THE VIKING LABELED RELEASE EXPERIMENT AND LIFE ON MARS

Gilbert V. Levin
Beyond Center
College of Liberal Arts and Sciences
Arizona State University
Tempe, AZ
gilbert.levin@asu.edu

THE VIKING MISSION AND LIFE ON MARS

Little did I know that the path I was taking in 1954, when I invented a rapid and sensitive method[1] for detecting bacteria, would cause me great travail on two planets – for 31 years - and still counting! After experiments at two sites on Mars in 1976 satisfied all the pre-mission criteria for the detection of microbial life, and after additional *ad hoc* experiments supported those findings, the experiments were, nonetheless, promptly discounted by the scientific community. Today, these experiments and their results are virtually unknown to the public, which believes that the Phoenix spacecraft just landed on Mars is the first endeavor to look for life, even though Phoenix contains no life detection experiment! Perhaps an account of this amazing journey from laboratory to instrument development to field experiments to Mars will be instructive to the reader by way of illustrating the importance of public outreach in gaining scientific acceptance of new discoveries.

The usual time to obtain bacteriological results by standard microbiological methods[2] is two to several days after the test is started. The new method required only several hours, which elapsed time was later reduced to minutes, with the test being capable of being conducted in the field. After several publications[3,4,5] in professional journals (but none in the public outreach media), the method was adopted for limited use by some states, but only as an emergency measure in the event of catastrophic contamination of potable water supplies. This restriction was imposed because the method used radioactive carbon, and any radioactivity was conceived of as highly dangerous in those early days of nuclear applications. It did not matter that the amount of ^{14}C used per test was below that amount which the U.S. Atomic Energy Commission (now the

Nuclear Regulatory Commission, which has relinquished authority to those states with their own regulations) permitted in drinking water.

After several years of frustrated efforts to overcome this impediment to adoption of the method that could render important public health benefits on Earth, I cast my eyes on another planet. As a child, I had always been interested in astronomy, and, in particular Mars, which I frequently gazed at from our back yard, wondering whether life might be there. Years later, after taking biology and biochemistry, it occurred to me that, if there were any form of life on Mars, there had to be microorganisms to recycle it, just as on Earth. Hence, when an opportunity arose through meeting the first Administrator of NASA, Dr. Kieth Glennan, in 1957, I asked him whether NASA might be interested in looking for microbial life on Mars. Surprisingly, he said they were just planning for that, and referred me to Dr. Clark Randt, newly appointed to head up NASA's biology programs. I met with Dr. Randt, and he suggested I submit a proposal, which I did, entitled "A Biochemical Probe for Extraterrestrial Life."[6] Just over one interminable year later, in 1959, just when I had almost given up hope, my proposal was funded. My trip from Earth to Mars had begun. Ironically, the results of my efforts were not accepted on either planet! Remembering Edison's "invention is one percent inspiration and 99 percent perspiration," I have not given up yet – on either planet. This paper will address my Martian adventures.

THE "STICKY STRING" EXPERIMENT

Things went almost too smoothly when I began my research. I quickly hired a very competent biologist, Mary-Frances Thompson, who had the laboratory experience and nimble hands needed. Within the first couple of months, we demonstrated that the method very quickly detected microorganisms in soils we brought in from various field locations. We also developed a control to determine whether any response was from living organisms or from some chemical or physical property of the soil. We next developed several candidate nutrient media and tested and selected substrates to obtain labeled with ^{14}C. We ran hundreds of tests with pure cultures and mixed cultures of a wide variety of microorganisms, including heterotrophs, chemotrophs, psychrophiles, halophiles, aerobes, anaerobes, etc. Sensitivity to as few as 10 cells within an elapsed period of several hours was demonstrated. Satisfied that we had a promising experiment, I then subcontracted with a near-by firm, AMF, Inc. to do the instrumentation. AMF assigned A. Wendell Carriker to be the project engineer. Together, we developed the concept for a simple field instrument which was promptly built, advanced through several generations, and tested with spectacular results[7,8].

Having no idea whether the ultimate spacecraft might obtain samples for its experiments, we took no chance, and designed the instrument to have its own sampling technique. We conceived the idea of having the instrument shoot out "bullet" with a string attached to be stretched out over about 100 feet of the landscape surface to be tested for life. The string was lightly coated with silicone grease so that, as it was reeled back into the instrument, it picked up soil granules. In early tests, the string wouldn't unwind quickly enough, and was broken by the pull of the bullet. To solve this problem, we resurrected an 1860 invention made to facilitate the uncoiling of the line attached to whaling harpoons. It worked, and we were able to reel the fully deployed string back into the instrument along with its precious cargo of soil particles. Inside the instrument, an ampoule containing the labeled nutrient was then broken in a programmed

sequence of events. The solution moistened the coiled up string and its stowaways. Any gas evolved promptly rose out of the moist "culture," there being no delay during a lag period since growth was not required. The gas was detected by an overhead Geiger counter which counted the radioactivity in 15-minute intervals.

Should a positive response result (as it always did, except for the one incident described below) the control was activated. This was performed by a duplicate instrument sampling the same landscape. However, an ampoule containing a powerful germicide was broken to douse the coiled up string prior to its receiving the nutrient solution. A sharply reduced response (which it always was) confirmed that the positive result had been caused by microorganisms and not an abiotic agent which the germicide should not have affected. NASA sent us bonded samples of soils from extreme locations around the world. Only once did we fail to get a positive response and a negative control. It was a soil from the Antarctic which showed no response to standard culture methods. We wondered whether there had been organisms present that had died in the interval between sampling and testing. A number of extreme environment samples showed positive responses even though the standard methods said they were "sterile." It is now known that such classical microbial detection methods find only less than one percent of soil microorganisms present. We think our work helped hasten the realization that virtually every region of the Earth's surface, above it and below it, harbors life. This goes to prove the force of Darwin's discovery. It makes me feel certain that, if life exists on Mars (which I claim it does), it is not confined to the underground "oases" proposed by some, but, like on Earth, is pervasive.

DIOGENES

While continuing to develop Gulliver, we conceived the idea and won NASA funding for an experiment looking for ATP in the Martian soil should there be a positive response for life. We did this by refining the firefly bioluminescent reaction, and creating a highly sensitive instrument, which we named "Diogenes". Thus, if Gulliver detected life, Diogenes[9] could then be flown on the next Mars mission to indicate whether the life detected was based on ATP as is Earth life. This would be a first step in inter-planetary comparative biology. The instrument was field-demonstrated at a local ball park in front of a NASA contingent. It picked up its own soil sample, and ran through the entire assay automatically. The positive response was immediate. However, further development was suspended by NASA on the grounds that we should await the first Mars mission results.

"GULLIVER" BECOMES "LABELED RELEASE"

Hoping that the instrument would make its way to Mars to seek out tiny beings, we named it "Gulliver," Jonathan Swift's adventurer who found strange miniature beings in the far off land of Lilliput. However, one concession we had to make was to change our name from Gulliver to the "Labeled Release Experiment (LR,)," which NASA somehow felt better described it to the public. Continued success made our experiment the darling of NASA, and we had many visits from its officials and their associates to witness laboratory experiments and field demonstrations of the nick-named "sticky string" instrument. It was this success that kept our little company funded through annual contracts, each of which we had to win against formidable competition. Many well-known universities and major corporations competed for the treasured opportunity of

initiating the search for extraterrestrial life. Along the years, NASA narrowed its support from well over a dozen efforts to just four selected for the Viking Mission: 1. its own Gas Exchange experiment (GEx) which sought to detect life by changes in headspace gas over a moist or wet culture, led by Vance Oyama of the NASA Ames Research Center; 2. the Wolf Trap which looked for an increase in optical density of a broth in which a sample was permitted to settle, led by Wolf Vishniac of the University of Rochester; 3. our own LR, of tiny Resources Research, Inc.; and 4. the Pyrolytic Release experiment (PR), a derivative of our LR that sought to identify photosynthetic organisms through their incorporation of ^{14} C-labeled CO and CO_2, led by Norman Horowitz of the California Institute of Technology, until then my NASA-appointed Co-experimenter on the LR. So intense was the competition that among the many winnowed out was Nobel Laureate Josh Lederberg's "Multivator."

DARK RELEASE

We were also successful in gaining NASA funding for our solicitously-named "Dark Release" experiment[10] to detect photosynthetic microorganisms in situ in the field by allowing them to fix ^{14} C-labeled CO_2 in the light, and detecting its evolution when light was excluded. We successfully transferred this experiment from the laboratory to the field. We merely covered a spot of soil with a small glass cylinder with a rubber stopper end through which we injected $^{14}CO_2$ after the cylinder was pressed firmly into the ground. After an hour's exposure, we removed the stopper, blew air into the cylinder, and fitted the end with a cap into which was placed a porous paper pad which was then wetted with a saturated solution of $BaOH_2$. A can was then placed over the cylinder, and we waited another hour. The pad was then removed and placed in a container for drying and counting for radioactivity back at the lab. Subsequent cycles could be made with the same set-up. Enthusiastic about this demonstration of the Dark Release's ability to detect indigenous phototrophs on the surface of the soil, we invited NASA officials to such a field demonstration. Alas, something went wrong, and the post-doc doing the test failed to demonstrate its effectiveness. The NASA people left the field, and, shortly thereafter, cancelled the program. My furnishing a complete report on subsequent successful tests and an invitation to witness another test failed to resuscitate the project. I think that was a major loss for Viking. Its result could have tipped the balance in favor of biology.

LR PROGRESSES

In 1969, to my great fortune, and that of the LR experiment, Dr. Patricia Ann Straat joined our project as senior scientist. Without her help in both the science and instrumentation, we would never have made it to Mars. With the repeatedly demonstrated effectiveness of the LR, as opposed to the other experiments' developmental efforts, the LR survived repeated assaults as NASA's best hope. Its position became even stronger when it was demonstrated that it could detect even a few photosynthetic microorganisms, as opposed to the PR's requiring an algal paste to produce a positive result (this was excused on the basis that things might be different on Mars). Problems also surfaced with the GEx when it registered positive for life on testing a sample of lunar dust (the LR reported a negative response for this sample). A two-fold difficulty arose with the Wolf Trap. With realization of how dry the surface of Mars is, NASA began to suspect that dunking any Martian microorganisms into the liquid culture of the Wolf Trap would likely kill the bugs before their presence could be detected. The second problem with Wolf Trap

was perhaps worse. When sterile dust was added to the water in the Wolf Trap culture chamber, it settled as anticipated, but then some of it rose from the sediment, setting off the signal for living microorganisms, a false positive result.

THE LR ON MARS

Viking 1 landed near the Valles Marianaris on July 20, 1976. The first LR experiment occurred July 30. We were all astonished at the results – an immediate strong positive. This was even more surprisingly followed by a negative control. Figure 1 presents all VL1 first cycle data and controls. Figure 2 does the same for VL2, landed at Planitia Utopia, 4,000 miles away. All data satisfied or were consistent with the established criteria for life. Indeed, improvised controls, seeking an even more refined distinction between life and chemistry, reinforced the criteria for life.

In summary, an "active agent" was repeatedly detected in the surface soil of Mars. The agent was shown to be destroyed at 160^{o}C; essentially destroyed at 51^{o}C; greatly impaired, but still active, at 46^{o}C; and fully inactivated after being held at 10^{o}C in the dark for either two months (at Viking site 1) or three months (at Viking site 2). All of the LR results are consistent with a biological response; none are contrary.

In terms of public outreach, the possibility that Viking had detected the Holy Grail of life on Mars was immediately played down by NASA. At the first and subsequent press conferences during and after the mission, I was told not to make any claims for having detected life. At an early press conference, when Jim Martin, the Viking Mission Manager, elbowed me in the ribs, saying, "Damn it, Gil, stand up there and say you detected life!," I demurred. I would have been immediately contradicted by NASA scientists and officials. Instead, the official NASA pronouncement was that some (still!) unknown property of the soil was "mimicking" life. Unfortunately, there was plenty of public outreach to those statements, frequently shown on TV and reproduced in all the news media in all the excitement over Viking.

Some astrobiologists immediately proposed the theory that the soil on Mars was "activated" by the UV light hitting it. We quickly eliminated this theory by moving a rock at dawn and testing the soil underneath. It was fully active (see Figure 2). More than 40 additional theories have since sought to explain away the possibility that life had been detected. The consensus was early established and then maintained that the LR had detected "chemistry not biology."

However, none of those theories has been sustained by experiment or by closer examination of the theories. Each proposed obstacle to biology has been rebutted. The wide-ranging key ones follow:

a. Failure to detect organics: the Viking organic analysis instrument ("GCMS" for Gas-Chromatograph Mass-Spectrometer)[11], was designed to identify the organic matter almost all scientists (still) believe to be present on Mars (from meteoric impacts and infall of interplanetary dust particles – just as on Earth). The GCMS Experimenter stated that the instrument was not a life detector since its detection limit required the amount of organic matter in 10^9 bacterial cells. To everyone's surprise, the GCMS detected no organic matter on Mars. Despite the eight orders

of magnitude difference in sensitivities between the GCMS and the LR, scientists proclaimed that the failure to find organic matter precluded the presence of life. Since then, however, the insensitivity of the Viking GCMS has been widely reported[12] along with the fact that it frequently obtained negative results[13] with live soils on Earth.

In addition, another Viking life detection experiment, the Pyrolytic Release (PR) demonstrated[14] that organic matter is continuously produced and preserved on Mars. Although the signal obtained by this instrument was insufficient to be considered evidence for life, it clearly showed the on-going formation of organic matter. The PR Experimenter stated, "The amount of product formed could be considerable over geologic time."

b. Strong Oxidant: many theories proposed there was hydrogen peroxide or other strong oxidants on the surface of Mars, destroying any life or organic matter. This was contended despite Viking's own Magnetic Properties experiment that demonstrated[15] the absence of strongly oxidizing conditions. This simple experiment found the Martian soil contained a high degree of magnetic particles, proof against strong oxidants.

Further evidence evolved. Two Earth-based telescopic observations[16] found no peroxide in the Martian atmosphere at an upper limit insufficient to produce the LR reaction. A recent effort to refute a biological interpretation of the LR results reported[17] finding hydrogen peroxide in the Martian atmosphere at a mixing ratio of 10^{-8}. BUT this is a miniscule amount and could not survive the Martian UV or contact with surface materials.AND it is less than the amount of hydrogen peroxide in the Earth's atmosphere! Finally, this contention ignores the biology of Earth which evolved to accommodate, even utilize, larger amounts of hydrogen peroxide. More recently, as seen in Figure 3, the Rover Opportunity confirmed that surface of Mars is not highly oxidizing. Its analysis of the soil supported the Viking Magnetic Properties experiment in that the majority of the iron is less than fully oxidized.

Even gratuitously granting the blatantly difficult case for an oxidant coating Mars, all these claims have carefully avoided the fact that such oxidants do not have the thermal sensitivity found by the LR controls. Peroxide is quite heat resistant[18]. It would not have been destroyed at any of the control temperatures over the times applied to the soil, and certainly not at merely being held at 10^{o}C for a couple of months. Besides, the soil that produced the active responses did so after being held at 10^{o}C for three days! It is certainly more likely that, held at constant temperature in a dark closed box, cut off from their environment for a couple of months, the microorganisms died.

c. "Too much too soon!"
Soon after the positive responses of the LR, the overly cautious Biology Team Leader dismissed them, saying "Too much, too soon". Figure 3 shows that this is not so. The Mars LR responses fall at the lower end of the range of responses from terrestrial soils, actually being quite close to the response from an Antarctic sample.

d. Second Injection of nutrient
Although not part of the official criteria for a biological response, a second injection of nutrient solution was made after completion of the nominal eight days of the LR experiment. There was

an immediate re-absorption of approximately 20% of the headspace gas, as seen in Figure 4. Saying that the second injection should have produced a new evolution of gas, many critics cited this as evidence against biology. However, as seen in Figure 5, one of the Antarctic soils tested by the LR showed a similar re-adsorption of headspace gas. This indicates that the organisms died during the test period, and that, when wetted again, the soil re-adsorbed gas.

e. “No liquid water, no life”
This is now the primary surviving obstacle to a biological interpretation of the Mars LR. But there is much evidence for sufficient liquid water to maintain microorganisms on the Martian surface. The first was given by Viking 2. Its footpad temperature was monitored in order to determine the surface temperature of Mars. As the sun rose, the temperature mounted, but, upon reaching 0^{o}C, the melting point of ice, the temperature rise halted. Only ice turning into liquid water could be responsible. Pictures of frost or snow, taken by the Viking 2 lander within camera-distance of where this measurement was made, support this conclusion.

Unfortunately, the rebuttals did not make exciting news and produced essentially no public outreach. I had relied on scientific publications of my findings to bring about the paradigm change now so overdue. The audiences for such publications were scientists who had already hardened their view on the LR Martian results. In 1997, I decided the accumulated evidence from Mars and Earth had become too strong to be ignored. I then first announced my conclusion that the LR had, indeed, detected life. Scant heed was paid to the publication[19] by its hardened audience. Skeptics continue to say, "We don't accept Levin’s conclusions, and the consensus is against him”. However, they never present supportable scientific reasons. In contrast, all my claims have been presented in scientific papers complete with supporting detail. In truth, consensuses are always wrong whenever a true change in paradigm impends! How else could anything be new?

My papers[20,21] have offered proof that liquid water exists on Mars. They cite theoretical proof (aided by my physicist son, Ron), and reference experimental proof by UCAL Berkeley and The Hamilton Standard Corp[22]. None of the contending scientists has ever rebutted the theory or the experiments. They merely state, "There can be no liquid water on the surface of Mars," or, finally retreating to "If there is any liquid water it is in brine, or in very tiny droplets,” as if these were not liquid water. Mostly, however, like the Principle Investigator for Odyssey, which found abundant evidence of water throughout and very near the surface of Mars, decline any comment on the significance for biology, stating they presume the water is in the form of ice. As Leo “The Lip” Durocher (the fabled manager of the Brooklyn Dodgers and other major league baseball teams) said, “Sometimes you can see a lot by just looking.” Figure 6 may be an outstanding example of this principle. It shows what looks like a simple mud puddle on Mars. No other explanation has been offered, but NASA scientists insisted it could not be mud, because there can be no liquid water on Mars. Then, the Rover Opportunity made (what looks like) tracks in mud seen in Figure 7. Well, there were times when The Lip was wrong, but, generally, not about seeing water.

The website, <gillevin.com>, carries each of the claims I and my colleagues have made concerning life, organic matter, water and even the still-debated issue of the true colors on Mars.

Anyone wishing to contend against any of these claims is obligated to provide their scientific rationale, not merely the statement, “I don’t believe that”. Generalities are not science.

Before and after Viking, I made three other attempts to discover life on Mars. First was my participation in the IRIS (Infrared Interferometer Spectrometer) experiment[23] aboard Mariner 9 in 1969. That produced no evidence of the sought organic gases in the Martian atmosphere, but did produce important evidence I later cited[24] against a strong oxidant on the planet. Next was a Post-Viking experiment aboard the Russian 1996 Mission to Mars. As a member of the MOx (Mars Oxidant experiment[25]) designed to find the putative oxidants on Mars) team, I managed to have two of the instrument’s optical fibers coated in a way to reveal the presence of microorganisms in the soil. Alas, the spacecraft never left Earth orbit and crashed in the Pacific or in Peru. Finally, I suggested to the TEGA investigators and NASA officials a slight modification[26] in two of the eight ovens of TEGA that, as in the case with the MOx experiment, added life detection capability to the instrument. Sometime after an inclusive meeting with them, I was unofficially told the modification had been added to the TEGA (Thermal Evolved Gas Analyzer) aboard the Surveyor 1998 Mission to Mars, but was unable to confirm that. In fact, it didn’t really matter, as the Surveyor never reported from Mars. Now, I am sad to say, however, that I feel certain my suggested modification is not in the TEGA that just landed on Mars aboard Phoenix.

This is written during an exciting time for Mars research. The Phoenix spacecraft has landed safely, and, after difficulty, has just filled one of its TEGA ovens with Martian soil granules. Should it find organic matter present, this should give a great boost toward acceptance of the LR having detected life. Having learned my lesson over some 30 years, I will now try for public outreach!

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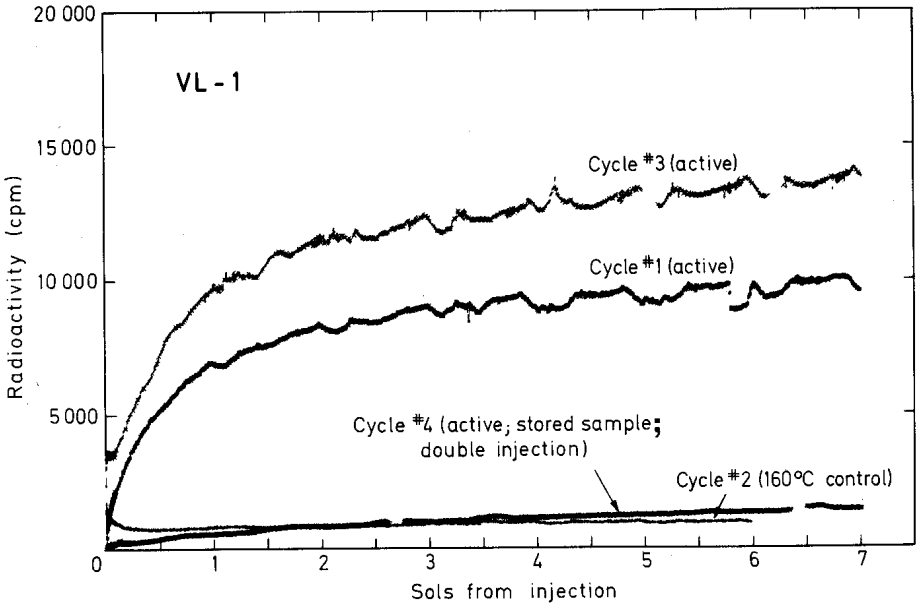
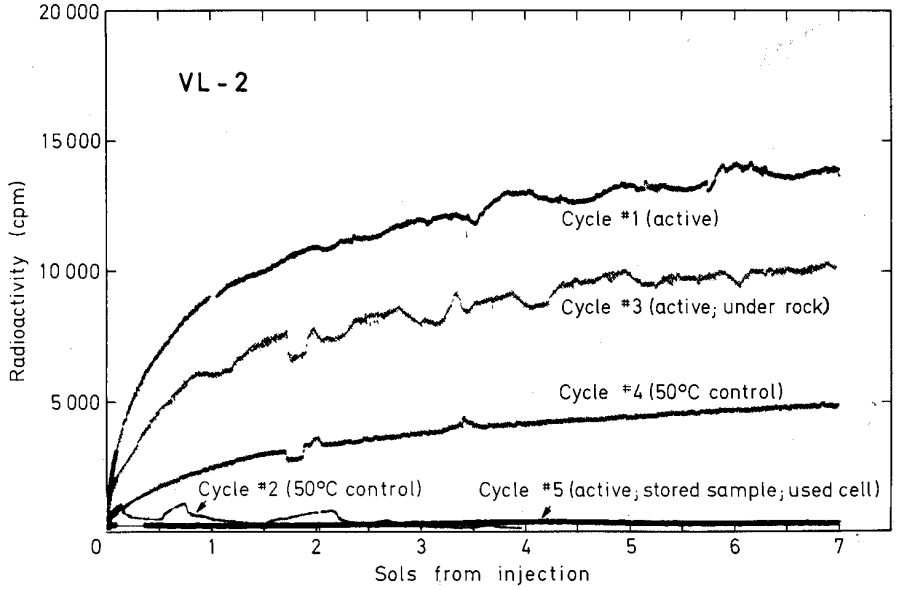
FIGURE 1
ALL VIKING 1 FIRST CYCLE RESULTS



Comparison of radioactivity evolved following the first injection of radioactive nutrient to each analysis cycle of VL-1. A fresh sample was used for the active sequences of cycles 1 and 3 whereas the sample used for active cycle 4 was stored for approximately 141 Sols at 10-26°C prior to use. For cycle 2, a stored portion of the same sample used for cycle 1 was heated for 3 h at 160°C prior to nutrient injection. All data have been corrected for background counts observed prior to nutrient injection.

FIGURE 2.
ALL VIKING 2 FIRST CYCLE RESULTS



Comparison of radioactivity evolved following the first injection of radioactive nutrient to each analysis cycle of VL-2. A fresh sample was used for each cycle except cycle 5 which used a sample stored approximately 84 Sols at 7°C prior to injection. The sample used in cycle 3 was obtained from under a rock. Cycles 1, 3, and 5 were active sequences, whereas cycles 2 and 4 were control sequences in which the samples were heated for 3 h at approximately 51.5°C and 46°C, respectively, prior to nutrient injection. Sample volumes were 0.5 cc except that for cycle 5 which contained 2.2 cc. All data have been corrected for background counts observed prior to injection.

FIGURE 3
"TOO MUCH TOO SOON"
LR Mars Response Compared to Terrestrial Responses

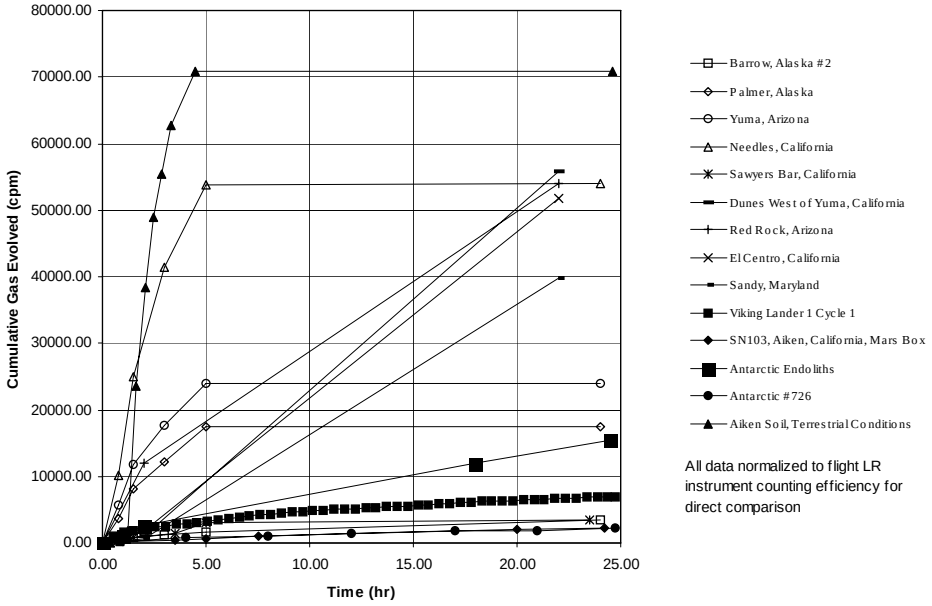


FIGURE 4.
LR SECOND INJECTION, MARS

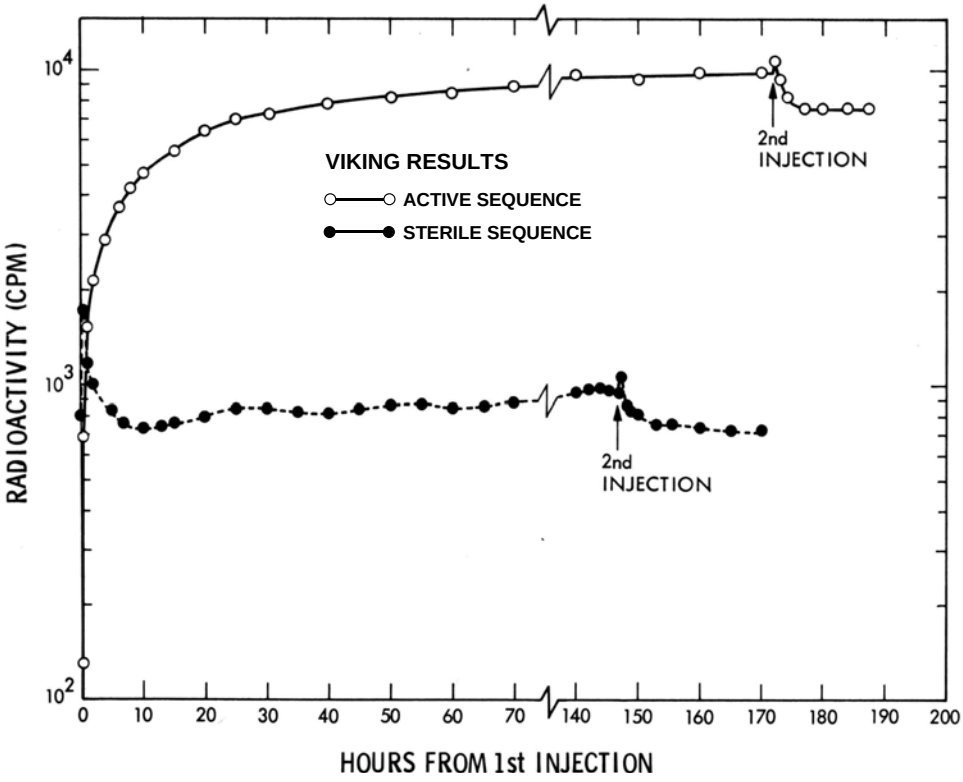


FIGURE 5.
SECOND INJECTION, EARTH

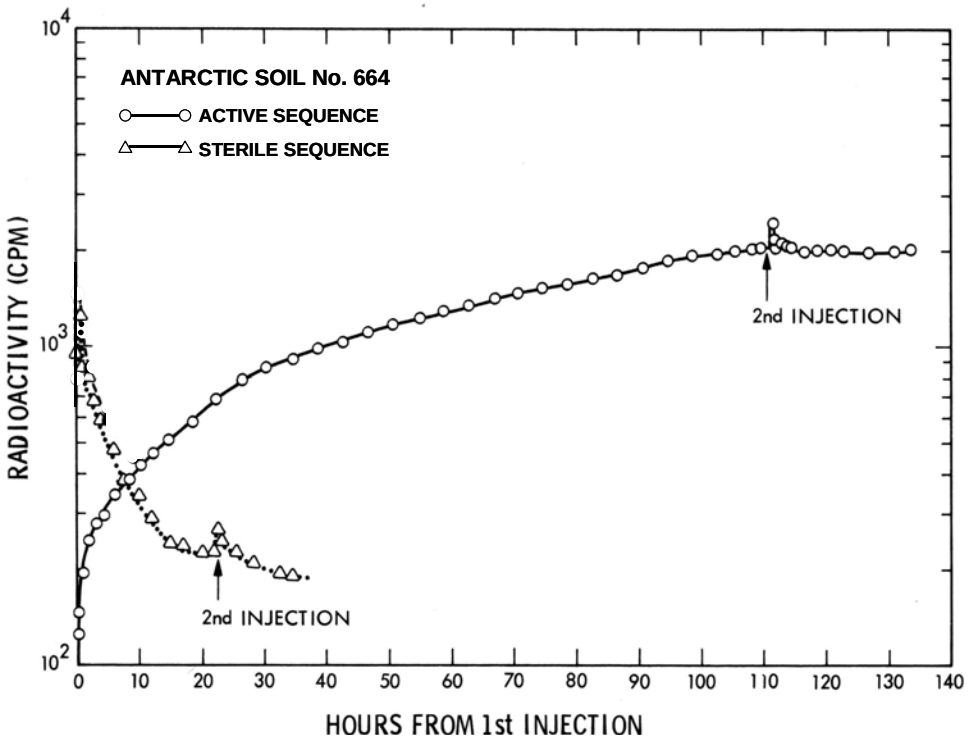


FIGURE 6.
SNOW OR FROST ON THE SURFACE OF MARS

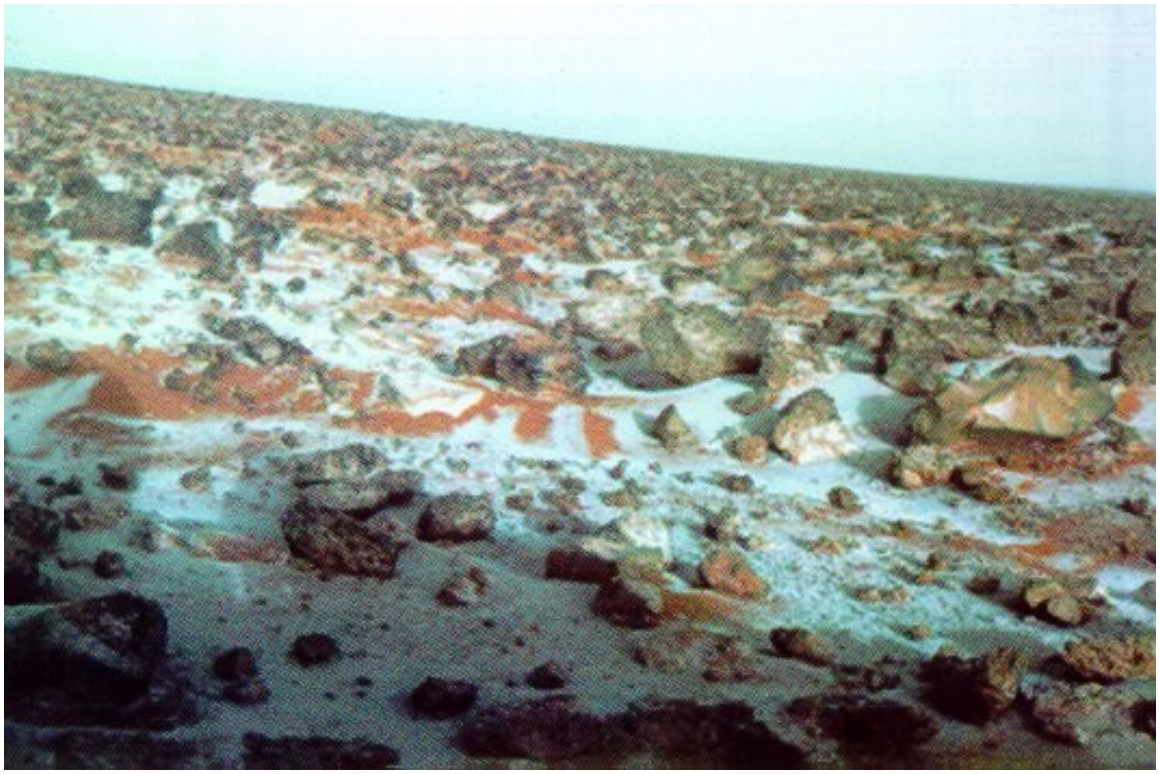


FIGURE 7.
POSSIBLE MUD PUDDLE ON SURFACE OF MARS