

Getter studies were next undertaken to determine whether low temperatures could enhance hydrogen peroxide stability on neutral or high pH soils. The soils selected for these studies are Mars analog B2 (pH 7.2) and the 15:85 w/w mixture of theta-iron/silica glass powder (pH 9.1). (Note that this is a different mixture than the theta-iron/silica sand mixture used for the Fig. 2 and Fig. 5 data. Hydrogen peroxide is more stable at room temperature on the lower pH silica sand mixture than on the higher pH silica glass powder mixture.) These two analog samples were of particular interest because of the observed instability of hydrogen peroxide added onto them at room temperature and because the Mars analog soil was tailored to the Viking analysis of Mars soil (see "Experimental").

Results obtained with the y-iron/silica glass powder mixture are shown in Fig. 9. In agreement with previous data, the iron in the mixture stimulates the reaction between hydrogen peroxide and LR nutrient when both are added together onto the analog at room temperature. Preincubation of hydrogen peroxide on this soil for 3 hr at room temperature destroys all subsequent activity with the LR nutrient. However, preincubation on the soil at dry ice temperatures (-78.5°C) for up to 6 hr preserves most of the hydrogen peroxide activity. If, after preincubation at -78.5°C for 3 hr, hydrogen peroxide is maintained at 10°C for an additional 3 hr prior to the nutrient addition, somewhat more than half of the hydrogen peroxide activity is lost. Essentially identical results were obtained with the Mars analog B2 when tested with LR nutrient immediately after warming from -78.5 to 10°C. However, in this case, most hydrogen peroxide activity disappeared after 3-hr incubation at 10°C following the -78.5°C exposure. With respect to flight data, this suggests that little if any, hydrogen peroxide, present in the Mars sample, would be expected to survive the 2-sol incubation at 10°C prior to LR nutrient addition.

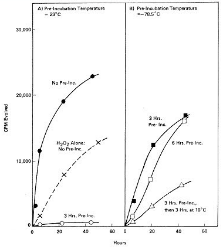


FIG. 9. Effect of freezing on hydrogen peroxide stability on γ-Fe_2O_3/ silica powder mixture. Screw cap vials containing 0.5-cm^3 samples of γ-Fe_2O_3/silica sand powder mixture (15:85 w/w) were equilibrated under nitrogen at 1 atm at room temperature (A) or on dry ice (−78.5°C) (B). After temperature equilibration for 15 min, 0.22 ml of a $1.5 \times 10^{-1} M$ hydrogen peroxide solution (pH 6.5) was added. For samples maintained at room temperature (A), 0.11 ml of LR nutrient was added with (○) and without (●) a 3-hr preincubation at room temperature. Reduced temperature preincubation regimes (B) were 3 hr at −78.5°C (■), 6 hr at −78.5°C (□), and 3 hr at −78.5°C followed by 3 hr at 10°C (△). Results are compared to the response (×) from H_2O_2 alone (no soil) at 23°C. Samples preincubated at reduced temperatures were raised to room temperature for approximately 10 min prior to nutrient addition. Radioactivity evolved following nutrient addition was monitored by the getter technique. All reactions were conducted in duplicate.

In a final attempt to ascertain hydrogen peroxide stability under conditions simulating those prevailing during the Viking mission, an additional getter experiment was conducted in which 0.22 ml of 1.5×10^{-1} *M* hydrogen peroxide was added separately onto the B2 Mars analog soil and onto the theta-Fe_2O_3/silica sand mixture (15:85 w/w) contained in 0.5-cm^3 portions in liquid scintillation vials. In this case, the theta-Fe_2O_3/silica sand mixture was selected to promote stability of hydrogen peroxide, whereas the B2 Mars analog was selected as the model closest to the known Mars surface composition. Both soils were preequilibrated under nitrogen at 1 atm on dry ice. The hydrogen peroxide solution froze instantly upon addition. The vials, still on dry ice, were placed in a glovebox under nitrogen and the entire arrangement transferred to a walk-in freezer maintained at -30°C. All samples were maintained for 19 days in the presence (caps removed) and absence (shielded) of ultraviolet radiation. At the end of the 19-day incubation, vials containing each soil were separated into four groups to receive one of the following treatments prior to addition of LR nutrient:

(1) Warm for 10 min at 10°C, then for 10 min at room temperature.

(2) Warm for 10 min at 10°C, then heat 3 hr at 50°C, then cool for 10 min at room temperature (simulates experiment 4 on Viking Lander 2).

(3) Warm at 10°C for 24 hr, then for 10 min at room temperature (simulates all Viking active runs, although on flight the storage time at 10°C was about 47 hr).

(4) Warm for 36 days at 10°C, then for 10 min at room temperature (simulates long term storage in Experiment 4 on Viking Lander I and experiment 5 on Viking Lander 2, although on flight these times were about 3 and 5 months).

As a control for this experiment, hydrogen peroxide was added to soil at room temperature and LR nutrient added immediately. This control was conducted just prior to beginning the 19-day -30°C storage using the same hydrogen peroxide solution used in the storage portion of the experiment.

The results are shown in Table III. For the theta-Fe_2O_3/silica sand mixture in the absence of uv, 20% activity remains after 19 days of cold incubation followed by 20 min of warming prior to addition of nutrient. Only 12% remains if this warming period at 10°C is extended to 24 hr. On Mars, the active soil samples were held for up to 47 hr at 10°C prior to nutrient injection. In order for sufficient hydrogen peroxide to be present after 47 hr on the theta-Fe_2O_3/silica sand at 10°C to give a response of flight magnitude, the initial concentration of the peroxide would have had to be considerably greater than that required to produce a 1.5×10^{-1} *M* solution upon nutrient injection. (Note that counting efficiency in the flight instruments was 3%; the active flight responses, ranging from 10,000 to 16,000 cpm, must be multiplied by a factor of 3 to relate them to the data presented in Table III).

TABLE III

EFFECT OF LOW TEMPERATURE IN PRESENCE AND ABSENCE OF UV RADIATION ON SUBSEQUENT STABILITY OF HYDROGEN PEROXIDE

Soil	Preincubation treatment of H_2O_2 on soil	Subsequent treatment	cpm Evolved[a] 20 hr after adding LR nutrient	Activity remaining (%)

theta-Fe_2O_3/silica sand (15:85 w/w)	None	None	80,000	100
	19 days at -30°C, no uv	20 min at 10°C	16,000	20
		3 hr at 50°C	4,000	5
		24 hr at 10°C	9,500	12
		36 days at 10°C	1,000	1
	19 days at -30°C, +uv	20 min at 10°C	4,000	5
		3 hr at 50°C	5,000	6
		24 hr at 10°C	5,000	6
		26 days at 10°C	1,000	1
Mars analog B2	None	None	16,000	100
	19 days at -30°C, no uv	20 min at 10°C	1,000	6
		3 hr at 50°C	500	3
		24 hr at 10°C	200	1
		36 days at 10°C	0	0
	19 days at -30°C, +uv	20 min at 10°C	1,000	6
		3 hr at 50°C	500	3
		24 hr at 10°C	200	1
		36 days at 10°C	0	0

a Data corrected for cpm from nutrient alone. Counting efficiency 10%, or approximately three times higher than on flight.

Note. Aliquots (0.5 cm^3) of the indicated soil samples were placed in screw cap vials on dry ice in a glovebox under nitrogen at 760 mm Hg. After temperature equilibration for 15 min, 0.22 ml of a 1.5×10^{-1} *M* hydrogen peroxide solution (pH 6.5) was added. The vials were capped, moved into a plastic portable glovebox under nitrogen at 760 mm Hg, and transferred to a cold room at -30°C for 19 days. Vials were stored either capped or uncapped and exposed to an ultraviolet source at 254 nm to give a calculated exposure of 1620 $\mu W/cm^2$. 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 min, 24 hr, or 36 days, or heat-treated for 3 hr at 50°C. After respective treatments, vials were stored at room temperature under nitrogen for 10 min prior to the addition of 0.11 ml of LR nutrient. Radioactivity evolved following the nutrient addition was monitored by the getter technique. Percentage hydrogen peroxide remaining after each treatment was calculated by comparing the 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.

Of the hydrogen peroxide activity remaining on the theta-Fe_2O_3/silica sand mixture after the 19-day storage at -30°C, more activity is lost by subsequently heating at 50°C for 3 hr than by storing at 10°C for 24 hr (95 vs 88%). However, despite these severe losses with respect to the original amount of hydrogen peroxide present, Table III shows that the 50°C heating loss was 60% of the nutrient surviving 24 hr at 10°C. On flight, the Mars sample was stored at about 10°C for approximately 12 hr prior to heating to 50°C for 3 hr (cycle 4 on Viking Lander 2). It was then stored approximately 25 more hours at 10°C before nutrient injection. This flight treatment resulted in approximately 60% loss of activity relative to that obtained from an active response after approximately 2 sols (48 hours) storage at 10°C. Had this flight regime been closely followed, the loss in activity in the simulation test would have been significantly greater. Thus, even without the instrument artifact mentioned earlier, the 50°C heating experiment run on Mars would probably have resulted in almost total loss of activity if the active agent were hydrogen peroxide unless some unknown soil property afforded protection.

Table III also shows that essentially all hydrogen peroxide activity was destroyed by 36-day storage at 10°C even in the absence of uv. If some survival of hydrogen peroxide after exposure to 10°C for 48 hr is conceded, these findings satisfy the third criterion. Coupled with the sensitivity of hydrogen peroxide to 160 and to 50°C, they demonstrate, for the first time, that in appropriate amounts with appropriate soil composition, the thermal responses of the active agent on Mars can be simulated by hydrogen peroxide.

The results with the B2 Mars analog (Table III), on the other hand, show that, even in the absence of uv, hydrogen peroxide reactivity with the LR nutrient on this soil is considerably less than on the theta-Fe_2O_3/silica sand mixture. This is despite the fact that both soil samples contain approximately 15% theta-Fe_2O_3. Finally, severe activity reductions occurred for both soils, under all conditions, when exposed to UV.

Hydrogen Peroxide Formation on Mars Analog Soil

The fourth prerequisite for hydrogen peroxide to be the active agent in the LR experiment is evidence for its formation under Mars environmental conditions. Mechanisms postulated include production in the Mars atmosphere coupled with freezing out on the planet surface (Parkinson and Hunten, 1972; McElroy *et al.,* 1977; Hunten, 1979), interaction of frost and surface material at low temperature (Huguenin *et al.*, 1979), and ultraviolet-induced formation by a reaction between water molecules and the Mars surface material (Huguenin, 1976).

Experiments in which oven-dried analog soil B2 was exposed in the Mars ultraviolet simulator for 712 hr under 6 Torr carbon dioxide at -35°C in the presence and absence of equilibrium water vapor failed to produce an agent that would react with the LR nutrient in the TSM (Fig. 10). Getter-type, experiments were conducted in further attempts to generate hydrogen peroxide from water in contact with Mars analog soil. In other tests of the ultraviolet-frost interaction theories, theta-Fe_2O_3/silica sand mixture was selected because it induced Mars-like thermal characteristics in hydrogen peroxide. Variable amounts of water (0, 50, 100, 200 µl) were added to this mixture and aliquots were incubated at dry ice temperatures in the presence and absence of ultraviolet radiation for 3 hr. In no case was any evidence obtained for formation on soils of an agent that would subsequently react with LR nutrient. Thus, it seems likely that any hydrogen peroxide present in the soil would have had to be produced in the Mars atmosphere (by one of the mechanisms cited above) and deposited on the soil. Rather than attempt the great difficulties required for experimental verification of these atmospheric mechanisms in the laboratory, the possibility that one would satisfy criterion 4 was acceded.

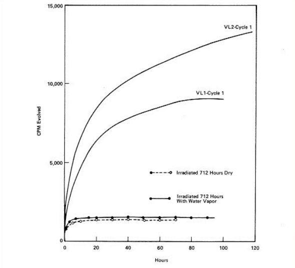


FIG. 10. Reaction between LR nutrient and Mars analog B2 following uv irradiation of analog soil. Two 0.5-cm^3 samples of Mars analog soil B2 were dried at 160°C for 24 hr and then exposed to 712 hr ultraviolet irradiation at −35°C in the presence and absence of water vapor (see "Experimental"). At the end of the exposure, the samples were stored at approximately −15°C for 3 months prior to testing for activity with the LR activity in the TSM using an active flight sequence at 10°C under 5 Torr carbon dioxide. The results are compared to active LR responses on Mars.

Accumulation and Survival of Hydrogen Peroxide on Mars AnalogSoil

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 coefficient for the destruction of hydrogen peroxide on the surface of Mars exceeds that for its formation through the entire column of the Mars atmosphere by a factor of 10^7 (Parkinson and Hunten, 1972; McElroy *et al.,* 1977). The possibility that freezing or complexing could permit hydrogen peroxide to accumulate protected against ultraviolet irradiation was examined in the experiment cited in Table III. However, as shown, in the two cases where any hydrogen peroxide activity remained after the storage regime in the absence of uv exposure (namely, for storage of 10 min or 24 hr at 10°C on the theta-Fe_2O_3/silica sand mixture), the uv exposure dramatically diminished hydrogen peroxide activity with LR nutrient. In all other cases, insufficient activity remained even in the absence of ultraviolet exposure to ascertain any further deleterious effect of uv. The uv light incident to the surface of Mars would seem to preclude any accumulation or preservation of hydrogen peroxide on Mars. Thus, hydrogen peroxide does not appear to fulfill the fifth criterion of survival on Mars in the presence of ultraviolet radiation. New data to support this contention are presented in the "Discussion."

DISCUSSION

Previous findings herein cited concerning the peroxides, superoxides and ozonides as possible LR reactants on Mars narrowed possible nonbiological candidates for the Mars active agent to hydrogen peroxide. Under the experimentally determined conditions reported herein, hydrogen peroxide on select analog soils can reproduce the kinetics and thermal information contained in the LR Mars data. However, the soil composition and chemical characteristics required differ from those of the Mars analog B2 soil prepared by the Viking 1norganic Analysis Team to match the Mars spectra. Thus, if the Mars analog B2 soil is a reasonable representation of the Mars surface material, the plausibility that hydrogen peroxide is the active agent becomes remote. However, should other Mars analog soils provide a good match to the surface of Mars and possess the requisite characteristics indicated by our stability studies, the hydrogen peroxide issue could remain open. Even so, a major problem would remain in that no mechanism has yet been found to permit hydrogen peroxide accumulation in the hostile Mars conditions.

However, assuming that the theta-Fe_2O_3/silica sand mixture is an acceptable Mars model soil, a rough calculation can be made of the hydrogen peroxide concentration that must be present in the Mars soil to produce a positive LR flight response. As seen in Table III, when 0.22 ml of 1.5×10^{-1} *M* hydrogen peroxide were added to 0.5 cm^3 of the theta-Fe_2O_3/silica sand mixture and the mixture held at -30°C under a uv flux equivalent to 190 days on Mars, then held at 10°C for 24 hr, subsequent injection with 0.11 ml of LR nutrient produced a response of 5000 cpm. Adjusting for the higher counting efficiency of the getter technique, this equates to a response in the Viking 1nstrument of approximately 1600 cpm, or about 1/10th that obtained on Mars. This indicates that, to produce a flight-type response, hydrogen peroxide in the soil must be sufficiently concentrated to produce a 1.5 *M* solution when 0.115 ml of nutrient was injected. This corresponds to a hydrogen peroxide concentration in the soil greater than 2% w/w. Since the 0.115 ml of LR nutrient may only partially wet the 0.5 cm^3 of soil, at least initially, the hydrogen peroxide concentration in the soil may have to be even higher than 2%. Because hydrogen peroxide on the surface is susceptible to destruction by ultraviolet radiation and by Mars temperatures approaching 10°C, hydrogen peroxide would have to be formed at considerably higher concentrations and replenished frequently in the course of a year. The only means of lowering the hydrogen peroxide concentration needed to produce a response of flight magnitude would be the existence of an extraordinarily effective catalyst in the Mars soil and/or formation of a highly stable complex to preserve hydrogen peroxide in the Mars surface material. Even then, the problem of survival of hydrogen peroxide under the Martian uv flux continues to cast doubt on the hydrogen peroxide theory.

Previous analysis of the LR flight data showed (Levin and Straat, 1979b) that essentially no decomposition of the Mars active agent would be expected over a 5-day storage period at 10°C. This is in sharp contrast to the nearly 90% activity loss of hydrogen peroxide (Table III) when stored for 24 hr at 10°C on the most stabilizing soil mixture yet devised in these experiments. The difference means that hydrogen peroxide must be sequestered in some highly efficient and yet unknown manner on Mars or that its possibility of explaining the LR results must be abandoned.

On the other hand, a biological explanation can also account for the observed flight data and we have previously discussed (Levin and Straat, 1979a, Levin *et al.,* 1978) 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 have been found beneath rock surfaces in Antarctica's dry valleys. Such models lend plausibility to the hypothesis that biological organisms could exist in the Mars environment. As cited and discussed previously, lichen are known which photosynthesize and grow when atmospheric water vapor is the sole water source. Anderson and Tice (1979) describe a hydrosphere at the Viking Lander sites which they believe could support endolithic organisms.

In pursuit of interpreting the Mars LR data, a study of the Viking Lander pictures was made (Levin *et al.,* 1978). Yellowish to greenish patches on some rocks near Viking Lander 1 and changes in color pattern with time were observed. The changes could have resulted from activities associated with the lander in combination with wind effects. On the other hand, the colored patches showed some similarities with lichens on rocks when images taken with the Viking Lander test camera were viewed and computer-analyzed.

One of the major obstacles to acceptance of a biological interpretation of the Viking LR results, namely the failure of the Viking Organic Analysis experiment to detect organic compounds in the Mars surface material (Biemann *et al.,* 1976, 1977), may reflect insufficient sensitivity of the instrument used on Mars. On theoretical grounds, we have estimated (Levin and Straat, 1980) that a soil must contain approximately 5×10^8 microorganisms per gram, or organic detritus and living cells, in combination, to provide the equivalent biomass to permit the detection of specific compounds or pyrolysis products at the sensitivity limits reported for that instrument. This estimate can be contrasted with the fact that positive LR responses have been obtained from a terrestrial sample containing as few as 10^2 living microorganisms per gram. LR responses of flight magnitude are estimated to require 10^4 living terrestrial cells per gram of soil. Thus, on theoretical grounds based strictly on instrumental sensitivity, the LR experiment should be able to detect a life response in a soil in which no organics can be detected by the organic analysis instrument.

Biemann (1979) has recently reported that a soil sample collected from Antarctica, identified (Lavoie, 1979) as Antarctic Soil No. 726, failed to indicate the presence of indigenous organic material when analyzed in a flight-like organic analysis instrument. Microorganisms had not been detected in this sample by classical methods (Cameron *et al.,* 1970). When tested in separate LR TSM experiments, this sample did indeed give a low-level positive response (Fig. 11). Details are being published elsewhere (Levin and Straat, 1981). (This and other low-population or sterile soils had been tested in the TSM program during the 1976 Viking mission and had been used as "library responses" for comparison to the flight response).

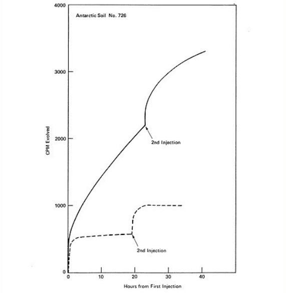


FIG. 11. Reactivity of Antarctic soil No. 726 with LR nutrient. On two consecutive TSM runs, a 0.5-cm^3 aliquot of Antarctic soil No. 726 was added to the TSM test cell and equilibrated at 10°C under 5 Torr carbon dioxide. One sample was heat sterilized for three hours at 160°C (---) and the other was stored in the test cell at 10°C for an equivalent length of time (——). A 0.115-ml aliquot of LR nutrient was added according to the flight sequence at the onset of each reaction and again at a latter time, indicated for each run by an arrow.

Various reports (for example, in the special Mars issue of the *Journal of Molecular Evolution,* December 1979) offer chemical, physical, or physicochemical models and data to explain the LR Mars results. While several of these experiments have elicited a positive reaction from the LR nutrient or a component thereof, none has duplicated the thermal sensitivities shown at 160, 50, and 10°C by the LR active agent on Mars. Banin and Rishpon (1979) report that montmorillonite (Wyoming bentonite) and nontronite clays have catalytic properties and produce an LR active response which heating the sample at 160°C drastically reduces (their Curve 5, Fig. 6). Although no loss of activity at 50 or 10°C is claimed, this work is especially interesting in view of the accumulating evidence (Toon *et al.,* 1977) indicating substantial quantities of such clays on Mars. However, not only does their Curve 5 show a drastic reduction in the evolution of gas, the kinetics of that evolution, being a straight line, are very different from the kinetics of the active LR Mars responses and from the LR Mars 50°C treatment response kinetics. The Curve 5 kinetics are similar to those shown for three of the other clays in Fig. 2 of the Banin and Rishpon paper. All four of these curves are typical of the evolution of minor amounts of carbon dioxide which occurs (Levin and Straat, 1970, 1976a) when LR nutrient or its constituents are applied to acid soils. The montmorillonite prepared by Banin and Rishpon for use in the Curve 5 experiment is stated to contain 30% H^+ in its exchange capacity. A pH of 3.6 is shown for a 4 wt% suspension. In the experiment cited, 0.1 ml of nutrient solution was added to 250 or 500 mg clay, thereby assuring even greater acidity. We believe that Curve 5 represents the response from an acid soil sample which, fortuitously, was sterile. The positive responses are best explained by the presence of microorganisms. Indeed, Banin reported (1978) microbial activity in some of the very samples used in this work.

Moreover, the "positive" responses with LR nutrient are also questionable in that Banin and Rishpon (1979) report a counting efficiency of 75%. The LR instrument counting efficiency was only 3%. Nonetheless, Banin and Rishpon compare the absolute magnitudes of the responses. It would appear that their LR nutrient reponses should be divided by 25, rendering them typical of the low level obtained in the LR TSM with analog B2 soil when LR nutrient was applied.

The present paper also examines the catalytic abilities of such clays to evoke LR responses since Mars analog B2 contains 17.5% nontronite and 29.8% Wyoming bentonite (Levin and Straat, 1979c). Using sterile techniques, we obtained no positive responses. However, in view of the important consequences of the Banin and Rishpon hypothesis, we suggest they duplicate their work under sterile conditions and in a manner to differentiate between the effects of pH and of the intrinsic properties of the clay.

Considerations of hydrogen peroxide as the active LR agent on Mars have assumed the presence of that compound on Mars. It occurred to us that a reexamination of the Mariner 9 IRIS data (Hanel *et al.,* 1972) on the Mars atmosphere might provide direct information. The spectra were examined (Hanel and Maguire, 1980). They found no signature for hydrogen peroxide and, based on instrument sensitivity, were able to calculate an upper limit for hydrogen peroxide through the column of the Mars atmosphere of 8×10^{-4} cm atm at STP, or 1.2×10^{-2} precipitable μm some two to three orders of magnitude below the atmospheric water abundance.

In review of the results presented herein, an objective view of the known facts, therefore, must allow for the possibility that the LR experiment detected biological activity in the Mars surface material. A return mission to Mars, with a more elaborate LR experiment and with additional chemical and physical analytical capability as components of an expanded instrument package, seems warranted for resolving this important matter.

ACKNOWLEDGMENTS

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