

The Biological Experiments

**Simulation of the Viking Biology Experiments:
An Overview**

Harold P. Klein

National Aeronautics and Space Administration, Ames Research Center, Moffett Field, California 94035, U.S.A.

Summary. Several ground-based investigations have been carried out since the Viking biology results were received from Mars. Many of these have resulted in reasonable simulations of the Martian data, using as analogues of Mars either strong oxidants, UV-treated materials, iron-containing clays, or iron salts. The ambiguity between the GCMS experiment, in which no organic compounds were found on Mars, and the Labeled Release experiment, in which added organics were decomposed, may well be accounted for by these simulations.

Key words: Viking biology experiments – Simulation of mars surface material – Extraterrestrial life detection

In a study published about a year before the two Viking landed spacecraft began their probing of the Martian surface, the Space Science Board of the National Academy of Sciences, in analyzing several possible Viking scenarios, stated that, "... a positive result from one or more biology instruments combined with failure of the GCMS to detect N-containing organic compounds in the soil – would seem at the present time to have no rational explanation other than instrumental error..If a result of this kind is transmitted by Viking, a fundamental reassessment of our models may be in order." (Anonymous, 1975). Yet, upon completion of the experiments on Mars by the Viking GCMS and biological instruments, the scientific community was faced with precisely this ambiguity. No indigenous organic compounds were detected in the samples assayed by the GCMS experimenters, using an instrument sensitive to parts per billion of organics (Biemann et al., 1977). On the other hand *one* of the three biology experiments (the Labeled Release (LR) experiment) consistently yielded 'positive' data (Klein et al., 1976; Levin and Straat, 1977a; Levin and Straat, 1977b) based on criteria that had earlier been developed for this experiment after extensive terrestrial testing (Levin and Straat, 1976).

Both of the other biology experiments, while failing to meet the criteria originally established for positive results (Hubbard, 1976; Oyama et al., 1976), nevertheless pro-

vided evidence indicative of highly reactive surface material at the two landing sites. Data from the Gas Exchange experiment (GEX), indicated the release of several gases (CO_2 , O_2 , N_2 and Ar/CO) whenever the Martian surface samples were humidified with water (Klein et al., 1976; Oyama and Berdahl, 1977; Oyama, et al., 1977). Analysis of these results suggests that the appearance of all of these gases except oxygen is readily explained by desorption from the surface material; however the amount of oxygen measured is anomalously high for any reasonable model of the Martian surface material (Ballou et al., 1978). On the basis of these results, as well as because other data on the Martian atmospheric constituents suggested the formation of hydrogen peroxide in the atmosphere (Hunten, 1974; McElroy et al., 1977), it seemed reasonable to speculate that the Martian surface material contained highly oxidizing compounds (Klein et al., 1976; Oyama et al., 1977; Ballou et al., 1978). Furthermore, it was postulated that these reactive species were derived from the interaction of ultraviolet light, solar wind, and/or cosmic radiation with water and inorganic constituents in the surface (Klein et al., 1976; Oyama and Berdahl, 1977; Oyama et al., 1977).

Other results, this time from the Pyrolytic Release experiment (PR), also pointed toward reactive chemical species in the Martian samples. In this experiment, surface material was incubated in the presence of the ^{14}C -labeled CO and CO_2 and, after incubation, the samples were examined for the incorporation of either of these gases into organic compounds (Hubbard, 1976). Samples from both landing sites yielded small, but significant, amounts of radioactive organics after incubation (Klein et al., 1976; Horowitz et al., 1976; Horowitz et al., 1977), but, since heating of one sample to 90°C for about 2 h did not affect the level of incorporation, the PR reaction on Mars is considered not to be indicative of a biological process but rather to be one involving inorganic surface constituents (Horowitz et al., 1977).

Several suggestions have been proposed to account for the non-biological incorporation of labeled CO or CO_2 into organic compounds in the PR experiment. One hypothesis, proposed by Oyama and associates, invokes the putative highly oxidizing surface compound(s) in initiating the formation of a polymer of C_3O_2 , with the subsequent incorporation, during the PR experiment, of labeled ^{14}C into this polymer (Oyama and Berdahl, 1977; Oyama, 1977; Oyama et al., 1978). Horowitz and his colleagues appear to favor a mechanism involving inorganic compounds(s) that are able to catalyze the incorporation of CO/ CO_2 into organic material (Hubbard, 1978) although the nature of this reaction is obscure as of this writing. This hypothesis is an extension of earlier work by that group showing that CO can be incorporated into organic matter under the influence of ultraviolet light under simulated Martian conditions (Hubbard et al., 1971). However, in the case of the experiments on Mars, there does not seem to be a requirement for light since the reaction leading to incorporation into organics also took place in the dark, although one sample incubated in the dark, from under a rock, gave a very low incorporation (Horowitz et al., 1977).

In addition to these working hypotheses, other explanations for the PR experiment have been proposed. One by Huguenin (1976 a and b), postulates the existence of UV-activated substances in the unweathered fraction of the Martian regolith, which lead to the formation of CO_3^{-2} on the surfaces of the "soil" particles, and the subsequent exchange of radioactive gas molecules during the PR experiment. With quite a different view, Banin (1977) has brought attention to the surface catalytic properties of

clay materials, and has proposed that metallic clays may be involved in the PR experiment by promoting the fixation of $^{14}\text{CO}_2$ into organic compounds, without a requirement for UV or oxidizing substances.

Returning now to the main issue of ambiguity in the Viking biology investigation – the LR experiment – the responsible principal investigators, acknowledging the weight of evidence for chemical explanations of the other experiments, have steadfastly maintained that all of the LR results are consistent with a biological interpretation (Levin and Straat, 1977a; Levin and Straat, 1977b). Their major arguments focus on the heat sensitivity of the LR process; on the fact that a UV-shielded sample (from under “Notched” rock on Mars) still produced a strong response; on the loss of activity when Martian samples were stored on the Viking lander for several months; and on their inability to simulate the LR data using irradiated terrestrial analogues of the Martian regolith. Other investigators have, however, been successful in obtaining data comparable to the LR data in many respects. For example, when hematite samples irradiated with UV were added to aliquots of the ^{14}C nutrient used in the LR experiment, these treated samples led to the release of $^{14}\text{CO}_2$. Furthermore, heat sterilization of the UV-treated material, prior to incubation with nutrient, destroyed its activity, in accord with the LR results on Mars (Ponnamperuma et al., 1977). Oyama et al. (1978) have been able to duplicate in fine detail the kinetics of ^{14}C release in the LR experiment using a mixture of H_2O_2 and $\gamma\text{Fe}_2\text{O}_3$ acting upon formate. However, in these experiments, there was no attempt to study the heat sensitivity of the reaction. Other support for the contention that oxidative chemical reactions are involved in producing the LR results comes from Huguenin who has proposed an explanation based on the photochemical production of OH^\cdot radicals (from surface frost), which then became adsorbed to the Martian surface grains (Huguenin, 1976a and b). According to this hypothesis, H_2O_2 is generated from the adsorbed OH upon the addition of water, thus leading to the oxidation of organic compounds upon contact with the LR nutrient solution.

Emphasizing the catalytic nature of certain iron oxides, Imshenetskii and Murzakov (1977) demonstrated that ordinary limonite readily decomposes formate, as well as other low-molecular weight organic acids, including several amino acids. Furthermore, these authors reported that when they heated their limonite sample following the Viking LR heat regimen, its catalytic activity was lost. Finally, they reported that UV-treatment of the limonite substantially reduced its ability to decompose formate, a finding consistent with the contention of Levin and Straat that UV irradiation of Martian surface material could not be directly responsible for the LR results.

Another study, by Banin and Rishpon (1978), has also resulted in the duplication of some aspects of the LR experiment without the intervention of UV or of strong oxidants. Using montmorillonite and nontronite clay samples saturated with iron (but not with Na or Ca), they showed that these materials rapidly decomposed labeled formate and labeled LR nutrient, yielding decomposition kinetics very similar to that observed in the LR experiments on Mars. They could not, however, duplicate the heat sensitivity of the LR experiments.

From this brief summary, it should be clear that several physico-chemical models for the LR experiment have been proposed. Many of these are currently being tested and evaluated. Some appear to duplicate many of the critical observations of this experiment, and come close enough to the Martian data to be regarded seriously in

assessing the validity of a biological interpretation for the LR data. In addition, the physicochemical simulations, together with all of the data from Mars, appear to account for the negative findings of the GCMS experiment (see also Oro and Holzer, 1979). Thus there is enough information in hand to ease considerably the ambiguity that appeared to confront us at the time that the Viking data were accumulating from the Biology and GCMS experiments. What emerges is the need to refine these studies in order to obtain a clearer picture of the nature of the Martian surface constituents.

References

- Ballou, E.V., Wood, P.C., Wydeven, T., Lehwalt, M.E., Mack, R.E. (1978). *Nature* **271**, 644–645
- Banin, A., Rishpon, J. (1978). Experimental simulation of the Viking Labeled Release (LR) results with iron adsorbed smectite clay minerals. Presented at the XXI Meeting of COSPAR, Innsbruck, Austria
- Biemann, K., Oro, J., Toulmin, P. III, Orgel, L.E., Nier, A.O., Anderson, D.M., Simmonds, P.G., Flory, D., Diaz, A.V., Rushneck, D.R., Biller, J.E., La Fleur, A.K. (1977). *J. Geophys. Res.* **82**, 4641–4658
- Horowitz, N.H., Hobby, G.L., Hubbard, J.S. (1976). *Science* **194**, 1321–1322
- Horowitz, N.H., Hobby, G.L., Hubbard, J.S. (1977). *J. Geophys. Res.* **82**, 4659–4662
- Hubbard, J.S., Hardy, J.P., Horowitz, N.H. (1971). *Proc. Natl. Acad. Sci. USA*, **68**, 574–578
- Hubbard, J.S. (1976). *Origins of Life* **7**, 281–292
- Hubbard, J.S. (1978). Laboratory simulation of the Pyrolytic Release experiments. Presented at the XXI Meeting of COSPAR, Innsbruck, Austria
- Huguenin, R.L. (1976a). *Icarus* **28**, 203–212
- Huguenin, R.L. (1976b). *Proc. Coll. Water on Planetary Regoliths*, Hanover, NH, 100–106
- Hunten, D.M. (1974). *Rev. Geophys. Space Physics* **12**, 529–535
- Imshenetskii, A.A., Murzakov, B.G. (1977). *Mikrobiologiya* **46**, 1103–1113
- Klein, H.P., Horowitz, N.H., Levin, G.V., Oyama, V.I., Lederberg, J., Rich, A., Hubbard, J.S., Hobby, G.L., Straat, P.A., Berdahl, B.J., Carle, G.C., Brown, F.S., Johnson, R.D. (1976). *Science* **194**, 99–105
- Levin, G.V., Straat, P.A. (1976). *Origins of Life* **7**, 293–311
- Levin, G.V., Straat, P.A. (1977a). *J. Geophys. Res.* **82**, 4663–4667
- Levin, G.V., Straat, P.A. (1977b). *Bio Science* **9**, 165–174
- McElroy, M.B., Kong, T.Y., Yung, Y.L. (1977). *Jour. Geophys. Res.* **82**, 4379–4388
- Oro, J., Holzer, G. (1979). The effects of ultraviolet light on the degradation of organic compounds. A possible explanation for the absence of organic matter on Mars. In: *COSPAR: Life Sciences and Space Research XVII*, Holmquist, R., ed., pp 77–86, Oxford: Pergamon
- Oyama, V.I. (1977). The chemical potential demonstrated in the Gas Exchange experiments and its meaning and confirmation of a model of Martian chemistry in the Biology experiments. Presented at A.A.A.S. Mtg., Denver, Colorado
- Oyama, V.I., Berdahl, B.J. (1977). *J. Geophys. Res.* **82**, 4669–4676
- Oyama, V.I., Berdahl, B.J., Carle, G.C. (1977). *Nature* **265**, 110–114

- Oyama, V.I., Berdahl, B.J., Carle, G.C., Lehwalt, M.E., Ginoza, H.S. (1976). *Origins of Life* 7, 313–333
- Oyama, V.I., Berdahl, B.J., Woeller, F., Lehwalt, M.E. (1978). The chemical activities of the Viking Biology experiments and the arguments for the presence of superoxide, peroxides, Fe_2O_3 and carbon suboxide polymer in Martian soil. In: *COSPAR: Life Sciences and Space Research XVI*, Holmquist, R. and Stickland, A.C., eds., p. 3, Oxford and New York: Pergamon
- Ponnamperuma, C., Shimoyama, A., Yamada, M., Hobo, T., Pal, R. (1977). *Science* 197, 455–457

Received December 22, 1978; Revised April 20, 1979