THE VIKING BIOLOGY EXPERIMENTS: EPILOGUE AND PROLOGUE*

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(Received August 26, 1991)

Abstract. In looking ahead to possibe new attempts to search for extant life on Mars, the history of the Viking biological investigations is reviewed here. Scientific considerations that led to the selection of specific experimental approaches for life detection are discussed, as well as the overall results obtained from that mission. Despite extensive preflight testing of the concepts that were to be used, unanticipated artefacts arose in the actual mission. These almost certainly reflect the fact that, at that time, there were many gaps in our understanding of the physical and chemical characteristics of the Martian environment. After Viking, many of these issues still remain unresolved, and future attempts to search for extant biology should be restrained until adequate new information about potential habitable microenvironments is obtained.

Introduction

After more than three decades of spaceflight activity, the Viking mission, in which two spacecraft were launched to Mars in 1975, remains as the only successful attempt to obtain direct information on the question of extant life on that planet. Now, while the data from that mission are still undergoing analysis, and when new missions to Mars are being discussed and planned, it may be of interest to review the general background history that led to the Viking biological experiments and what was learned from these experiments – with a view toward helping to formulate strategies for any future attempts to search for living organisms on Mars.

The prospect of conducting *in situ* experiments on Mars in order to obtain evidence for extant biology became a dominant theme in planning for solar system exploration in the early 1960's, as many scientific advisory groups declared that the search for life on Mars would be one of the most important and exciting challenges for NASA to undertake (cf. Pittendrigh *et al.*, 1966; Ezell and Ezell, 1984). During this period, numerous investigators were involved in perfecting assay systems and techniques to detect the presence of an extant biota on Mars. (For reviews, see Lederberg, 1960; Anonymous, 1963; Quimby, 1964; Lederberg, 1965; Bruch, 1966; and Imshenetsky, 1970). Additional descriptions of specific 'life detection' methods were given by Vishniac, 1960; Lederberg, 1961; Levin *et al.*, 1962; Soffen, 1963; Young *et al.*, 1965; and McLaren, 1966.

The various approaches suggested by these authors were predicated on many different assumptions about the nature of Martian organisms. At the time, this

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* Presented at the International Symposium on the Biological Exploration of Mars, October 26–27, 1990, Tallahasee, Fla., U.S.A.

Origins of Life and Evolution of the Biosphere **21**: 255–261, 1992.

wide spectrum of ideas for 'life detection' experiments largely reflected the fact that there was only limited knowledge of the overall physical and chemical environment of Mars and no information about its possible surface microenvironments.

Approaches to Life Detection

The approaches that were proposed for determining the presence of an extant biota on Mars can be grouped into three major categories (Table I). At one extreme were those that made no, or only minimal, assumptions about the chemistry and physiology of Martian organisms, while at the other extreme were those that were based on the idea that chemical evolution on Mars would have produced a biota with biochemical properties similar to those of terrestrial organisms. In between these were concepts that assumed a Martian biota based on organic compounds, but with organisms whose detailed biochemical pathways could have evolved differently from those of terrestrial organisms.

It might be useful here to examine some of these notions, since it is conceivable that some of them might be revived in the event that another *in situ* mission is contemplated in the future. If this were to happen, it seems reasonable that one would want to utilize an experimental playload that was based on the fewest and simplest assumptions. This being the case, schemes that do not rely on any assumptions about the chemistry or biochemistry of Martian organisms would appear to have an advantage. Intuitively, it would seem that simply looking for organisms employing appropriate imaging systems would be most satisfying. After all anyone can see the difference between a small pebble and a mouse! And one needn't assay the amino acid composition of the mouse to know that it is a biological structure. But, in looking at a picture of these two objects in the field, how could one be

TABLE I

In situ life detection concepts for Mars

Minimal assumptions on chemistry	Carbon-based chemistry	Carbon-based chemistry; analogous to terrestrial
Imaging (Viking Lander Cameras)	Organic C Compounds (Viking GC-MS)	Enzyme Analyses
Calorimetry	Macromolecules	Specific Bio-Chemical Markers (e.g. ATP, DNA)
Turbidimetry	Optical Activity	Metabolism (Viking LR Expt.) (Viking GEX Expt.)
	Gas Disequilibria	
	Metabolism (Viking PR Expt.)	

sure that the pebble wasn't really a small mushroom? The point here is that pictures can be conclusive, but – especially in an alien world – they may also be misleading. This is particularly true for minute or microscopic objects. Even in terrestrial geological samples, distinguishing biological from inanimate matter is often fraught with difficulties. Non-biological filaments, globules, and other 'life-like' objects are not uncommon in terrestrial samples (Bramlette, 1967) and, especially in ancient terrestrial sediments, such objects have long been the subject of debate as to their biogenic or abiogenic origins (cf. Schopf and Walter, 1983 and Hofmann and Schopf, 1983, for example).

The Viking Mission

As specific concepts were being developed for the scientific payload of the Viking mission, the inclusion of experiments to obtain evidence for extant biology necessitated the adoption of several guidelines. First, the strategy was to concentrate on the detection of microbial forms of life, but not to limit the scientific payload to this objective. Second, recognizing the many gaps in our understanding of the Martian environment, and to minimize the chances of reporting false negative results, the search strategy was to include several experimental approaches rather than relying on any single life detection instrument. That is, it was desirable to test many different assumptions about Martian biology, if possible. Third, in order to minimize making conclusions based upon false positive results, the strategy called for including the capability of heat 'sterilization' of samples in the event that any individual experiment gave a presumptive positive result. [As events showed during the actual Viking mission, the inclusion of several life detection experiments clearly prevented the drawing of erroneous conclusions from the data; had any of the three Viking biological experiments been flown in isolation, the derived conclusions would have been misleading. On the other hand, it also transpired that the capability to subject samples to heat sterilization, while useful in interpreting some of the 'positive' results that were obtained, was not a sufficient critical determinant in one of the experiments.]

For the Viking mission, an elegant imaging system was included as part of the lander spacecraft. This system (Huck *et al.*, 1972) was capable of producing pictures in black-and-white, as well as in color, and was capable of resolving objects as small as a few millimeters in size near the footpads of the landers. The cameras, thus, while incapable of detecting microscopic organisms, could, in principle, see other, larger, organisms. [For a discussion of the use of this imaging system as a life detection instrument, see Levinthal *et al.*, 1977]. During the years of imaging of the Martian surface, and after sending back more than 4500 pictures, no 'macrobes' were seen or reported. However, to illustrate the possible ambiguities with imaging as a technique, it has been claimed that some of the colorations on the Martian rocks seen in Viking pictures could be due to lichen-like growths (Levin *et al.*, 1978).

One approach to life detection on Mars – not requiring any assumptions about the biochemistry of its biota – was developed by Vishniac (1960) for inclusion as part of the Viking payload. Here, he argued that organisms in their natural environment were likely to be found closely associated with their 'normal' nutrients, whatever these might be, and that extracting the 'soil', and incubating the resultant mixture, one might be able to detect the growth of the indigenous population. In his approach, he proposed to assay this process by following the turbidity of such suspensions over a period of time. Of course, one major problem with this idea is to know what to use as the extractant. Water may seem to be an obvious choice, but on an extremely dry planet like Mars, extraction with water could well destroy any indigenous organisms (that presumably were adapted to an arid existence). Ultimately, this experiment was dropped from the Viking science payload, when cost and complexity issues forced reductions in the payload.

In the category of approaches that assume a carbon-based biology, but which make minimal assumptions about the chemistry of hypothetical Martian organisms, there were suggestions to analyze surface material for the presence of organic compounds. For this purpose, each Viking lander carried a sophisticated gas chromatograph-mass spectrometer capable of detecting organic residues down to parts per billion for compounds containing three or more carbon atoms, and to parts million for compounds containing 1 or 2 carbon atoms (Biemann *et al.*, 1977). These methods had, as a potential drawback, the fact that organic compounds, including macromolecular substances, were also known to be formed non-biologically (Miller and Urey, 1959; see also Fox and Dose, 1977). Furthermore, in studies that preceded the Viking mission, Horowitz and his collaborators had shown that organic compounds were produced when a simulated Mars atmosphere was exposed to simulated solar radiation (Hubbard *et al.*, 1971). [Indeed, during the actual Viking mission, this group obtained data supporting their earlier ground-based experiments (Horowitz *et al.*, 1977).]

Among the experiments that tested directly for the presence of metabolizing organisms was the Pyrolytic Release experiment (Horowitz *et al.*, 1977), which made no specific assumptions about the biochemistry of Martian organisms, being based on the assumption that the biota on Mars involved the metabolism of carbon compounds and that it would include organisms capable of incorporating carbon dioxide and/or carbon monoxide (known to be present in the Martian atmosphere) into organic compounds.

Two of the biological investigations that were finally chosen for the biology investigation on the Viking landers fall into the third category. One (the 'Gas Exchange' experiment (Oyama and Berdahl, 1977)), went much further in assuming an Earth-like biota on Mars than the other (the 'Labeled Release' experiment (Levin and Straat, 1977)). The former incubated samples of the Martian surface either in a humid or frankly wet environment, with or without the addition of a rich, complex, mixture of organic nutrients, including some vitamins. By contrast, the latter experiment was more constrained in its assumptions – exposing the samples to a gradient of moisture and to a mixture of a few very dilute, simple organic compounds. Both of these experiments, as well as the experiment of Horowitz and his colleagues mentioned above (the 'Pyrolytic Release' experiment), called for incubating surface samples for varying periods of time – up to many months – so that any initial living entity, present in the samples, could multiply and thus amplify the biological signal. All of them were predicated on the assumption that Martian biology would be based on carbon chemistry.

All three of the biology experiments gave results indicative of active chemical processes when samples of Mars were subjected to incubation under the conditions that were imposed on them. However, no clear evidence was obtained that could reasonably be ascribed to biology. (For overall summaries of these Viking biological investigations, see Horowitz, 1977; Klein, 1977, 1979).

Taken as a whole, the results obtained after 26 separate incubations of Martian surface samples indicate that adherence to the initial set of guidelines for the biological investigations did not adequately rule out artefacts. Thus, in the Pyrolytic Release experiment, small amounts of atmospheric CO/CO_2 were apparently incorporated into organic compounds both in the lights and in the dark, initially suggesting some kind of biological synthetic activity. However, similar levels of incorporation were seen even after prior heating of the samples at 90 °C for 2 hr. In the Labeled Release experiment prior heating of samples eliminated, or greatly reduced, the observed rapid decomposition of added organic compounds to carbon dioxide, thereby satisfying the initial guidelines for a 'positive' result. Nevertheless, in conjunction with the data from the Gas Exchange experiment (which suggested that strong oxidants were present in the surface samples), and from the gas chromatograph-mass spectrometer experiment (which failed to detect organic compounds), the results of the Labeled Release experiment almost certainly represent another artefact. These results merely underscore the fact that the Viking experiments were conceived and performed in the absence of adequate information about potential microenvironments on Mars. Furthermore, no one should be surprised to learn that Mars' surface material is probably much more complex than the simulated Martian 'soils' that have been formulated on the basis of spectroscopic observations and Viking chemical analyses of the Martian surface. Such analogs, while useful for many purposes, may be inadequate for duplicating the characteristics of actual Mars samples.

After Viking — What

At the time that the Viking playload was being determined, the information with which to plan for life detection experiments was much more fragmentary than it is now. For example, there was no data on the presence of nitrogen anywhere on the planet; virtually nothing was known about the composition of its regolith; the radiation flux at the surface was poorly understood; and, most importantly, were questions about the availability of waster for metabolic processes. Speculations about the possibility of extant life on Mars were thus predicated upon many uncertainties (Klein, 1976). As a result of the Viking mission, some of the gaps in our knowledge have been filled in. Nevertheless, despite the enormous amount of new data about Mars (for a comprehensive summary of Viking results, see the special issue of *J. Geophysical Research*, Vol. 82, No. 28, 1977), many important gaps still remain to be considered before future searches for extant life are contemplated. The availability of water remains a key issue. The apparant presence of strong oxidants in the regolith needs to be investigated and, if confirmed, extended to include information about their chemical properties, topographical distribution and distribution with depth.

From the foregoing discussion, it should be evident that we remain rather poorly informed about microenvironments within which a Martian biota – if it exists – must operate. For these reasons, it is ill-advised to plan for new *in situ* experiments to search for extant life until the major issues of concern are resolved and understood. In this regard, it has been suggested (Space Sciences Board, 1977) that a sample return mission would be more useful than further *in situ* missions. The arguments that support such an approach center around the substantially greater level of sophistication and instrumentation that can be brought to bear on this problem, as well as the expected ability to deal much more effectively with unexpected or puzzling data. As has been amply demonstrated with returned samples from the moon, both the US and Soviet returned sample mission yielded a wealth of scientific data, using 'state-of-the-art' techniques and often requiring only exquisitely small samples of material. In considering this alternative, it should be recalled, however, that the lunar samples were collected only after an extended period of prior observations and exploration, resulting in carefully selected sites for later analytical probing. A sample return mission from Mars, in which one or more random samples were collected for analysis, could prove to be only marginally useful from the point of view of searching for life on that planet.

By analogy with terrestrial ecosystems, proposals have also been made that extant life might be found on Mars in very specialized habitats, in which organisms could be sheltered from the UV radiation flux, protected from the effects of the postulated oxidants, and at the same time exist in environments that are (even intermittently) wet. One such suggested possibility is that of 'cryptoendolithic' organisms living inside of rocks as is the case for mixed bacterial/lichen communities found in certain rocks in Antarctic desert areas (Friedmann and Ocampo, 1972). More recently Ivanov (1988) has proposed that ancient hydrothermal vents may be present on Mars within which an ecosystem could exist based upon the anaerobic oxidation of reduced compounds emanating from volcanic activity below such areas. While of theoretical interest, in neither of these cases is there any evidence now for such specialized microenvironments anywhere on Mars. Once again, considerable precursor investigation is necessary to locate, or at least to demonstrate the feasibility of, such specialized niches before undertaking experiments to test these ideas. Successful completion of the prospective US Mars Orbiter and USSR Mars '94/96 missions will undoubtedly raise our level of understanding of what is and what isn't possible on Mars. As with previous missions to Mars, they may also raise intriguing new questions.

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