Implications of Curiosity's Findings for the Viking Labeled Release Experiment and Life on Mars

Gilbert V. Levin

Adjunct Professor, Beyond Center for Fundamental Concepts in Science, College of Liberal Arts and Sciences, Arizona State University, Tempe; Honorary Professor, Centre for Astrobiology, University of Buckingham (UK)

> SPIE Optics + Photonics Conference Optical Engineering and Applications Instruments, Methods, and Missions for Astrobiology XVI Tuesday 27 August, 2013 Convention Center San Diego, CA

ABSTRACT

Curiosity's latest reported findings, or lack thereof, are interpreted from the standpoint of their implications for the Viking Labeled Release experiment, and for life on Mars in general. As of the writing of this abstract, Curiosity has reported no findings related to those anticipated by the author's last year's paper, "Stealth Life Detection Experiments Aboard Curiosity." However, Curiosity scientists have stated that soil and rock samples have been taken and analyzed, and abundant images have been downloaded. The only (indirectly) relevant reports issued by Curiosity scientists concern small-molecule organics found in a soil sample, which simple compounds they suggest might be terrestrial contamination, and images of rocks with colored (green) patches, the latter not of sufficient resolution (of which the cameras are capable) to detect possible evidence of biology. Hopefully, by the time of preparation of the body of this paper, more information will be available.

1. Background

The Mars Science Laboratory (MSL), "Curiosity," is widely cited by NASA as having no life detection capability. Its primary mission is to determine whether Mars was once habitable in ancient times. Just seven months into a two-year mission, Curiosity's chief scientist, John Grotzinger, announced¹ that this foremost goal has already been achieved, stating, "Strong evidence for past habitability has been found."

This author, however, has contended² the MSL has several experiments that, while not direct life detectors, can bear on and even confirm the possibility that the Viking Labeled Release (LR) experiment did discover microbial life in the topsoil of Mars in 1976. Some of those MSL results have already been reported as will be discussed herein. Others are anticipated. Meanwhile, a review of the 37-year-old Viking LR evidence for life may help interpret the existing and anticipated data from the MSL.

2. The Viking Labeled Release Life Detection Experiment

The Viking LR experiment³ on Mars was essentially one of the analyses to test for microorganisms described in the Standard Method for the Examination of Water and Wastewater⁴ in use by public health departments worldwide. In this test, an aqueous solution of lactose in a clear test tube is inoculated with the sample suspected of the microbial contamination. If present, microorganisms metabolizing the lactose will expire gas accumulating into bubbles, the visual observation of which is the evidence of living microorganisms. The LR merely adds several additional organic nutrients, each widely tested for microbial responses over a wide range of species, and all of which are Miller-Urey products. These are organic compounds thought to have formed on early Earth, some of which are believed to have participated in the genesis and evolution of life unto the current era. The additional compounds in the LR broaden its appeal to possible alien microorganisms on Mars, where Miller-Urey products are also likely to have formed and been available to any independent genesis. Very dilute solutions were used to prevent possible toxicity.

To increase the sensitivity and rapidity of the method, so that the experiment might be completed before possible spacecraft or communication failure, the nutrients were lightly, but uniformly, tagged with radioactive carbon, ¹⁴C. Accordingly, any gas produced from the nutrient solution would be radioactive, permitting its detection long before visible bubbles form. Thus, the LR is a Standard Method, merely enhanced by augmenting the nutrients and substituting the form of read-out. But the LR goes one step further. It introduces the concept of a control against the possibility that some exotic chemical on Mars might cause a false positive. On Earth, the method has proven so reliable that no control is used.

3. The LR on Mars

Viking 1 landed on Mars on July 20, 1976. On July 30, the first LR test was run. It immediately produced a positive response, rapidly evolving radioactive gas, and continuing at a diminishing pace for the seven sols of the experiment. The 160 deg. C control was than run and produced a background level, nil result. As seen in Figure 1, the pre-mission requirements for the detection of microbial life were met. Viking 2 landed, some 4,000 miles away on September 3. It, too, produced a positive response. In total, four LR tests for life were made. All were positive. A total of five control runs were run on duplicate samples of each soil that had tested positive. All of the controls supported the positive results⁵.



Fig. 1. The VLR Response and Control at Viking 1 Site.

The amplitudes and kinetics of the positives were virtually the same as produced by several of the thousands of tests of soils performed on Earth – which tests had never produced a false result, attesting to the reliability of the method. All of the positive Mars LR results fell within the range of those from LR tests performed on terrestrial microbial inhabited soils.

4. Interpretation of the Viking LR Results

Nonetheless, the LR Mars results were largely discounted. The principal reasons initially given were the failure of the Viking organic analysis instrument (GCMS – gas chromatograph mass-spectrometer) to find any organic matter: "No organics – no life," was the consensual verdict. Two additional major barriers to life were soon cited: the presumed ubiquitous presence of a highly oxidizing, organic-destroying and lethal substance in the Martian soil, and the presumed lack of that primary essential for life, liquid water.

5. Oxidative State of Mars Regolith

The Mariner 9 IRIS experiment⁶ that orbited Mars in 1971, several subsequent Earth-based spectroscopic observations, and more recent results from soils analyzed by Pathfinder, Phoenix, and, now, Curiosity have demonstrated the absence of the initially supposed H₂O₂, or any other strong oxidant, dominating the Martian surface. Perchlorates have been found by Phoenix and confirmed by Curiosity, but in concentrations too small to account for the LR response, and not reported at the Viking sites. Furthermore, perchlorates would easily have survived the control thermal regimens of the Viking LR experiment. Curiosity has verified that original conclusion⁷ of the Viking Physical Properties experiment that the surface was not highly oxidizing. As seen in Figure 2, the new data emphasize that the surface could have sustained "ancient microbes."

All these results confirm the Viking PR experiment's finding that the organic matter abiotically formed survived the experiment's five-Martian-day exposure to the presumed organic-destroying oxidant in the Martian soil. Further, this is consistent with the fact that the active agent in the Viking LR experiment retained essentially the same activity whether held in the LR test chamber for as little as two or up to five Martian days before being inoculated with the radioactive nutrient solution.

"The chemical analysis of the sample also revealed compounds in varying states of oxidation – for example both sulfate and sulfide. *This is significant because it demonstrates that the environment was not violently oxidizing* (emphasis added). All these factors point to a habitat in which ancient microbes could conceivably have survived." Joe Michalski, as reported in *Chemistryworld*, by Simon Hadlington, Mar. 18, 2013.

FIG. 2. The Surface of Mars at Curiosity is not Highly Oxidizing.

Why the surface could have sustained ancient microbes, but not still do so is not stated. However, this re-visitation of the oxidative state of the surface brings forth an important, perhaps biologically-related matter. The Martain atmosphere is in disequilibrium in that the sun's UV light should long ago have destroyed the CO₂ in the atmosphere, rendering large quantities of CO as a product. Such a disequilibrium in a planetary atmosphere has been theorized⁸ as constituting evidence for biology. On Earth, CO₂ is maintained in the atmosphere by its release from the movement of tectonic plates, emissions from volcanoes and by the respiration of living organisms. Mars has virtually no tectonic plate movement or volcanic activity, leaving microorganisms as the possible source maintaining atmospheric CO₂ by recycling the CO. Previously, the strong oxidant mistakenly presumed on the surface or in the dust of Mars was theorized as the recycling force re-converting CO to CO₂. Thus, Curiosity's confirmation of the mild surface

conditions on Mars supports both the likely survival and presence of organic material and life.

6. Liquid Water

Recently, Curiosity has supported Viking's initial, but ignored, finding of liquid water in the Martian soil. This discovery occurred at the Viking 2 landing site, where the lander footpad recorded the soil temperature. Rising with the sun, the soil temperature paused at 273K, a "fingerprint" of ice absorbing the heat of fusion required to melt into liquid water. As seen in Figure 3, Curiosity's thermal analysis of water vapor coming from the first Rocknest surface dust sample shows water vapor emerging from the sample even at just above freezing, 0 deg. C.



FIG. 3. Gases Released from Surface Dust.

This water vapor could have come only from liquid water or sublimated ice. Hydrated minerals would not yield water vapor at those low temperatures. In either case, liquid water would form films in the mineral interstices, as they do in permanently frozen South Polar regions and elsewhere on Earth. That the water vapor coming from the Rocknest sample was not merely taken in as atmospheric vapor in the sampling process is shown by the fact that CO₂, which would have been taken in similarly, did not appear until the sample was heated to approximately 90° C. It is also interesting that the slope of increasing water vapor evolved from approximately 100° to 200° C is essentially a straight line. This corresponds to the region of thermal sensitivity shown by the Viking controls, and raises the issue of some possible identity or association, such as water vapor coming from disrupted microorganisms. The constant rate of issue from a mineral hydrate is difficult to imagine since the hydrate should issue most of it water when its bulk remains greatest, issuing declining quantities as the bulk becomes lesser through the continued heating and evaporation.

Figure 4 shows the same type of data for the 4th John Klein drilled rock sample.



FIG. 4. Gases Released from 4th John Klein Drilled Rock Sample.

As in Figure 3, water vapor is seen evolving at just above freezing, but, as would be expected from rock dust, apparently in lesser amounts (although neither figure gives quantitative data). Again, between approximately 100° C and 200° C, water vapor increases with temperature in essentially a straight line, raising the same issues as in Figure 3. In each event, a strong case is made for the presence of at least microbial supportive films of liquid water in the samples. However, do these conditions exist on Mars, or were they imposed by the analysis?

This matter is resolved in Figure 5, which presents air and surface temperature data recorded by Curiosity over a 200-sol period.



FIG. 5. Air and Surface Temperatures Recorded by Curiosity.

It is seen that the temperatures frequently rose above freezing diurnally. Surface ice has long been seen at several sites on Mars. Odyssey detected⁹ vast areas of ice just several centimeters below the surface. Thus, over vast areas of Mars, liquid water must exist as interstitial films, available for microbial use, as occurs in frigid climes on Earth. However, the presence of high concentrations of salts and other minerals on Mars significantly reduces the evaporation rate for liquid water. In addition, the difference in gravity between Earth and Mars renders the rate of water evaporation¹⁰ on Mars 30 times less than on Earth. The reference cited points out that ice has been detected over most of Mars, covered by shallow layers of regolith that protect and stabilize the ice by providing a barrier to sublimation. Under conditions that may obtain fairly commonly on Mars, this stabilization could result in liquid water widely distributed in the soil.

7. Organic Matter

This sole remaining critical objection to acceptance of the Viking LR results has been saved for last. Several publications^{11,12,13} since Viking have found a variety of faults with the Viking GCMS, effectively impugning its results. Moreover, the Viking Pyrolytic Release (PR) experiment showed¹⁴ that organic compounds are being formed and accumulating on Mars today. On Earth, this experiment had produced organic matter when its simulated sunlight shone on its simulated Martian atmosphere even under sterile conditions. The PR experimenters reported¹⁵ that organic matter formed abiotically in amounts sufficient to have an accumulative influence on the evolution of biology. To prevent such a false positive on Mars, an optical filter was inserted in the Viking PR instrument to remove the offending UV spectrum from the simulated sunlight. Despite this precaution, some organic compounds did appear in the terrestrial PR testing, making it necessary that a very strong response be obtained on Mars to view it as evidence for life. On Mars, the PR did produce positive results, but only in amounts no greater than in sterile runs on Earth, high enough to demonstrate the formation of simple organics, but too little to allow a claim to life. However, this PR result does demonstrate that organics are present on Mars, constantly forming in daylight, despite the failure of the Viking GCMS to find them.

Curiosity's Mars Science Laboratory (MSL) Surface Analysis at Mars (SAM) instrument reported finding several low molecular weight organics: chloromethane, dichloromethane and trichloromethane. Figure 6 shows them. However, the possibilities were raised that the chlorohydrocarbons detected by Curiosity were either contaminants brought from Earth, or were thermally synthesized from Martian sample ingredients in the act of analysis.



 $\begin{array}{l} CH_4+Cl_2\rightarrow_CH_3Cl+HCl\\ CH_3Cl+Cl_2\rightarrow_CH_2Cl_2+HCl\\ CH_2Cl_2+Cl_2\rightarrow_CHCl_3+HCl\\ CHCl_3+Cl_2\rightarrow_CCl_4+HCl\\ This complete suite of products was not detected in the assay. \end{array}$

FIG. 6. Chlorohydrocarbons in John Klein Rock Sample.

Countering that possibility, Figure 6 shows that the amounts of chlorohydrocarbons detected were less in the pre-sample blank runs than in the first sample. This would seem to rule out terrestrial contamination, at least as a major contributor. There is also a problem with respect to the synthesis of the chlorohydrocarbons from indigenous ingredients in the sample. As seen in the Figure Note, the suggested synthesis process also produces trifloromethane and carbon tetrafloride. This complete suite of products was not detected in the sample. Thus, a case can be made that the organic compounds were indigenous to the sample. However, to be truly supportive of the Viking LR results, the organic compounds found should be biologically complex. If so, this would remove the last obstacle to acceptance of microbial life on Mars. For this reason, the results from surface material analyses by the MSL liquid extraction method have been eagerly awaited. While recent reports from Curiosity¹⁶ have stated that such analyses have been run and others are at the ready, no such data have been reported. Since the finding of complex organic compounds by the Viking GCMS would have led to instant acceptance of the LR results in 1976, it might seem only logical that, should Curiosity find them now, the same conclusion should result.

8. Visual Evidence for Biology

Another very important assay of Curiosity remains to be reported. The suite of cameras includes the hi-resolution color Hand Lens Imager (MAHLI) designed for close ups of rocks and regolith soil, with resolution of 14.5 um. Its Principal Investigator had agreed¹⁷ with the author's suggestion of taking close-ups of the greenish spots seen in many color images taken by the lessor-resolving cameras, such as seen in Figure 7.



FIG. 7. Mars Curiosity Mastcam Soil 999 of 1542, Aug. 17, 2012.

9. Status of Life on Mars

To date, of the countless experiments performed and theories proposed to explain away the Viking LR test and control data as evidence for life, none has been scientifically tenable. While some experiments have shown that a variety of oxidants can react with one or more of the LR nutrients to evolve gas, none of these chemicals has duplicated the Viking LR thermal control data. The recently proposed perchlorates are likewise excluded. Thermally stable until about 400 deg. C, they would easily have survived the control regimens. It is puzzling why none of the proposers of abiotic explanations of the Mars LR results addresses all of the control data, especially the one most difficult to explain – how the proposed chemical oxidant lost its activity after storage for two months isolated from its environment in a dark box at the modest constant temperature of approximately 10 deg. C, a temperature frequently reached diurnally at the Martian surface. This was also the temperature the samples were held at prior to injection of the R nutrient solution. No difference in activity showed between samples stored 2 and five days. This loss of activity over longer periods seems far more likely attributable to the death of organisms isolated from their environment than from the decay of any known strong chemical oxidant.

Yet, these failures to find a chemical agent to duplicate the Viking LR results do not constitute the proof of the LR claim¹⁸ to life. No proof by elimination of alternatives is asserted. The claimed proof is the hard positive data of the Viking LR, adequately replicated at two Martian sites, and confirmed by its range of controls. These data are supported by, or are consistent with, virtually every new finding about the habitability of Mars and the astonishing number of extremeophiles being found in the most Mars-like environments on Earth.

10. Summary

So, in summary, we have:

- 1. Positive results from a universally accepted microbial test;
- 2. Negative responses from a range of strong controls;
- 3. Duplication of the results at each Viking lander, and duplication at sites 4,000 miles distant;
- 4. No finding on Mars of any factor inimical to life;
- 5. The absence of any scientifically sustainable experiment or theory to provide a non-biological explanation of the Viking LR results.

11. The Future

To those still not accepting this evidence as proof, the author has long proposed (unsuccessfully) a chiral version¹⁹ (Figure 8) of the Viking LR. This would seek chiral specificity in the on-going reaction of the active agent on Mars, a sign of metabolism. Many astrobiologists have approved this idea as capable of resolving the life issue.

Confirmation may come sooner should Curiosity, as the author predicts, find complex organic matter in the Martian soil, or biological evidence in close-up images of rocks or surface regolith. Ultimately, science will prevail, and the paradigm will change, alas, once more deflating man's ego regarding his place in the Cosmos.



ACKNOWLEDGEMENTS:

I wish to acknowledge my Viking LR Co-Experimenter, Dr. Patricia Ann Straat, for her ten years of hard work in helping develop the science and hardware of the Viking LR, and for her support to this day.

I also wish to acknowledge the help and support of my son, Dr. Ron L. Levin, in particular with respect to his thermodynamic presentation that showed that liquid water is feasible on Mars today. His help in preparing the PowerPoint slides for this paper is also much appreciated.

REFERENCES:

- 1. "After Finding Mars Was Habitable, Curiosity Rover to Keep Roving," quoted by Nola Taylor Redd, SPACE.com Contributor, March 18, 2013.
- 2. Levin, G. V., "Stealth Life Detection Instruments Aboard Curiosity," Instruments, Methods, and Missions for Astrobiology XV, SPIE Proc., 8521, 852102-1, 2012.
- 3. Levin, G. V. and P. A. Straat, "Labeled Release An Experiment in Radiorespirometry", Origins of Life, 7, 293-311, 1976.
- 4. Standard Methods for the Examination of Water and Wastewater, American Public Health Assn., American Water Works Assn., Water Environment Fed., 2005.
- 5. Levin, G. V. and P. A. Straat, "Completion of the Viking Labeled Release Experiment on Mars," J. Mol. Evol., 14, 167-183, 1979.
- 6. Levin, G. V., "The Oxides of Mars," Instruments, Methods, and Missions for Astrobiology, SPIE Proceedings, 4495, 131-135, 2001.
- Hargraves, R.B., D.W. Collinson, R.E. Arvidson and C.R. Spitzer, "The Viking Magnetic Properties Experiment: Primary Mission Results," J. Geophys. Res. 82, 4547, 1977.
- Lovelock, J. E., "A physical basis for life detection experiments," Nature 207 (997), 568–570, 1965. doi:10.1038/207568a0, 1965.

- 9. Levin, G. V., "Odyssey Gives Evidence for Liquid Water on Mars," Instruments, Methods, and Missions for Astrobiology, SPIE Proceedings 5163, 16, 2003.
- 10. Chittenden, J. D., <u>Investigation of the Effect of Dissolved Salts, Soil Layers, and</u> <u>Wind on the Evaporation Rate of Water on Mars</u>, Ph.D. Dissertation, University of Arkansas, 2007.
- 11. Navarro-González *et al.*, "The limitations on organic detection in Mars-like soils bythermal volatilization–gas chromatography–MS and their implications for the Viking," 2006.
- 12. Benner, S. A., *et al.*, "The Missing Organic Molecules on Mars," 6, 2425-2430, PNAS, 2,000.
- 13. Glavin, D., *et al.*, "Investigating the Origin of Chlorohydrocarbons Detected in the Sample Analysis at Mars (SAM) Instrument at Rocknest," Lunar Planet. Conf. 44 Sci., 1080-2013, 2013.
- 14. Op. Cit. 9.
- 15. Hubbard, J.S., J.P. Hardy, G.E. Voecks and E.E. Golub, "Photocatalytic Synthesis of Organic Compounds from CO and Water: Involvement of Surfaces in the Formation and Stabilization of Products", J. Mol. Evol., 2, 149-166, 1973.
- 16. Kramer, K., http://www.universetoday.com/104012/curiosity-conducts-scienceon-the-go-and-zooms-to-stunning-mount-sharp/,Aug.21.2013.
- 17. Levin, G. V. and M. Malin, private communications Nov. 27/28, 2011.
- Levin, G.V., "The Viking Labeled Release Experiment and Life on Mars," Instruments, Methods, and Missions for the Investigation of Extraterrestrial Microorganisms, SPIE Proc., 3111, 146-161, July, 1997.
- 19. Levin, G. V., et al., "Detecting Life and Biology-Related Parameters on Mars, IEEE Aerospace Conf., 1, 1-15, DOI 10.1109/AERO0.2007.352744, 2007.