

## The Labeled Release Experiment – Past and Future

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**Abstract:** The primary focus of NASA's Mars and other planetary exploration programs, such as Titan, Enceladus, and Europa, "is to determine if life is or was present." The author suggests that NASA's stated primary focus should, therefore, include a re-examination of the data from the 1976 Viking Mission Labeled Release (LR) life detection experiment. That experiment obtained repetitive strong positive signals supported by a variety of controls, altogether signifying the detection of microbial metabolism in the top few centimeters of the surface of Mars. The data fall well within those obtained from hundreds of terrestrial LR tests of soils and microbial cultures that made up the response library assembled for the Viking LR experiment. No physico-chemical theory or experiment of the many attempted over the years has duplicated or explained away the Viking LR results as indicative of life. Together with pertinent findings on Mars and Earth since Viking, the possibility of microbial life on Mars has become a singular scientific issue warranting the herein requested re-examination of the Viking LR data.

An elaboration of the Viking LR could seek to confirm the Viking results. The experiment would probe the active agent on Mars for a chiral preference in reacting with the LR nutrients. Chiral preferences are a strong characteristic of all known life, but play no role in chemical reactions. A finding of chiral metabolism would unambiguously prove the existence of extant life, even to the most critical observers. Moreover, were a chirality found that was different to that now known for terrestrial life, the finding would be strong evidence for an independent origin of that life form, even if it were found on Earth.

### **1. Introduction**

It has been more than a third of a century since an extraordinary activity was discovered on the surface of Mars. When dosed with simple  $^{14}\text{C}$ -labeled organic compounds, the soil reacted immediately, producing a strong, continuing evolution of one or more  $^{14}\text{C}$ -

labeled carbon gases. This same reaction occurred at the two Viking landing sites 4,000 miles apart. The data, themselves, are incontrovertible, and there has been no disagreement in the scientific community about these findings. The disagreement is about the interpretation – for life or not – of the data. Yet, despite numerous additional missions to Mars since Viking, there has been no attempt made to investigate the nature of the reaction. This lack of scientific pursuit is particularly disconcerting since the experiment that made the discovery satisfied the pre-mission criteria for the discovery of microbial life. Instead of pursuing the scientific method, officials directing the U.S. and European space agencies made the unwarranted presumption that the reaction was caused by a strong oxidant in the surface material of Mars. As a result, no life detection experiment has been sent to Mars since Viking. Moreover, no follow-up experiment has sought to identify the putative chemical to which the startling reaction has been attributed.

## **2. Background**

In 1976, NASA's Viking Mission to Mars conducted a series of Labeled Release (LR) experiments (Levin and Straat, 1976) seeking microbial life in the soil of the red planet. In the LR, a tiny drop of an extremely dilute aqueous solution of low specific activity  $^{14}\text{C}$ -labeled organic nutrients, shown in Table 1, was added to the center of a soil sample. The solution spread across the sample chromatographically, producing a range from wet to moist soil.

**TABLE 1. VIKING LABELED RELEASE SUBSTRATES**

Labeled Substrate	Structure and Label Position (*)	Concentration (x 10 <sup>-4</sup> M)	μCi mL <sup>-1</sup>	Specific Activity (Ci/Mole)
<sup>14</sup> C-glycine	NH <sub>2</sub> *CH <sub>2</sub> *COOH	2.5	4	16
<sup>14</sup> C-DL-alanine	*CH <sub>3</sub> *CH (NH <sub>2</sub> )*COOH	5.0	12	48
<sup>14</sup> C-sodium formate	H*COONa	2.5	2	8
<sup>14</sup> C-DL-sodium lactate	*CH <sub>3</sub> *CHOH*COONa	5.0	12	48
<sup>14</sup> C-calcium glycolate	(*CH <sub>2</sub> OH*COO) <sub>2</sub> Ca	2.5	4	16

The rate and amount of any <sup>14</sup>C gas evolved from any reaction between the soil sample and any of the nutrient(s) were to be monitored by a beta detector. Should a positive response occur, the experiment called for a control designed to determine whether the response were of biological or chemical origin. The NASA-selected control consisted of pre-heating a duplicate sample of the soil to a temperature designed to destroy or severely impair metabolism, but not high enough to destroy chemical reactants that might have caused the positive result.

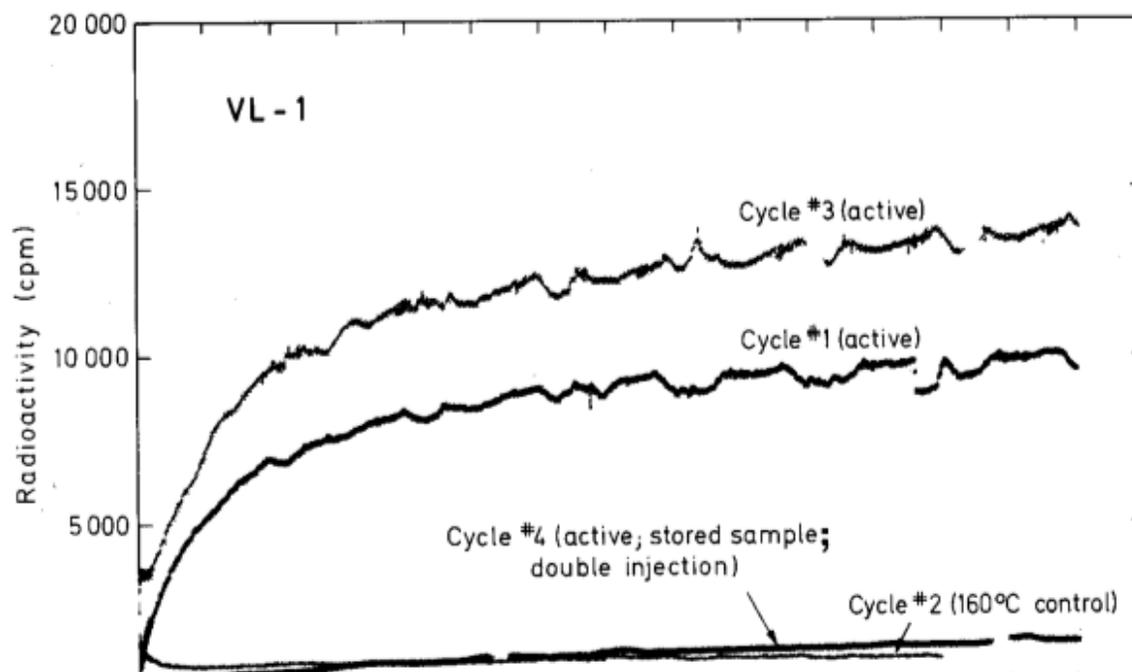
### **3. The LR on Mars**

After a flawless landing in Chryse Planitia, Viking 1 performed the first LR experiment on July 30, 1976. The soil tested had been trenched by the Viking sampling arm from the surface to a depth of about four cm, placed in the distribution box and then dispensed to the LR test cell. Immediately upon injection of the LR nutrient, <sup>14</sup>C-labeled gas began evolving. The cumulative amount of gas rising to the detector chamber was measured by its radioactivity, recorded every four minutes initially and then at 16 minute intervals for the remainder of the eight-sol experiment. After about three sols, the volume of the accumulating gas approached a plateau, but continued to show a very slight, steady increase for the remainder of the experiment. The response clearly called for a control run. A duplicate soil sample was inserted into a fresh test cell, heated for three hours at 160° C to sterilize it, allowed to cool and then was tested. It produced virtually no response, thus

completing the pre-mission criteria for the detection of microbial life. All VLR1 Cycle 1 results, both test and control, are shown in Figure 1.

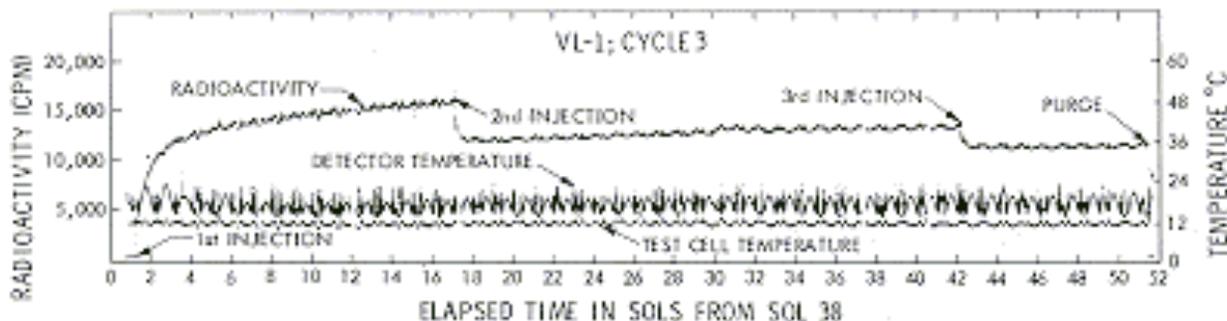
### Viking Labeled Release Experiment on Mars

1:



**FIGURE 1. ALL VLR1 CYCLE 1 RESULTS**

Additional, *ad hoc* experiments were then made. At the end of the eight-sol VLR1 Cycle 3 test, second and third injections of nutrient were made, as seen in Figure 2. Each of these later injections produced a surprising and precipitous fall in radioactivity in the headspace of the detector cell, indicating a similar percentage decline in the total amount of radioactive gas that had been evolved (held in the test cell, the detector cell and the interconnecting tube). While the diurnal temperature changes in the test cell correlated with the fluctuations in the detector cell, the changes in CO<sub>2</sub> solubility produced by the temperature changes could account for only about one-third of the observed changes in gas volume (Levin and Straat, 1979).



**FIGURE 2. EFFECT OF 2<sup>ND</sup> AND 3<sup>RD</sup> INJECTIONS ON VL1 CYCLE 3**

The precipitous drops in headspace gas were followed by gradual release of the absorbed gas, as indicated by its approaching the original maximum amplitude after the sharp declines.

While an increase in evolved gas would have supported a biological conclusion, the lack of such an increase was taken by some as evidence against biology. However, a search of the Viking LR library of terrestrial responses found a low-population Antarctic soil (NASA-Ames bonded Antarctic Soil 664) that produced a positive response on first injection, and, very similar to the Mars LR response, upon second injection immediately re-absorbed headspace gas. A negative response to the heated control demonstrated that the initial positive response was biological. Figure 3 presents these data in semi-log plot.

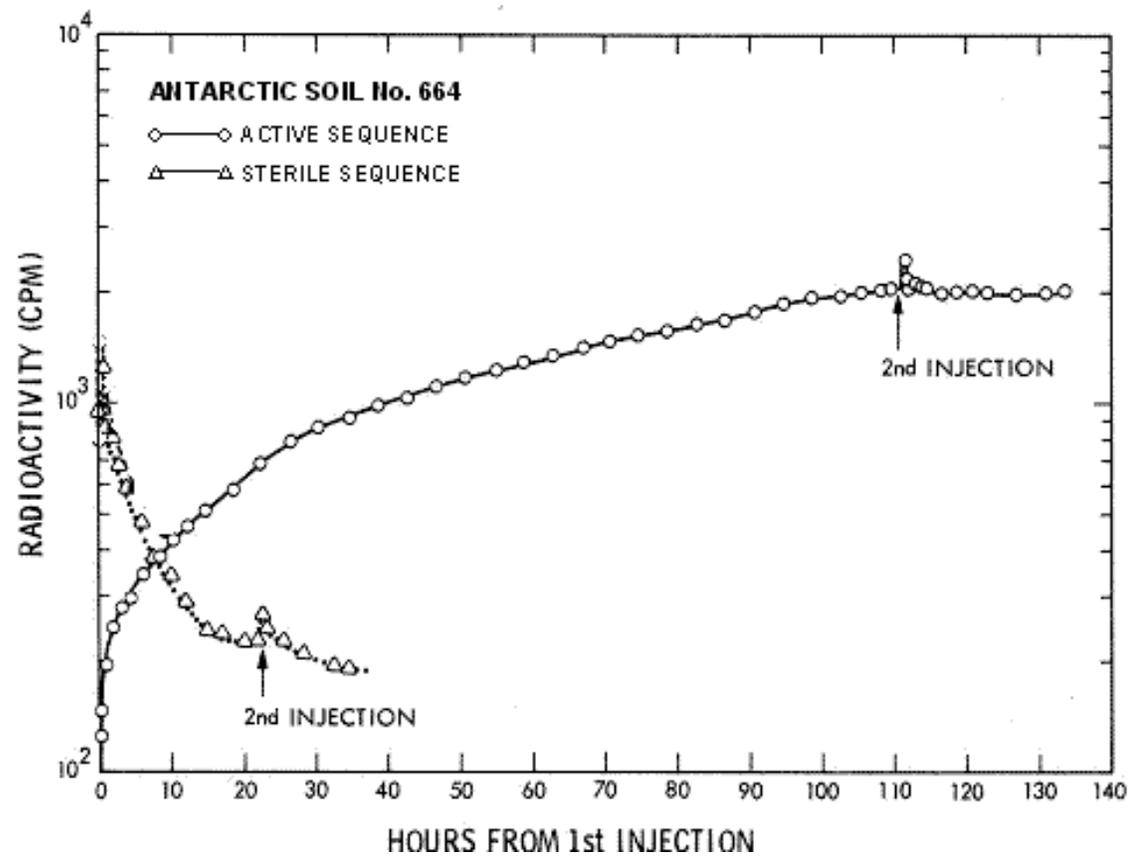
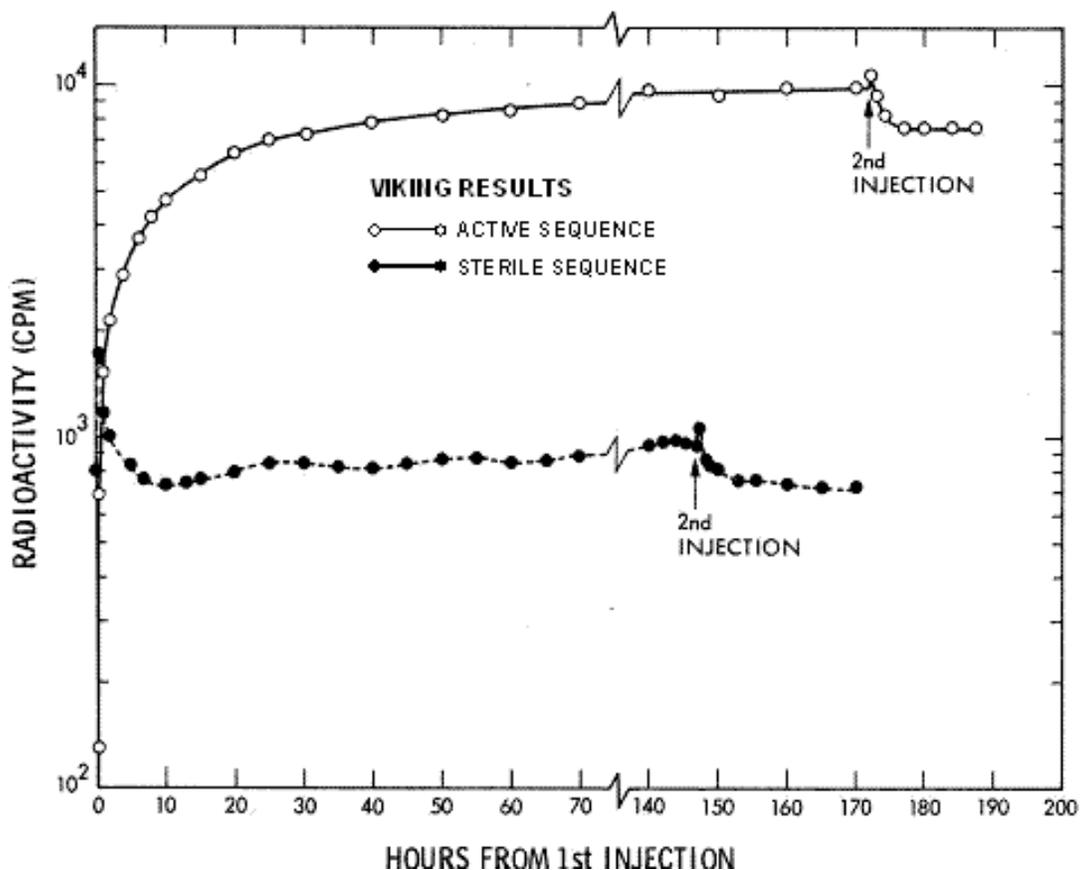


FIGURE 3. EFFECT OF 2<sup>ND</sup> INJECTION ON ANTARCTIC SOIL NO. 664

To facilitate comparison, the Viking data are also shown in semi-log plot in Figure 4.



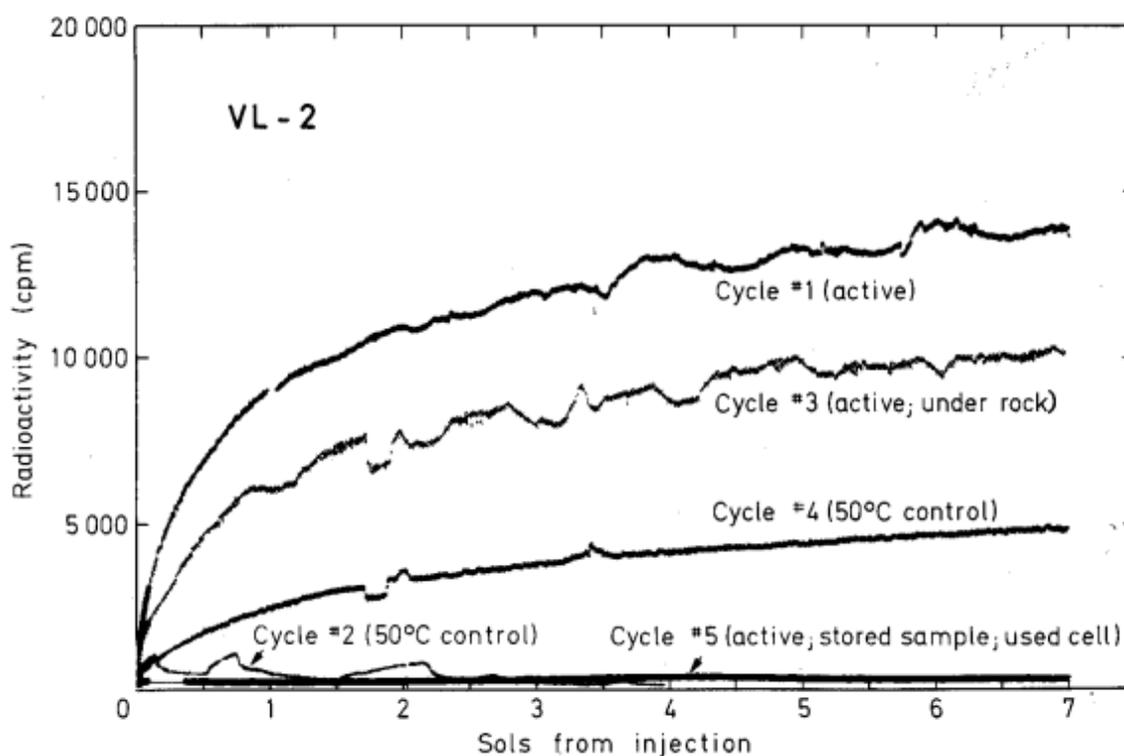
**FIGURE 4. SECOND INJECTION VLR1, CYCLE 1**

The kinetics of the Mars and Antarctic responses are strikingly similar, thus supportive of, or, at least, consistent with the LR response on Mars being of biological origin. (The high initial cpm of the sterilized Antarctic soil reflects residual gas remaining after incomplete purge of a previous test. This does not negate its serving as a valid control, nor the demonstration of the effect of the 2<sup>nd</sup> injection.)

Failure of the 2<sup>nd</sup> injections on Earth and Mars to elicit a response can be attributed to the organisms in the active samples from each planet having died sometime after the 1<sup>st</sup> injections. The effect of the 2<sup>nd</sup> injections was to wet the soils, causing them to absorb headspace gas physically. A gradual re-emergence of the gas into the headspace in the Viking LR occurred as the system equilibrated over time.

Four thousand miles away, Viking 2 landed in colder, high latitude Utopia Planitia. The LR results there were very similar to those of VL1. Based on knowledge gained from the

Viking 1 LR results, more definitive controls were run to further discern the nature of the active agent. These included moving a rock just after dawn to take a soil sample not exposed to UV light for geological time. The light-denied soil responded with a strong positive, thus invalidating an initially prevalent theory that the LR response was caused by UV activation of the soil. Another control run demonstrated that even modest heating of the soil significantly depressed its response. The active agent in the soil, initially responsive at 10° C, was greatly inhibited or inactivated by heating to 46° C or 51° C. This thermal differentiation is exhibited by a variety of terrestrial microorganisms (e.g. *E. coli* v. other coliforms). All VLR2 Cycle 1 results are shown in Figure 5.



**FIGURE 5. ALL VLR2 CYCLE 1 RESULTS**

As the LR experiments continued on Mars, it was found that samples isolated in the dark sample distribution box and held at ~ 10° C lost their activity over storage periods of three and five months, respectively, at the two Viking lander sites. No means of attributing this loss of activity to a chemical agent under such benign conditions has been advanced.

As with VL1, the VL2 LR data satisfy the pre-mission criteria for life. All results either support, or are consistent with, the presence of living microorganisms.

A description of the Viking Mission LR experiment and all data obtained by it are now available on-line (NASA, 2009).

### 4. Critiques

Immediately following the first positive Viking LR response, the statement was made that the response was “too much, too soon,” even before the control result was obtained (which was subsequently ignored). However, the pre-mission library of responses showed this statement to be in error. Figure 6 shows the Mars positive response at the lander 1 site to fall within the low end of the terrestrial soil responses, as did the subsequent positive responses from the Viking 2 lander site.

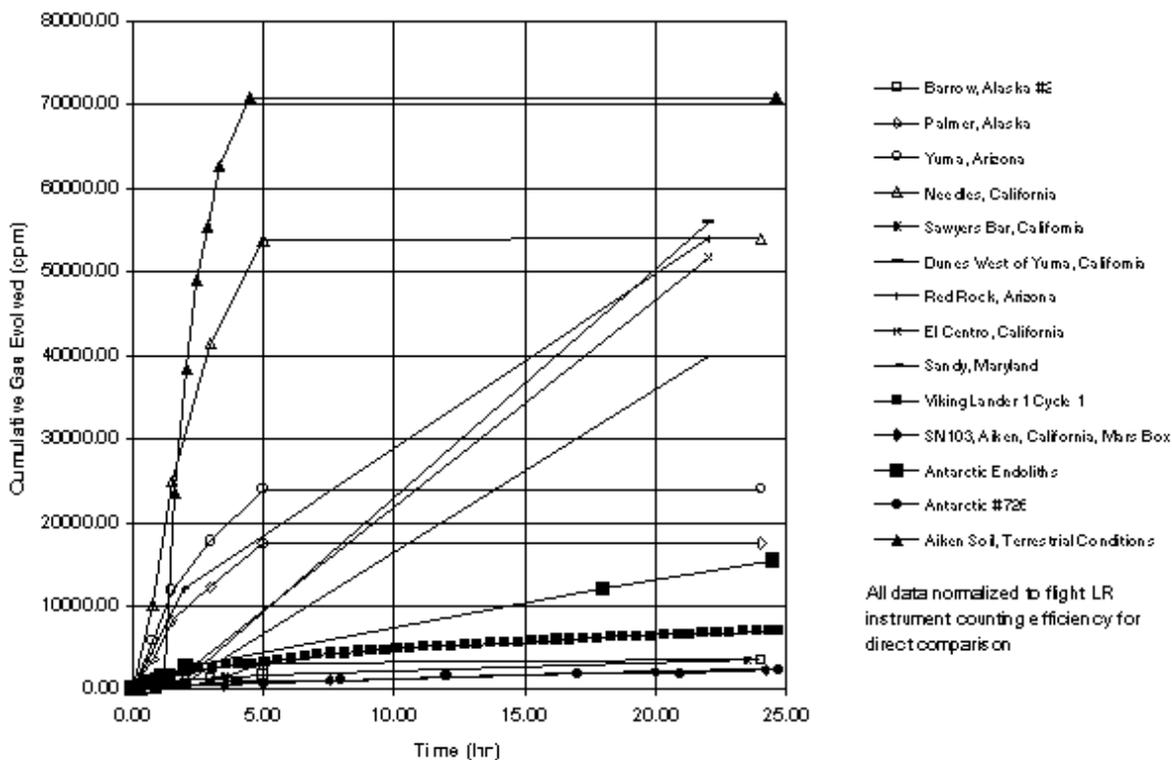


FIGURE 6. COMPARISON OF TERRESTRIAL AND MARS LR ACTIVE RESPONSES

In summary, the agent detected in the soil samples maintained in test chambers under Martian atmosphere at approximately 10° C, immediately responded with an evolution of <sup>14</sup>C-labeled gas upon injection of the nutrient solution. After approximately three sols, the evolution of gas approached plateau for the remainder of each positive run. The control LR tests run on duplicate soil samples of those that had responded positively. produced a nil response after having been heated at 160° C; essentially nil after heating at 51° C; greatly impaired at 46° C; and nil after samples at each landing site were held at 10° C in the dark for three and five months in their respective distribution boxes. In active LR runs, the Martian soil samples were held in the test chambers at approximately 10° C for up to 10 sols before nutrient injection, indicating the time of inactivation was somewhere between 10 sols and three months. This rapid deterioration rules out the possibility that a strong oxidant was responsible for the LR positive results, since all samples, obviously, had been “stored” on Mars for more than several months before they were tested.

Despite these findings, which, at a minimum, if the pre-mission criteria for life were now rejected, were, at least, completely consistent with the positive responses being attributed to a living agent. Nonetheless, because of the great significance of finding extraterrestrial life, the author was cautious. When called on to give a summary of the LR findings in a press conference at JPL, he said that, although the results on Mars were similar to those of viable terrestrial soils, it was too early to say that life has been detected on Mars. He advised “There are too many factors that have yet to be weighed and tested. All we can say at this point is that the response is very interesting. Be it biological or non-biological, it is unanticipated.”

### **5. The Other Viking Life Detection Experiments**

The Gas Exchange (GEx) experiment (Oyama and Berdahl, 1979) was administered in two phases. Its first phase allowed water vapor alone to contact the soil sample while the headspace gas above the sample was monitored for changes. In phase 2, the sample was dosed with a “chicken soup” containing a mixture of amino acids, sugars, vitamins, and other organic and inorganic compounds in aqueous solution. As with phase 1, the composition of the headspace gas above the sample was monitored for changes in composition and concentrations indicative of life. At Viking site 1, upon humidification of the soil sample, an immediate evolution of gas, identified as O<sub>2</sub>, was detected. The evolution lasted three sols,

and abruptly ceased. When a duplicate sample was heated and tested as a control, it issued about half as much O<sub>2</sub> as the original sample. Applying the dose of chicken soup to the sample that issued O<sub>2</sub> when humidified, no further evolution of O<sub>2</sub> occurred. The results were interpreted by the GEx Experimenter and most of the scientists as supporting the belief that the release of O<sub>2</sub> was caused by a strong oxidant in the soil. This same oxidant, they contended, was also the source of the positive LR reaction. The possibility of life was rejected.

The Pyrolytic Release (PR) experiment (Horowitz and Hobby, 1977) was the inverse of the LR, but was based on the LR's technique of <sup>14</sup>C-labeling. Trace-labeled CO<sub>2</sub> and CO, as components of a simulated Martian atmosphere, were placed in a test chamber. A soil sample was introduced to the chamber and a sun lamp was then turned on to induce any photrophic microorganisms that might be present to fix the labeled carbon molecules. After an incubation period of five sols, the sample was heated moderately to drive off any labeled gas that had been physically adsorbed on the soil. Then, the soil was heated to a temperature that would pyrolyze any microorganisms present, thus liberating any labeled carbon that had been photosynthetically fixed. A positive response, confirmed by a negative or greatly diminished response from a heated control would be evidence for life. Data reported for the PR experiment were confounding. Once again, a "positive" reaction had been detected, but it was very small, a second pulse of only 96 cpm over a background of 15 cpm. This "positive" response compared poorly to responses from live soils in terrestrial PR tests achieving second pulses of several thousand cpm. The Experimenter said, "There's a possibility that this is biological, but there are many other possibilities that have to be excluded." In an attempt to validate the "positive" response as evidence for life, it was counted for 24 hours to determine its statistical significance. This was announced as significant to three sigma. However, this determination merely proved statistical significance above the level of background radiation, and in no way proved the response was significant as being of biological origin. In developing the PR in the laboratory, positive

responses of the magnitude of the Mars response, and exceeding it, had been obtained in tests of sterilized soil (Hubbard, J. S., *et al.*, 1973). Responses from viable soils were orders of magnitude larger than that obtained on Mars. However, the Martian PR results did prove something very important – that sunlight shining on Martian atmosphere synthesized organic matter (which produced the PR response). What’s more, this organic matter persisted over the five sols of the PR test, thus proving that no strong oxidant was present in the Martian soil, as had been proposed to explain away any LR evidence for life. Were an oxidant present, it would have destroyed the organic matter formed, providing the PR with a nil response. The survival of the organic matter was strong evidence against the negative results reported by the organic analysis (GCMS) instrument.

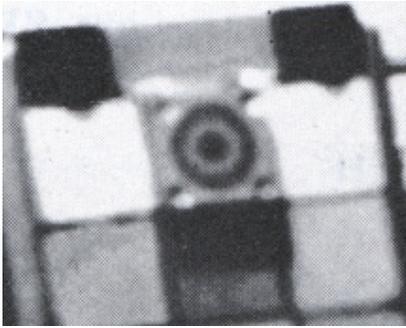
### **6. A History of Proposed Abiological Theories**

It was quickly proposed (Oro, 1976), and has been widely accepted ever since, that some ubiquitous oxidant, ( $\text{H}_2\text{O}_2$  was initially proposed) coated the surface of Mars and destroyed any organic matter including life. This was shown unlikely by analyses of the Martian atmosphere, cited in the bullets below, which reported the absence of  $\text{H}_2\text{O}_2$  (down to upper limits well below the amount required to coat soil with an amount that could produce the LR positive responses) before and after Viking. These findings, along with the survival of organic matter formed in the PR experiment were largely ignored. In addition to  $\text{H}_2\text{O}_2$ , many other potential oxidants were proposed. Among them, one proposed (Yen *et al.*, 2000) that superoxide radical ions ( $\text{O}_2^-$ ) form directly on Mars-analog mineral surfaces exposed to ultraviolet radiation. It was claimed that these oxygen radicals explained the reactive nature of the soil, and the apparent absence of organic material “as the most straight-forward explanation for the unusual reactivity of the Martian soil; The stability, motility and reactivity of  $\text{O}_2^-$  are all consistent with the Viking Lander results.” However, it was shown that Fig. 3 in the paper made this theory untenable (Levin and Straat, 2001a). The figure showed that superoxide ions degraded only 10% after 1 hr at  $100^\circ\text{C}$ , and remained 30% active after an additional 1 hr at  $200^\circ\text{C}$ . This heat resistance differs sharply enough from the LR Mars samples’ inactivation at lesser temperatures (Levin and Straat, 1979b) to disqualify

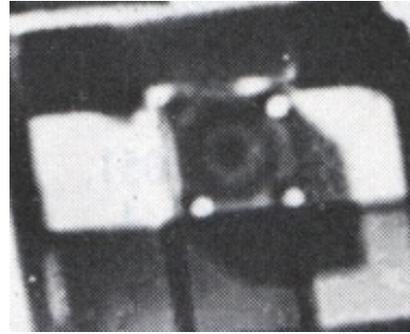
superoxide ions as the source of the LR Mars response. Another proposed abiotic explanation of the LR results (Tsapin *et al.*, 2000) claimed that ferrate (VI) reacts with water in a manner “qualitatively similar to the Viking Gas Exchange Experiment (GEx) results,” and produces a reaction “in line with the results of the Viking Labeled Release (LR) Experiment.” This theory was also shown unlikely (Levin, 2002) based on a number of theoretical and experimental inconsistencies with the LR results. The many other oxidant theories proposed over the then quarter century since Viking were also examined and found wanting (Levin, 2001c).

In addition, a long series of positive observations have been made against the surface of Mars being coated with a strong oxidant, including:

- The IRIS experiment (Hanel *et al.*, 1972a; 1972b; Hanel and Maguire, 1980) found no evidence of H<sub>2</sub>O<sub>2</sub> in the Martian atmosphere to an upper limit of several ppb. Relatively strong concentrations (approximately 2%) of H<sub>2</sub>O<sub>2</sub> are required to be applied to the LR labeled nutrients to emulate the amplitude and kinetics of the LR Mars positive responses.
- The above cited Viking PR experiments which formed organic matter when simulated Martian sunlight was shone on simulated Martian atmosphere, organic material which survived seven sols’ contact with the putative oxidant in the soil (Hubbard *et al.*, 1973; Horowitz, N.H., Hobby G.L., Hubbard J. S., 1977).
- The Viking Magnetic Properties experiment found much magnetic material in the Martian top soil as evidence that it was not fully oxidized (Hargraves, *et al.* 1977), based on the results shown in Figure 7;



Reference test chart magnet image for VL-1 on sol 31.



Reference test chart magnet image for VL-2 on sol 42.

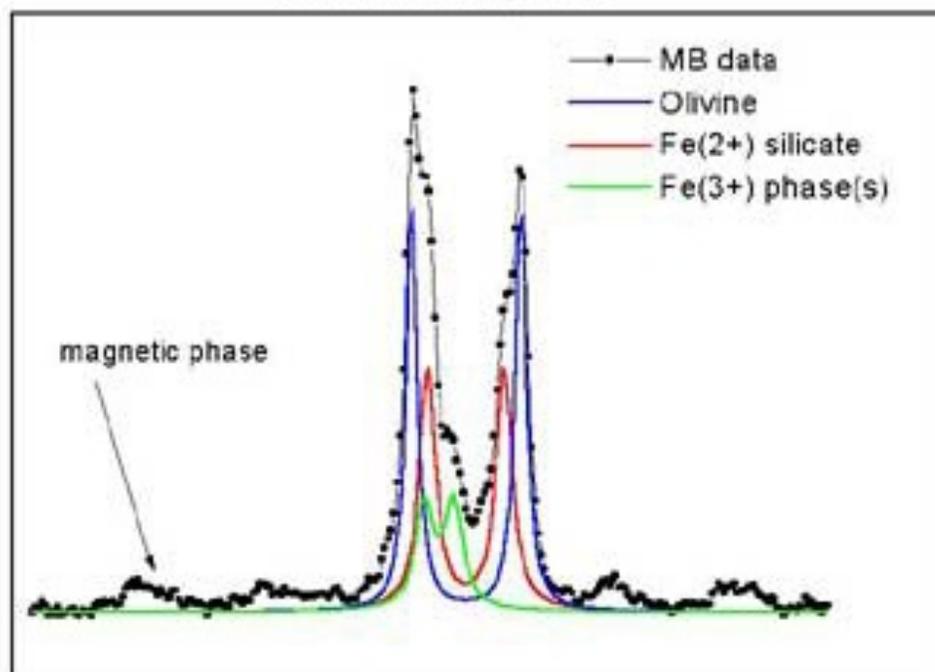
2 mm to 4 mm of surface material were picked up by each magnet.

*“If there is a lot of material adhering to the magnet, it would certainly say that whatever the surface processes are on Mars, they are not innately highly oxidizing.”* Robert Hargraves, Viking Magnetic Properties Experimenter

#### FIGURE 7. THE VIKING MAGNETIC PROPERTIES EXPERIMENT

- Pathfinder confirmed that Martian soil was well below full oxidation of its metallic soil particles (Hviid *et al.*, 1997);
- two attempts to find  $H_2O_2$  in the Martian atmosphere found none at the very limit of sensitive observations (Krasnopolsky 1993; Krasnopolsky *et al.*, 1997) while a third observation (Encrenaz *et al.*, 2004) found a stringent upper limit of the  $H_2O_2$  abundance, at ppb levels far too low to have caused, directly or indirectly, the LR responses;
- The MER Rover, “Opportunity,” analyzed the oxidation of metals in the Martian soil, and found that the relatively reduced ferrous state exceeds the fully oxidized ferric state of iron as shown by the spectra in Figure 8;

#### Mössbauer Spectrum on Soil at Meridiani Planum, Sol 11, MER Rover Opportunity



**FIG. 8. Mars Soil Minerals Not Completely Oxidized**

[http://www.jpl.nasa.gov/mer2004/rover-images/feb-04-2004/mb\\_soil\\_opportunity-380.jpg](http://www.jpl.nasa.gov/mer2004/rover-images/feb-04-2004/mb_soil_opportunity-380.jpg).

- Direct soil analysis by the Phoenix Scout Mission lander found the oxidant, perchlorate. However, perchlorates are much too resistant to destruction by heating to qualify as the LR active agent. With respect to the life issue, it was stated (Renno *et al.*, 2009), “Phoenix results found no chemicals detrimental to all microbial life.” Perchlorates constitute generous sources of chemical energy utilized by numerous species of terrestrial microbes genetically able to produce a perchlorate reductase enzyme (Nozawa-Inoue *et al.*, 2008; Wang, Lippincott and Meng, 2008; Coates and Achenbach, 2004, Coates *et al.*, 1999). On Phoenix, the very sensitive Microscopy, Electrochemistry and Conductivity Analyzer (MECA) reported no other strong oxidant. In addition, Phoenix confirmed the reports of Viking, Pathfinder and MER that the Martian surface was not fully or predominately oxidized.

To this date, 2011, no oxidant found or proposed satisfies the thermal profile recorded for the active agent that the Viking Mission LR test and control data detected on Mars. In 1997, after a decade of reviewing the Viking LR data against the constantly evolving new information on environmental conditions and possible habitats on Mars, and after the finding of extremophiles in the most Mars-like places on Earth, Levin (1997) finally came to the conclusion that life had been detected on the surface of the red planet. A paper refuting Levin’s conclusion (Klein, 1999) took issue with many of the points made, principally that contending the presence of liquid water.

## **7. Liquid Water**

As on Earth, liquid water is deemed essential for life on Mars. Addressing this requirement, Klein stated that Mars’ surface is “devoid of liquid water,” rendering the positive LR response chemical rather than biological. However, a paper by Levin and Levin (1998), that, independent of any claim for life, provided a theoretical thermodynamic calculations that liquid water must exist on present-day Mars.

The Odyssey mission made the startling discovery (Feldman *et al.*, 2002) that vast regions of Mars, including the sites of both Viking landers, contain a subsurface hydrogen compound. The material constitutes from two to up to 10 percent of the soil weight of the top several cm of the surface. This compound was interpreted by the Odyssey scientists as water in the form of ice.

The Viking Orbiter Infrared Thermal Mapper (NASA, 1994) had detected that the soil around the equator in the Martian summer reached a maximum temperature of 27 °C. Pathfinder determined ground temperatures as high as in the 20's °C (Sawyer 1997), indicating that some ice could diurnally become liquid water. The Spirit rover (Smith *et al.*, 2004) detected summer air temperatures as high as 5 °C. Low-lying fogs and wispy white clouds of water ice crystals were reported sometimes falling as snow (Whiteway *et al.*, 2009). The Phoenix Science Team (Smith *et al.*, 2009) reported “stickiness” of the surface soil samples, hardly accountable unless attributed to moisture. Finally, visual proof of liquid water was obtained in photographs of droplets in liquid and frozen states, depending on the time (temperature) of day on a strut of the Phoenix lander (Renno *et al.*, 2009). Furthermore, the range of ice table depths measured at the Phoenix landing site (Smith *et al.*, 2009) is consistent with Odyssey's finding.

New thermodynamic calculations offer additional evidence that salty liquid water can exist where Phoenix landed and elsewhere on Mars (Marion *et al.*, 2009). The calculations predict a water droplet growth rate consistent with that observed on the Phoenix strut. Further, this article disproves an hypothesis offered that ice had sublimed from the cold ground just under the strut of the lander's leg and was then deposited on a (warmer) strut. The deduction that the Martian atmospheric water vapor is concentrated near the planetary surface making for a diurnal liquid water – water ice equilibrium (Levin, 2007, ) of high atmospheric moisture content near the Martian surface was empirically confirmed by Phoenix (Moore *et al.*, 2011).

In keeping with the Viking LR analysis, Phoenix confirmed (Smith *et al.*, 2009) that the soil analyzed was alkaline, pH = 7.7 (previously thought by many to be acidic, despite the Viking LR's second injections indicating an alkaline soil). Viking found chlorine in the soil, not ascertaining if it came from perchlorates or another source. Phoenix identified calcium carbonate (thought to have formed by the interaction of atmospheric carbon dioxide with liquid water films on particle surfaces, perchlorate and chlorate salts, likely including magnesium and calcium perchlorate, hydrates that lower the freezing point of water (Boynton *et al.*, 2009; Smith *et al.*, 2009). A study (Möhlmann, 2010) shows how layers of liquid water form in the upper sub-surfaces of snow/ice-packs on Mars even to within 2 cm

of the surface, where they are protected from UV radiation. Terrestrial microorganisms are reported (Hansen *et al.*, 2009) to survive under this same circumstance.

In September, 2009, additional evidence for near-surface water ice and for liquid water was reported, included at the Viking 2 lander site, (Byrne, S., *et al.*, 2009). Experimental and theoretical evidence have now made it extremely difficult to deny that liquid water in amounts sufficient for microbial activity exists at the Viking landing sites and over broad areas of Mars. The concept of Mars as a habitat has drastically changed.

### **8. Changing Perceptions of the Viking LR**

An increasing number of definitive reports upsetting previous assumptions about the inhabitability of Mars have been published. A panorama of them is found in the papers, abstracts and summaries presented at the 40th Lunar and Planetary Science Conference (Lunar and Planetary Institute, 2009). Of high significance is the finding of microorganisms thriving in the South Polar Cap in ice, where the temperature never exceeds minus 15 °C (258 °K, slightly colder than Phoenix's site), Microorganisms were found in active metabolism and thriving (Carpenter, Lin, and Capone, 2000.) It is thought that equilibrium between ground ice and atmospheric water (Sizemore *et al.*, 2009) provides liquid water in amounts sufficient for active microbial life.

On Earth, unfrozen water exists at  $-30^{\circ}\text{C}$  and lower (for a summary see Möhlmann and Rempel, 2010, section 2.2), direct evidence for metabolism in a variety of constantly frozen environments has pushed temperature limits for bacterial activity to increasingly lower temperatures, so far to  $-20^{\circ}\text{C}$ . Biotas in permafrost and its cryopegs (the latter biotas, thus, inherited from previous marine sediments) are known to contain cell numbers ( $10^7$  cells/ml) comparable to non-psychrophilic communities. Some of their species enter dormancy (but reversibly) only around to  $-35^{\circ}\text{C}$  (Panikov and Sizova, 2007), and the ability of cold-adapted organisms to move, to remain metabolically active and incorporate amino acid substrates into macromolecules, to produce cryoprotectant polysaccharides, and even to recover from deep-freezing to  $-80^{\circ}\text{C}$ , is well documented nowadays (Margesin, 2009; Marx, Carpenter and Deming, 2009; Junge *et al.*, 2006). Evidence that microorganisms are capable of protecting themselves against freezing by use and production of teichoic acid was recently reported (Rice, R. *et al.*, 2009).

On Mars, Phoenix measured diurnal variations in water vapor pressure, in amounts suggesting its adsorption onto regolith grains, or control through hydrated perchlorate salts, or both (Levy *et al.*, 2009). It is now accepted that films of liquid water existing between crystals of ice or minerals are sufficient for microbial life, thus accounting for its existence in permafrost.

### **9. Other Key Findings**

The Viking LR results, test and control, have never been duplicated by physico-chemical simulations, even after more than three decades of continuing efforts by many researchers. This is not an “argument by elimination,” but merely points out that all attacks made upon the biological source of the response have failed. The true case made is not based on eliminating other hypotheses, but upon the extensive amount of evidence from hundreds of terrestrial tests and the Martian LR test and control data described above.

The Viking GCMS sensitivity has been (Benner, S., *et al.*, 2000), by demonstrating that the Mars instrument could have missed biological evidence by not pyrolyzing its sample at a high enough temperature. The reported ppb-sensitivity applied only to vapors resulting from the pyrolysis of the soil sample. However, as Benner reported, the pyrolysis temperature of 500°C was found insufficient to vaporize the organics in soils containing low populations of some extremophiles and thus to have permitted their detection by the Viking GCMS. In addition, an experiment (Navarro-Gonzalez, *et al.*, 2006) indicates that the perchlorate found at the Phoenix site, if present at the Viking sites, would have oxidized the organic matter in the soil samples during the GCMS pyrolysis step, thereby accounting for the failure of that instrument to detect organics.

Importantly, none of the literature contending against a biological source of the Mars LR response paid attention to the published (Levin and Straat, 1986) fact, that a terrestrial soil was tested in an exact duplicate of the Viking LR instrument. The chamber containing the soil was pumped down and maintained at simulated Martian atmosphere and pressure, including the He overpressure (to prevent boiling in the event Martian atmospheric pressure was below 6.1 mb, the triple point, which it proved not to be during the entire Viking mission) for three days, then injected with the LR nutrient and monitored for an additional eight days, exactly as on Mars. The microorganisms in the soil survived the shock and produced results (Levin, 2001b) like those subsequently obtained on Mars. However, the

terrestrial organisms responded positively to a second injection of the nutrient. On Mars, the second injection produced a re-absorption of about 25% of the headspace gas, deemed by some as strong evidence against biology. Examination of the LR terrestrial response library found this same response to a second injection with the re-absorption of headspace gas in a NASA-bonded soil from the Antarctic (Levin 2006, Figs. 10a and 10b; see Figs. 4 and 5 above).

### **10. Chirality**

Viking was too constrained in weight and space to allow for the instrumentation needed for the separate administration of the two enantiomer solutions. The only way to avoid a possible false negative (should Martian life not respond to D-carbohydrates or L-amino acids) was to use a single solution, containing racemic mixtures of those selected nutrient compounds having chiral isomers. These were combined with the selected non-chiral nutrients (Levin and Straat, 1976), as shown in Table 1.

In continuing pursuit of the key issue of life on Mars, in August 1995 the author and a colleague (Christopher McKay) proposed to NASA a Chiral LR experiment. It was designed to determine possible chiral specificity or preference by the agent in the soil. Such chiral preference would be strong evidence for life, especially buttressed by a negative response from a heat-treated control sample. This proposal was not funded for the U.S. missions then in planning, but an opportunity to conduct a chiral experiment came soon after. It was on the Mars Oxidant experiment (MOx) designed by a NASA-appointed team for inclusion on the Russian 1996 Mission to Mars (McKay *et al.*, 1998; Zakharov, A., 1996). The Chiral LR proposers, MOx Team Member and Team Leader, respectively, had that instrument enhanced to include chiral life detection capability. This was done by having one of the many MOx optical fibers coated with L-cysteine, and another with D-cysteine. In the MOx experiment, any reaction between each coating and the soil into which it was thrust would be detected by measurement of reflected light from laser pulses delivered to the coating. An unequal response between the cysteine isomers supported by a control would be strong evidence for life. Unfortunately, the spacecraft failed to leave Earth orbit and crashed.

Seeking unequal chirality in amino acids and carbohydrates has become increasingly prominent in the search for alien life, both on extraterrestrial objects and, more recently, even on Earth as reviewed in a popular article by P.C. Davies (2007). Such chiral compounds

have been named “biosignatures” or “biomarkers”. When detected in unequal isomeric amounts in a given compound, it was thought to be evidence for life. A variety of means have since been developed for detecting chiral amino acids and determining their enantiomeric ratios. However, it is the authors’ position that the mere presence of more of one isomer than the other in a compound does not constitute proof of life, either extant or extinct. Occam’s Razor would find such claimed biosignatures to be more likely the result of fortuitous chemical reactions in the soil than of the far more complex alternative of life.

Nonetheless, in recent years, interest in the use of chirality to detect and identify alien life, as such, has intensified. Many authors (e.g., Sims, *et al.*, 2002; Nikolaev, *et al.*, 2007; Rodier, *et al.*, 2005; also now propose to analyze samples for such evidence). In addition, as Davies, *et al.*, (2009) point out, scientists have begun to look for “alien” life on Earth, by seeking microorganisms that exhibit chirality different from that found in all life forms examined so far. A slight excess of L- over D-amino acids was reported (Cronin and Pizzarello, 1997) in the Murchison meteorite. Nevertheless, recent work (Glavin and Dworkin, 2009; Botta *et al.*, 2008) shows that the small imbalance of L- and D- isomers common in meteorites may not be indicative of life. They state that long-time exposure to liquid water can amplify any minute racemic inequality in amino acids.

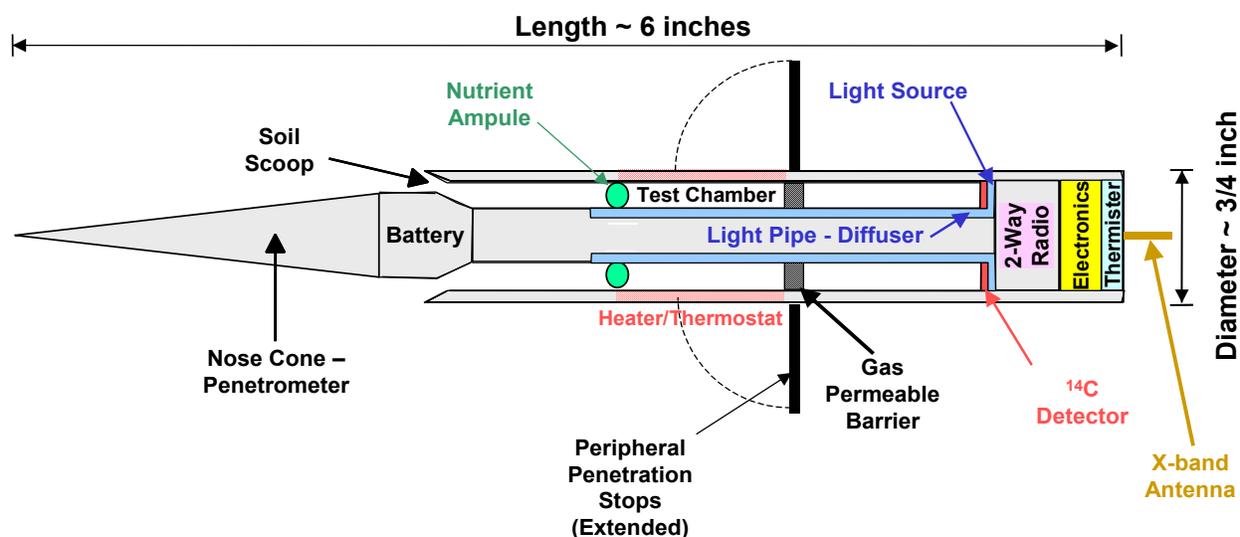
In 2007, the Chiral LR was adapted to test samples of Atacama Desert soil for comparison with the results of the Viking LR on Mars. However, the report (Quinn, 2007) of the Atacama work showed that  $^{13}\text{C}$  was used as the label rather than  $^{14}\text{C}$ , which substitution greatly reduced the sensitivity of the method, requiring significant departures from the Viking LR regimen. While, for this reason and others (including failing to consider key LR control experiments), the results are not comparable to those of the Viking LR, the authors, nonetheless, concluded that the use of chirality to distinguish between chemical and biological reactions is valid.

Another recent article (Wu, 2007), discussing the results of Viking’s test for organic matter on Mars, cites an ESA mission slated for launch in 2013. It will seek chirality in any organic matter found, as evidence for its biological or chemical origin. However, only one-time analysis of a given sample will be made. It has been demonstrated (Hazen and Sholl, 2003) that modest amounts of chiral selection can be achieved abiotically when a racemic mixture of an amino acid is washed across the surface of a calcite crystal. The selectivity of

this method will likely be improved, and other abiotic methods will be developed to select between and concentrate chiral isomers. Thus, the mere “snap shot” determination of chirality will not likely be accepted as proof of life.

### 11. The Multi-Metabolism Probes

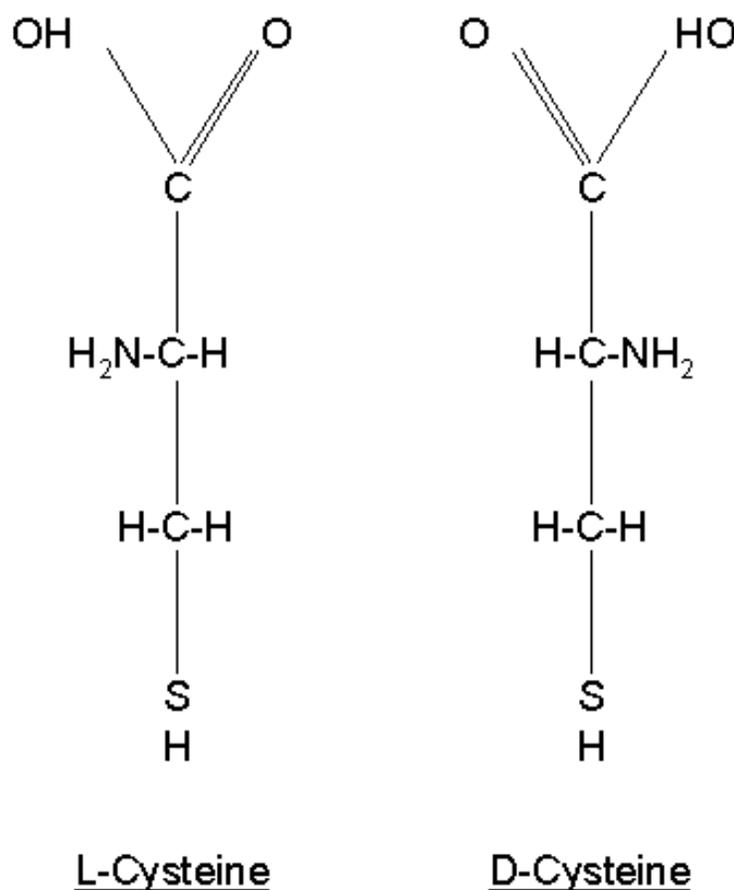
In response to the ever-tightening specifications restricting weight and power for instruments on spacecraft missions, the author and colleagues have made efforts (e.g., Levin *et al.*, 2002) to reduce the size, weight and complexity of life detection experiments, while increasing scientific content. The objective is a major improvement to the present state of the art of robotic extraterrestrial life detection. Each Multi-Metabolism Probe (MM) provides a combination of tested metabolic experiments, each in a miniaturized probe that is self-contained and, except for communications, operates essentially free of lander or orbiter support. The probes are packaged in a hermetically sealed cocoon that is terminally heat-sterilized prior to launch. The cocoon can be piggy-backed onto any lander or orbiter. The individual probe is shown in Fig. 9.



**Note** Sterilized canister contains multiple probes that are ejected away from spacecraft after landing.

**FIG. 9. The MM Chiral Probe. Sterilized Canister Contains Multiple Probes That are Ejected Away from Landed or Orbiting Spacecraft.**

Essentially, the probe is a dart approximately six inches long and three-quarters of an inch in diameter (15 cm by 1.7 cm). Each dart contains two soil sample chambers. The darts are launched from a landed spacecraft or from an orbiting spacecraft. They land nose-first, the force of the impact collecting soil in the testing chambers. Detent-deployed stops prevent the probe from penetrating more than about four inches should the soil be soft. As soil is forced into each chamber, an ampoule containing an aqueous solution of the test  $^{14}\text{C}$  tagged substrate breaks, moisturizing the soil. Any gas produced by reaction with the soil rises into a beta counting chamber. Each probe contains batteries to maintain the temperature above freezing and to power the monitoring of the experiment for several sols. A miniature FM radio transmits the data to the spacecraft or orbiter for relay to Earth. The proposed complement of substrates for individual probes is:  $^{14}\text{C}$ -L- and D- lactate;  $^{14}\text{C}$ - L- and D- alanine (racemic mixtures of lactate and alanine were in the Viking LR);  $^{14}\text{C}$ -L- and D- cysteine (Figure 11):  $^{35}\text{SO}_4$ ;  $^{14}\text{CO}_2$ .



**FIGURE 10. AN EXAMPLE OF CHIRAL SUBSTRATES, L- AND D-CYSTEINE**

Cysteine and sulfur are included because of the high percentage of sulfur (including sulfites and sulfates) found in the Mars surface material; the biological sulphur cycle on Earth includes sulfur-oxidizing and sulphur-reducing microbiota, and sulfur is also suspected involvement in chemoautotrophic early life on Earth (Wickramasinghe, 1973; Canfield, Habicht and Thamdrup, 2000; Grassineau *et al.*, 2005; Gallardo and Espinoza, 2007). The use of  $^{35}\text{SO}_4$  results in the production of  $\text{H}_2^{35}\text{SO}_4$  which is monitored in the same manner as  $^{14}\text{CO}_2$ . The exposure of the Martian soil to  $\text{CO}_2$  in alternating light and dark periods provides a test for photosynthesis. Also, because degraded organic matter might outcrop in such a way that organisms could eat “wrong” handed material, future development should envisage to get a suite of compounds analyzed, the putative nutrients always being kept at minimum concentrations and offering, if feasible, variable concentrations within a range. Nevertheless, it should be emphasized that the negative responses to the offer of nutrients lack in scientific significance. Results from a laboratory experiment are seen in Figure 11.

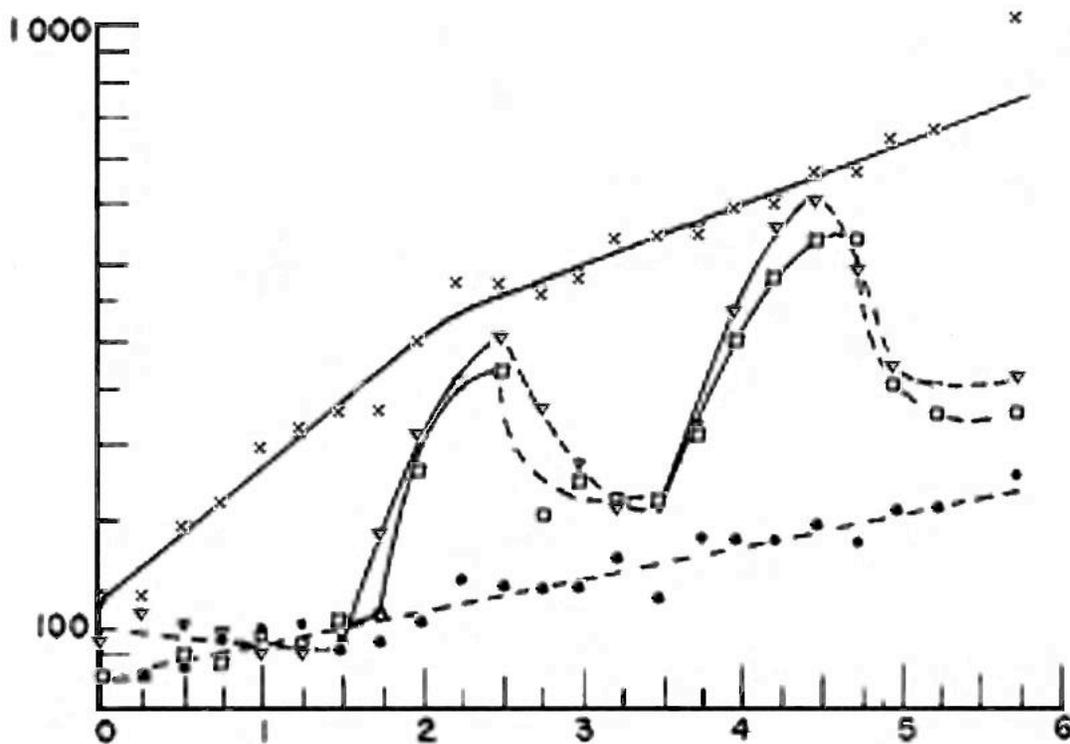


Figure 11. Detection of photosynthesis of metabolically derived  $^{14}\text{CO}_2$ . *Chlorella pyrenoidosa* evolve or absorb  $^{14}\text{CO}_2$  in response to light and dark growth cycles (Levin, *et al.*, 1964). Continuous line, dark cycle; dashed line, light cycle; y-axis, headspace  $^{14}\text{CO}_2$

(cpm); x-axis, time (hr). Symbols: circles, crosses, triangles and squares are individual measurements. Medium: urea agar labeled with  $l\text{-}^{14}\text{C}$  sodium lactate ( $2 \times 10^{-3}$  M).

The multiple, single substrate probes reinforce each other, but, should some of the probes hit rocks or otherwise fail, each survivor would provide strong complementary evidence with those surviving, and even by itself.

While detecting a difference in chiral preference of on-going reactions is strong evidence for biology, in itself being a controlled experiment, further confirmation would be sought in the nature of separate heated controls, as with the Viking LR experiment. Should the reaction in a control probe be significantly less than that in a test probe, metabolism in the test sample will have been demonstrated. Such a result, reinforcing a preferred chirality response in the test probe, would virtually preclude a non-biological explanation.

## 12. Conclusions

A successful Chiral LR experiment on Mars could remove any remaining doubt concerning the existence of Martian microbes. Moreover, it could reveal important information about that life, suggesting if, and perhaps where, one biosphere would branch from the other's tree – if at all. Should the chirality on Mars be the same as that on Earth, this would suggest that both life forms may stem from a single source. Should the chiralities differ, this would be evidence that the two life forms had separate origins. This would imply that, even though based on our total sample of only two planets, life is widely distributed in the cosmos. Should the Mars samples show no chiral preference, the distinction between chemistry and biology can still be made by judicious application of thermal controls and the use of anti-metabolites as controls. These intriguing possibilities can be realized only if metabolic analyses are applied in an on-going temporal method in order to overcome the objections raised against biological interpretation of proposed static “biosignatures.” Much has changed since Carl Sagan used to frequently remark in this regard about extraordinary claims requiring extraordinary evidence. With our new knowledge of terrestrial extremophiles, what we have learned about Martian habitats, and how the two planets have been exchanging ejecta with the potential for viable organisms that can survive the journey through space, the claim of life on Mars has become ordinary, and the evidence for it has become extraordinary. Now it is more difficult to imagine a sterile Mars than a live one.

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