Viking on Mars: The Carbon Assimilation Experiments

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A fixation of atmospheric carbon, presumably into organic form, occurs in Martian surface material under conditions approximating the actual Martian ones. The reaction showed the following characteristics: The amount of carbon fixed is small by terrestrial standards; highest yields were observed in the light, but some dark activity was also detected; and heating the surface material to 90°C for nearly 2 hours had no effect on the reaction, but heating to 175°C for 3 hours reduced it by nearly 90%. New data from Mars do not support an earlier suggestion that the reaction is inhibited by traces of water. There is evidence of considerable heterogeneity among different samples, but different aliquots from the same sample are remarkably uniform in their carbon-fixing capacity. In view of its thermostability it is unlikely that the reaction is biological.

INTRODUCTION

With respect to its surface environment, Mars is more earth-like than any other extraterrestrial body of the solar system. Of particular importance from a biological point of view is the fact that Mars possesses both an atmosphere of light elements and a temperature regime that is compatible with the existence of organic matter and even of some terrestrial life forms. These characteristics make Mars the most plausible habitat of extra-terrestrial life in the solar system, but at the same time it is clear that they represent necessary, not sufficient, conditions for life. In particular, the absence of liquid water on the Martian surface excludes the possibility of terrestrial types of organisms on Mars. All known terrestrial species have high and, within narrow limits, apparently irreducible requirements for water. On this ground alone it has been evident for some time that Martian life could not be built on a terrestrial model [*Horowitz et al.*, 1972a; *Horowitz*, 1976a, b].

The carbon assimilation experiment (also known as the pyrolytic release experiment) is designed to perform a biological test on Mars under Martian conditions of temperature, pressure, water activity, and atmospheric composition. Although these conditions depart from our geocentric sense of what constitutes a proper biological environment, it is obvious that Martian life, if any, is adapted to Martian conditions, not terrestrial ones. By preserving these conditions as far as possible we maximize the chance of survival of indigenous life and make reasonably sure that whatever events we may detect are not artifactual. In actual practice, our experiment has operated on Mars under a reasonable approximation of local conditions except for temperature, which, owing to heat sources within the spacecraft, has been consistently higher than the ground temperature.

Preliminary accounts of the data returned from Mars have been published [*Klein et al.*, 1976; *Horowitz et al.*, 1976]. In this paper we summarize all of the data, including the results of two new experiments on Mars, and we conclude that they are unlikely to have a biological explanation.

EXPERIMENT DESCRIPTION

The carbon assimilation experiment is designed to detect the synthesis of organic matter in Martian surface material from atmospheric CO or CO_2 or both. The experiment assumes that Martian life would be based on carbon and that this carbon would necessarily cycle through the atmosphere. The rationale for these assumptions has been presented [*Horowitz, 1976a*]. Descriptions of the experiment, including the results of tests carried out on a variety of terrestrial soils, have also been published [*Hubbard et al.*, 1970; *Horowitz et al.*, 1972b; *Hubbard*, 1976].

In brief, 0.25 cm^3 of Martian surface material ('soil') is enclosed in one of the 4-cm^3 test chambers of the instrument under Martian atmosphere at the ambient pressure. The chamber is closed by a window that transmits wavelengths longer than 320 nm originating in a 6-W high-pressure xenon lamp mounted above the chamber. The radiant energy entering the chamber, integrated between 335 and 1000 nm, is about 8 mW cm^{-2}, or approximately 0.2 times the energy in the same wavelength interval at the Martian subsolar point. Although solar wavelengths as short as 200 nm reach the surface of Mars, we have excluded wavelengths shorter than 320 nm from the test chamber in order to prevent a nonbiological, photocatalyzed synthesis of organic compounds from CO and adsorbed water vapor that we find to occur on silicate and other mineral surfaces irradiated with ultraviolet shorter than 300 nm [*Hubbard et al.*, 1971, 1973, 1975]. Since these short wavelengths are generally destructive to organic matter except under the special conditions of the synthesis, we assume that Martian life, if it exists, must also avoid this spectral region; its deletion from the radiation entering the test chamber therefore does not constitute a significant change in the biological environment. The use of the lamp during any experiment is optional and is commandable from earth. Also commandable is the injection of approximately 80 μg of water vapor (or integral multiples thereof) into the test chamber at the start of an experiment.

The atmosphere in the test chamber is labeled by the injection of 20 μl of a mixture of $^{14}CO_2$ and ^{14}CO (92:8 by volume, total radioactivity 22 μCi) at the start of an experiment. The resulting pressure increase is 2.2 mbar over ambient, which was 7.6 mbar initially at both landing sites. The Martian atmosphere contains about 95% CO_2 and 0.1% CO. The injection of radioactive gases increases the partial pressures of CO_2 and CO by 28% and 23-fold, respectively.

The sample is incubated for 120 hours at temperatures which have ranged from 8°C to 26°C in the experiments

conducted to date. The lamp is then turned off, and the chamber is brought to 120°C while the radioactive atmosphere is vented. The chamber is next heated to 635°C to pyrolyze organic matter in the sample. The volatile products, together with a large amount of $^{14}CO_2$ and ^{14}CO desorbed from the soil grains and walls of the chamber, are swept by a stream of He into a column packed with a mixture of 25% cupric oxide and 75% Chromosorb-P (a form of diatomaceous earth). The column, which operates at 120°C, retains organic molecules larger than methane but allows all but a small fraction of the CO_2 and CO to pass into a radiation counter, where their radioactivity is measured. This count is referred to as peak 1.

The column temperature is then raised to 640°C, the high temperature causing the release of organic compounds and their oxidation to CO_2 by the CuO in the column. The radioactivity of this gas is peak 2; it represents organic matter synthesized from ^{14}CO or $^{14}CO_2$ during the incubation. Peak 2 also contains the small fraction of CO and CO_2 which failed to elute with peak 1, presumably because of the presence of some high-affinity sites in the column. The radioactivity of this fraction, referred to as peak 2(0), must be subtracted from peak 2 in order to estimate the amount of C fixed in organic matter. The size of peak 2(0) is known from laboratory tests carried out with heat-sterilized soils or with no soils in the test chamber by using flight-configured columns. These measurements show that for values of peak 1 less than 7×10^5 dpm (disintegrations per minute), peak 2(0) is only weakly dependent on peak 1, i.e., that it is almost constant. It can be estimated from the regression line peak $2(0) = 28.8 + 2.84 \times 10^{-5}$ peak 1, peak heights being expressed in disintegrations per minute. The standard deviation of peak 2(0) is 27 dpm. The regression line is plotted in Figure 1.
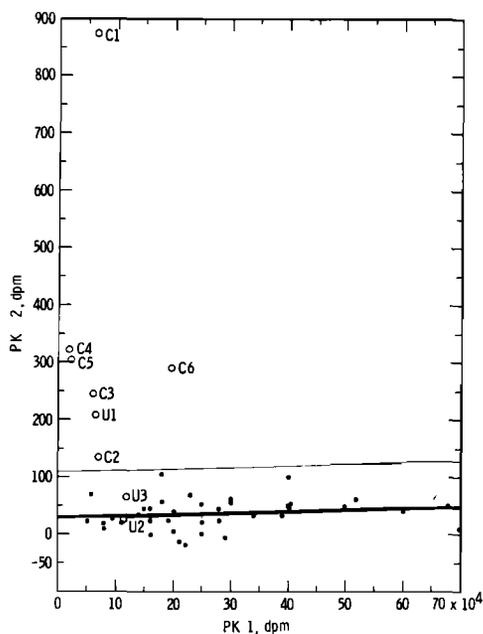

Fig. 1. ^{14}C fixation results from Mars (open circles) compared with laboratory data obtained with heat-sterilized soils or no soils (solid circles) The laboratory data are fitted with the regression line peak $2(0) = 28.8 + 2.84 \times 10^{-5}$ peak 1. The light line marks the 3σ level. The high point at peak $1 = 41 \times 10^4$ dpm was not used in computing the regression line. C is Chryse; U, Utopia; and dpm, disintegrations per minute.

RESULTS

Ten experiments were performed on Mars, six at the Chryse site (numbered chronologically C1–C6) and four at the Utopia site (U1–U4). Of these, U4 is not usable owing to an apparent valve failure which allowed peak 2 to escape before it could be counted. Attempts to correct or circumvent the failure proved ineffectual. A summary and a statistical analysis of all usable results are shown in Figure 1 and Table 1. Descriptions of all experiments but C5 and C6 have been given in previous reports and will not be repeated here.

Experiment C5. C5 was performed with a surface sample that had been acquired before the solar conjunction and stored in the hopper of the soil distribution assembly for 69 sols before C5 was initiated (a sol is a Martian day, equal to 24.65 hours). Since the three test chambers of the Viking Lander 1 (VL 1) instrument had already been used, it was necessary to run the experiment in the 'soil-on-soil' mode, as was also the case with C4. The purpose of C5 was to determine the effect of water vapor injection, followed by evaporation of the water at an elevated temperature, on the soil activity. Since the test chamber now contained two soil aliquots, two injections of water vapor were made. After 4 hours the chamber was vented, and the temperature was brought to 120°C for 1 or 2 min before it dropped to about 90°C, where it was held for approximately 112 min. The temperature was then lowered to 17° ± 1°C for the rest of the experiment. Three 20-μl injections of radioactive gas were made to compensate in part for the reduced pressure in the VL 1 gas bottle, which, after 160 sols of use and normal leakage, should by now have dropped to about 10% of its initial value. The remainder of the experiment proceeded normally.

The results of C5 are hardly distinguishable from those of C4. The unusually low first peaks of both experiments are explained by the depletion of radioactive gas mentioned above. The second peaks of C4 and C5 are statistically identical, an important result in view of the different thermal histories of the two samples. This finding will be considered further in the discussion section below.

Experiment C6. This experiment tested the effect of water vapor injection without heating or evaporation. It repeated U2, the result of which had suggested that water vapor inhibits the fixation reaction [*Horowitz et al.,* 1976]. It differed from U2 and C5 in that the order of addition of water vapor and radioactive gases was reversed, the latter being added first in C6 in consideration of their low pressure in the gas bottle. The change is probably not significant. The surface sample used in C6 was an aliquot of the same material that had been used in C4 and C5 (see Table 1). This material had by now been stored in the soil hopper for 139 sols at temperatures from 5°C to 24°C. Two injections of water vapor and six injections of radioactive gas were made; incubation was performed with the lamp on.

The high value of peak 1 (Figure 1 and Table 1) confirms that water vapor injection occurred. (In C5 the water was evaporated before the addition of radioactive gas, and a high first peak was not obtained or expected.) Of great interest is peak 2, which is seen not to differ significantly from peak 2 of C4 or of C5. We have to conclude, despite earlier indications to the contrary, that water vapor does not inhibit the fixation reaction. It should be noted here that two anomalous data segments, identifiable by their non-Poisson statistics, were edited from the C6 data. Similar anomalies had been encountered in the C2 data [*Klein et al.,* 1976]. Despite these episodes we do not doubt the validity of the results.

TABLE 1. Carbon Fixation Statistics

Experiment	Sample	Conditions	Incubation Temperature, °C	Disintegrations per Minute ± Standard Error				
				Peak 1	Peak 2	Peak 2(0)	Peak 2 − Peak 2(0)	P
C1	Sandy Flats 1, fresh	light, dry	17 ± 1	67,464 ± 536	873 ± 10	30.7 ± 27	842 ± 29	~0
C2	Sandy Flats 1, stored 19 sols	light, dry, 175°C heat treatment	15 ± 1	69,536 ± 545	136 ± 12	30.8 ± 27	105 ± 29.5	2×10^{-4}
C3	Sandy Flats 2, fresh	light, dry	13–26	61,027 ± 527	245 ± 8.9	30.5 ± 27	214.5 ± 28	10^{-14}
C4	Sandy Flats 3, fresh	light, dry	16 ± 2	18,545 ± 381	318 ± 15	29.3 ± 27	289 ± 31	6×10^{-21}
C5	Sandy Flats 3, stored 69 sols	light, H_2O, 90°C heat treatment	17 ± 1	20,295 ± 395	304 ± 11	29.4 ± 27	275 ± 29	10^{-21}
C6	Sandy Flats 3, stored 139 sols	light, H_2O	15 ± 2.5	193,803 ± 864	289 ± 15	34.3 ± 27	255 ± 31	10^{-16}
U1	Beta 1, fresh	dark, dry	15 ± 3	64,845 ± 527	209 ± 15	30.6 ± 27	178 ± 31	5×10^{-9}
U2	Beta 2, fresh	light, H_2O	18 ± 1.5	113,845 ± 690	25 ± 8.4	32.0 ± 27	−7 ± 28	0.5
U3	Under Notch Rock, fresh	dark, dry	10 ± 2	118,309 ± 400	68 ± 23	32.2 ± 27	36 ± 35	0.15

Samples are identified by sampling location, acquisition number, and age at the start of the experiment. Conditions indicate whether the lamp was on or off, whether water vapor was injected, and whether the sample was heated before starting incubation. P is the conventional one-tailed probability that a positive deviation from peak 2(0) as large as or larger than that found would be obtained by chance.

DISCUSSION

The data show that a fixation of atmospheric carbon occurs in the surface material of Mars under conditions approximating the Martian ones. The highest activity was seen in experiment C1, where approximately 10 pmol of CO (or 30 pmol of CO_2, which has a lower specific activity in the gas space) was fixed. This activity is quite small by terrestrial standards, but it is significant. Indeed, when it is recognized that the only identified nonbiological organic synthesis, the surface-catalyzed photoreaction referred to earlier, had been eliminated in designing the experiment, the results are startling. Nevertheless, a biological interpretation of the results is unlikely in view of the thermostability of the reaction. Thus the second peaks of experiments C4, C5, and C6 are not significantly different from one another, although the thermal histories of the samples are very different. The three samples were aliquots from the same scoopful of surface material (Table 1). In C4 the material was tested fresh from the surface. In C5 it had been stored for 69 sols at temperatures between 10°C and 24°C before the experiment was initiated, and it was heated for nearly 2 hours at 90°C before incubation began. In C6 it had been in storage for 139 sols at temperatures from 5°C to 24°C before the start of the experiment. Given these histories and the observation that the temperature of the ground, the habitat of any presumed organisms, does not reach 0°C at any time of the year at either landing site [Kieffer, 1976], it is difficult to reconcile the constancy of the responses with a biological origin.

Further evidence is found in experiment C2, in which a sample from the same dig that had supplied the C1 sample was heated at 175°C for 3 hours before starting the incubation. The activity was reduced considerably but not to the level of sterile soils; the peak 2 count is 3.5σ above the mean of such soils. It appears that the agent responsible for the reaction is somewhat heat labile but not as labile as we expect living organisms to be.

In an earlier report we drew the inference from the results of experiments U2 and U3 that the fixation reaction is inhibited by traces of water [Horowitz et al., 1976]. Unless the two landing sites are fundamentally different in their surface chemistries (an assumption that is opposed by all the available

evidence), we are forced by the outcome of the C6 experiment to conclude that the results of U2 and U3 are related not to the water content of the samples but more probably to sample heterogeneity. That significant heterogeneity exists can be seen in experiments C1, C3, and C4. These experiments were run in the same way with different samples collected at the Sandy Flats site. The peak 2 counts all differ significantly from one another, and they vary over a 3.5-fold range. These results are in striking contrast to the uniformity seen in C4, C5, and C6, the samples for which were all aliquots from the same dig.

The Utopia samples are known to be heterogeneous with respect to at least one relevant characteristic: their irradiation histories. Sample U3 was obtained from under Notch Rock and presumably had not been exposed to solar radiation in recent times. If the plausible assumption is made that only recently irradiated surface material can fix atmospheric carbon, then the difference between experiments U1 and U2 could reflect variations in the quantity of such material transferred to the incubation chambers; a similar explanation is possible for the other differences noted above.

If organic matter is being synthesized on Mars, it does not accumulate above the sensitivity threshold of the GCMS (gas chromatograph mass spectrometer), the Viking organic analysis instrument [Biemann et al., 1976]. The amount of carbon fixed even in experiment C1 is well below the detection limit of this sensitive instrument. A low-level, steady state synthesis like that described by Hubbard et al. [1971, 1973, 1975] would be compatible with the observations, providing it could operate under the conditions described in this paper. We are investigating the possibility that organic compounds synthesized by the Hubbard reaction on Mars analog surface materials can exchange with ^{14}CO or $^{14}CO_2$ under the conditions of the carbon assimilation experiment [Hobby, 1977].

Other mechanisms that have been suggested to explain the results described here include carry-over of particulate matter from the incubation chamber to the column [Huguenin, 1977], incorporation of ^{14}CO into carbon suboxide polymer preformed on the Martian surface [Oyama, 1977], and reduction of ^{14}CO by H_2O_2 in the surface material [Hobby, 1977]. It remains to be seen whether any of the proposed mechanisms can account for the intriguing observations.

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