The Viking Biological Investigation: General Aspects

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The Viking biological investigation has tested four different hypotheses regarding the possible nature of Martian organisms. While significant results were obtained for each of these, tests of three of the hypotheses appear to indicate the absence of biology in the samples used, while the fourth is consistent with a biological interpretation. The original assumptions for each experiment and the experimental procedures that were utilized to test these assumptions are reviewed.

Introduction

Before the two Vikings reached Mars, speculation about the prospects for life on that planet ranged from extreme pessimism to optimism. At that time, when the biology experiments were actually selected for final inclusion on Viking, our information about Mars was far from complete. Under these circumstances it is not surprising that different ideas emerged concerning what Martian organisms might be like and what procedures or techniques would best elicit evidence of their metabolism. As a result of these diverse ideas it was decided to incorporate several different biological experiments into the Viking payload in order to test a number of different, and sometimes conflicting, assumptions about the characteristics of Martian organisms [*Young*, 1976]. Indeed, had it been possible to fly additional biological experiments and thereby extend the number of different ideas and techniques that could be tested, this would have been a valuable thing to do. However, the biological portion of the payload was constrained to approximately 0.03 m^3 of volume and 15 kg of weight [*Klein*, 1976].

By supplying four different sets of environmental conditions within which to conduct incubations (Table 1), the three experiments that were finally selected for the Viking biology instrument actually tested four different hypotheses. One of these assumed that active Martian metabolism was limited by the availability of water. Another assumed that biological activity would best be seen under conditions approximating those on Mars. The remaining two tested for heterotrophic metabolism, one by using a very dilute aqueous solution of simple organic compounds, the other by utilizing a concentrated mixture of many organic compounds. All of these have now been tested, and the results from these are the subjects of the ensuing papers.

As of this writing the two Viking landers have been operating on the surface of Mars for 8½ and 7 months, respectively. All during this time the Viking biology instrument has performed exceptionally well, with few instances of instrument anomalies, and those that have occurred are sufficiently well understood so that they do not interfere with the interpretations of the data. Accordingly, for the papers that follow one can safely assume that all of the information is based in statistically significant data with strong 'signal-to-noise' ratios and that we are not engaged here in describing or explaining artifacts produced by the instrumentation.

Initially, in the so-called 'nominal' mission we expected to perform a total of 13 or 14 separate experiments on the two spacecraft. With no a priori knowledge of the local Martian surface environments our overall initial strategy was essentially based on the concept that there might be local heterogeneity in the surface of Mars in the vicinity of the spacecraft. We planned therefore to perform the biological experiments in a 'survey' mode, testing sample after sample by using the same experimental sequences until any one of the experiments yielded a 'presumptive' positive result. (For a discussion of the criteria that were to be used to arrive at this judgment, see *Hubbard* [1976], *Levin and Straat* [1976a], and *Oyama et al.* [1976].) Once such a presumptive positive result was observed, our strategy called for repeating that particular experiment by using a heat-'sterilized' sample. This procedure, of using heated 'controls' to confirm biological processes, is deeply rooted in biological tradition. More important, it was based on hundreds of ground-based tests of the Viking concepts using terrestrial soil samples (from desert soils to rich garden soils), lunar samples, and pure cultures of microorganisms, the results of which enabled us accurately to determine the presence or absence of living organisms.

In point of fact, our intended nominal strategy was quickly discarded as the very first sample was analyzed. Two of the three experiments yielded presumptive positive results, while the third produced evidence for oxidizing surface material at that site [*Klein et al.*, 1976]. This combination of findings, together with the demonstrated lack of organic compounds [*Biemann et al.*, 1976], required substantial changes to the original experimental strategies and resulted in major departures from experiment sequences that we had anticipated using in these experiments. In all cases every element of flexibility inherent in the instruments was called into play in an effort to use the equipment both as biological and as chemical laboratories in order to discriminate between the two mechanisms that might be responsible for the presumptive positive results.

By now, considerable progress has been made in resolving the issues raised by the first set of analyses. To achieve this, we have exceeded the number of experiments initially planned, having carried out 26 experiments. A few additional experiments still remain to be performed, but on the basis of all the information now available, it is likely that the results of one of the two experiments that yielded presumptive positives, the Pyrolytic Release experiment [*Horowitz et al.*, 1976], are non-biological in origin. The Labeled Release experiment, which also has consistently yielded presumptive positive biological results [*Levin and Straat*, 1976b], remains ambiguous.

In arriving at any final judgment on the fundamental

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TABLE 1. Environmental Parameters in the Viking Biology Investigation

Experiment	Nutrients Added	Water Added	Illumination
Pyrolytic Release	None*	none	light and dark
	none	traces§	light and dark
Gas Exchange	none	moist‖	dark
Labeled Release	dilute solution of simple organic compounds	moist¶	dark
Gas Exchange	concentrated solution of organic and inorganic compounds	wet**	dark

*A mixture of CO_2 and CO was introduced into the incubation chamber.
†See *Levin and Straat* [1976b].
‡See *Oyama* [1972].
§Approximately 80 µg of H2O was injected into an incubation chamber (2.6-cc volume) containing a 0.25-cc sample.
‖Approximately 0.5 cc of nutrient solution was added below the 1-cc sample in the chamber (8.7-cc volume).
¶Approximately 0.115 cc of nutrient solution was added to the 0.5-cc sample in the chamber (3.25-cc volume).
**Approximately 2.5 cc of nutrient solution was added to the 1-cc in the chamber (8.7-cc volume).

question, Is there life on Mars?, we must carefully consider not only the experimental results that have been obtained but also the context within which these data were obtained. We must examine the assumptions on which each of the experimental techniques was based, the assumptions under which the [p.4678] experiments were actually carried out, and the data themselves.

THE GAS EXCHANGE EXPERIMENT (HUMID NONNUTRIENT MODE)

As is indicated in Table 1, the Gas Exchange (GEX) experiment tested two different concepts of Martian biology. In one mode the fundamental assumption was that the sole limiting factor to growth of Martian organisms is water. Here it was assumed that nutrients, perhaps in the form of simple organic compounds formed photochemically as is described in *Hubbard et al*. [1971], already are present in the Martian surface and that organisms would be dormant in the dry Martian environment until enough moisture became available to stimulate the dormant organisms into metabolic activity, which was to be measured by analyzing the atmosphere above the incubating system with a gas chromatograph system.

Now let us examine some of the experimental conditions under which this assumption was actually tested. First, the Martian samples were incubated in the presence of the Martian atmosphere to which additional carbon dioxide, krypton, and helium were added in order to bring the total pressure to approximately 200 mbar to facilitate subsequent gas sampling [*Edelson et al*., 1975]. After introduction of approximately 0.5 cc of nutrient solution into the incubation cell, under conditions in which the nutrient does not come into contact with the samples, the atmosphere rapidly becomes saturated with water at the incubation temperature of 8-15°C. After an incubation period for this phase of the experiment of approximately 7 days the experiment was terminated.

In this experiment, which was performed twice, once at each of the landing sites, the findings for both were essentially the same. While physical (desorption of some gases) and chemical (generation of oxygen) phenomena were noted, there was nothing in the data to suggest the presence of metabolic activity on the basis of the criteria that had been developed for this experiment, and therefore the results of this experiment can be said to be negative with regard to biology.

In terms of interpreting the results obtained, if we regard both the assumptions and the experimental conditions to be valid, we must conclude that the samples that were assayed did not contain metabolizing organisms. However, the original assumption may be incorrect in that some source of energy may be a requirement to stimulate metabolic activity of organisms on Mars. In this case a negative result would not preclude the existence of 'life' in the samples tested. Alternatively, it is possible that one or more of the experimental conditions employed during these tests prevented the accumulation of biological signals. For example, in this experiment, as in all of the biological experiments, incubation temperatures ran some tens of degrees warmer than ambient surface temperatures at the two sites [*Kieffer*, 1976]. Another issue is whether the total incubation period of 7 days was sufficient to demonstrate metabolic activity in view of tests with Antarctic soils that required months of incubation to produce presumptive positive results [*Oyama et al*., 1976]. Other potential sources of possible inhibition of metabolism include the high pressure and alteration of the incubation atmosphere, as indicated above.

THE GAS EXCHANGE EXPERIMENT (WET NUTRIENT MODE)

In running the GEX experiment, in the presence of added organic compounds and inorganic salts the fundamental assumption made was that a significant fraction of the Martian 'biota' is composed of heterotrophic organisms [*Oyama et al*., 1976]. Therefore the addition of organic compounds was deemed necessary to elicit metabolic response. Furthermore, it was assumed that this response would be expressed only in an aqueous environment, and finally, the presence of a large number of different organic and inorganic compounds [*Oyama*, 1972] was assumed not to be inhibitory to the expression of this metabolism.

This experiment was performed three times, for periods of 200 (Viking 1), 31 (Viking 2), and 116 (Viking 2) sols. However, Martian atmosphere was present for only a portion of these incubation periods, 13, 19, and 78 sols, respectively. For the remainder of the time the atmosphere consisted of carbon dioxide, krypton, and helium. Once again the total atmospheric pressure was approximately 200 mbar, and incubations temperatures were in the 8-15°C range.

While some gas changes were noted in the three trials of this experiment [*Oyama*, 1977], none of these fit the criteria for biological activity. Consequently, on the basis of the original assumptions for this experiment and provided that the conditions under which they were tested were adequate, we can conclude that no viable organisms were present in the samples. On the other hand, a negative finding for this experiment does not rule out the possible presence of autotrophs (i.e., chemosynthetic organisms) in the samples. In addition, the corollary assumptions concerning the nutrient mixture used, as well as the high water activity under which these tests were conducted, must also be assessed in arriving at the biological significance of the data from this experiment. Finally even if

the original assumptions are correct, some of the experimental conditions (temperature, pressure, and 'artificial' atmosphere) may have precluded positive biological findings in this experiment.

THE PYROLYTIC RELEASE EXPERIMENT

The fundamental assumption for this experiment stems from considerations of the characteristics of the planet Mars. [p.4679] Since both carbon dioxide and carbon monoxide were known to be present in the atmosphere of Mars, it was assumed that organisms in the local ecology of that planet would have developed the capacity to assimilate one or both of these gases [*Horowitz et al*., 1972] and convert these to organic matter. A basic tenet of this experiment is that metabolic activity would best be demonstrated under conditions approximating the ambient conditions on Mars as closely as possible.

The actual Pyrolytic Release (PR) experiments were conducted under conditions which, in many aspects, did approximate those on Mars, but in some they did not. Incubations were carried out either in the light or in the dark for 5-day periods. In those cases in which illumination was used, wavelengths below about 320 nm were filtered out in order to avoid false positives [*Hubbard*, 1976]. The incubation temperatures again were in the 10-18°C range.

As has been reported [*Horowitz et al*., 1976], initial results of the PR experiment, as well as later attempts to repeat the original conditions, resulted in weak but significant presumptive positives. However, later experiments designed to elucidate the mechanisms yielding these results appear to rule out a biological explanation for these results.

As with the GEX experiments, it is possible that in the case of the PR experiment the basic assumptions are not correct. The photochemical synthesis of simple organic compounds has not been ruled out on the surface of Mars. If in the steady state this process were to supply organic matter to the surface, the need for autotrophic fixation in the Martian ecology may be obviated, and only heterotrophs may be present. In this case a negative result in this experiment would not preclude the existence of life in the samples tested.

In examining the experimental conditions under which the PR tests were conducted, the duration of the incubation periods, the incubation temperatures, and the fact that short-wavelength solar radiation was unavailable to the surface samples all raise questions regarding the adequacy of these experiments in providing the requisite conditions for measuring this type of metabolism even if Mars should have indigenous autotrophic organisms.

THE LABELED RELEASE EXPERIMENT

This experiment [*Levin*, 1972] makes the assumption that heterotrophic organisms are present on Mars and that these organisms would be capable of decomposing one or more simple organic compounds of the type reported to be produced from the so-called 'primitive reducing atmosphere' in laboratory simulations [*Miller and Urey*, 1959] and including some that have been found in carbonaceous chondrites [*Kvenvolden et al*., 1970]. For this experiment, even more than for the others, the inclusion of a heated control was deemed absolutely necessary in order to avoid the possibility of obtaining false positive results. The conditions under which samples that were not heat sterilized were incubated, now four times, for periods of about 13, 52, and 90 days, were Marslike with the exception that incubation was carried out at around 10°C; and of course, there was the addition of a small volume of water containing the dilute solution of organic substrates [*Levin*, 1972]. As with the GEX experiment, the incubation cell was somewhat pressurized (to approximately 60 mbar) during incubation. Heat sterilization was carried out on three samples, first at approximately 160°C, and later at 50°C and 44°C.

TABLE 2. Comparison of Data From the LR and GEX Experiments

Sample	Oxygen Released (GEX)*	Carbon Dioxide Produced (LR)*
Viking 1 (surface)	770	~30
Viking 2 (surface)	194	~30
Viking 2 (subrock)	70	~30

*Nanomoles per 1-cc sample.

What has been observed in all of the analyses performed on nonsterilized samples is an active initial decomposition of the nutrient, the reaction levelling off, in each case, at a time when about 95% of the radioactivity still remained in the surface material. Prior heating of the Martian samples had substantial effects on this process, 160°C for 3 hours completely abolishing the reaction [*Levin and Straat*, 1976b].

On the basis of all of the experiments performed to date, the labeled Release (LR) experiment, unlike the other biological experiments, yielded data which met the criteria originally developed for a positive. On this basis alone the conclusion would have to be drawn that metabolizing organisms were indeed present in all samples tested. Can we believe such a conclusion? Clearly, we must be wary of this in the face of information indicating that all of the samples tested yielded oxygen in the GEX experiment upon introduction of water. The evidence for strongly oxidizing chemicals in these samples is quite convincing. However, it should also be recognized that the two phenomena are not directly related (Table 2). From the relatively constant amount of decomposition that took place in all cases in the LR experiment, it would appear that all of the Mars samples contained excess oxidants. Thus it is reasonable to assume either that the factor limiting the LR reaction in each case was the depletion of some constituent in the mixture of substrates supplied in the nutrient or that the samples analyzed in these experiments contained at least two kinds of oxidants.

While a nonbiological (i.e., a chemical 'oxidant') theory may well explain the LR data, it does not seem likely that the ambiguity in interpreting this experiment will be resolved on Mars by the remaining Viking experiments.

SUMMARY

We have tested a number of different concepts about the nature of hypothetical Martian organisms over the course of the past few months. We have seen significant and quite reproducible results in each of our experiments. For each experiment, except for the LR experiment, we must conclude that there were no organisms present within the limits of detectability for these experiments and that all of the observed reactions for these were the result of nonbiological phenomena. Otherwise, we must question the fundamental assumptions made for these experiments and the conditions under which they were actually carried out. In the case of the LR experiment, from the very beginning of operations on Mars we recognized the possibility that the striking changes seen during incubation could be the result of nonbiological processes, but our attempts to discriminate between biological and non-

biological mechanisms by manipulating sterilization temperatures and the length of incubation have not resolved the issue.

Finally, we must not overlook the fact, in assessing the probabilities of life on Mars, that all of our experiments were conducted under conditions that deviated to varying extents from ambient Martian conditions, and while we have accumulated data, these and their underlying mechanisms may all be coincidental and not directly relevant to the issue of life on that planet.