Measurement of $^{14}$CO$_2$ Assimilation in Soils: an Experiment for the Biological Exploration of Mars

JERRY S. HUBBARD, GEORGE L. HOBBY, NORMAN H. HOROWITZ, PAUL J. GEIGER, AND FRANK A. MORELLI

Bioscience Section, Jet Propulsion Laboratory, California Institute of Technology, Pasadena, California 91103

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A method is described for the measurement of $^{14}$CO$_2$ assimilation by microorganisms in soils. A determination involves exposing soil to $^{14}$CO$_2$, pyrolyzing the exposed soil, trapping the organic pyrolysis products on a column of firebrick coated with CuO, combusting the trapped organics by heating, and measuring the radioactivity in the CO$_2$ produced in the combustion. The detection of significant levels of $^{14}$C in the trapped organic fraction appears to be an unambiguous indication of biological activity. The $^{14}$CO$_2$ which is adsorbed or exchanged into soils by nonbiological processes does not interfere. The method easily detects the $^{14}$CO$_2$ fixed by $10^8$ to $10^9$ algae after light exposure for 3 to 24 hr. Assimilation of $^{14}$C is also demonstrable in dark-exposed soils containing $10^8$ to $10^9$ heterotrophic bacteria. Possible applications of the method in the biological exploration of Mars are discussed.

A number of experiments have been proposed for the detection of microbial life on Mars (6–8, 10). In general, these methods involve the measurement of growth or metabolic activities in samples of Martian soil incubated in aqueous nutrient media. Obviously, these conditions are geocentrically oriented and may not be optimal for Martian species. For example, the low partial pressure of water vapor in the Martian atmosphere makes the existence of appreciable quantities of liquid water on the surface seem unlikely. Thus, there is reason to question how organisms in the soil of that planet would respond to liquid water. If life has arisen and evolved on Mars, its biochemical and functional adaptations should be uniquely Martian. Among these specialized adaptations should be its manner of utilizing water from the arid environment.

An additional problem is the preselection of the nutrients for the media. At present, components can be selected only on assumptions derived from experience on Earth. Conceivably, such components could be toxic or not metabolized by Martian species.

This paper describes a test for Martian microorganisms based on the assimilation of $^{14}$CO$_2$ into organic compounds. It assumes that Martian life, if any, is carbon-based and that this carbon cycles through the atmosphere (3). The test was originally designed to detect photosynthesis, but it also effectively measures dark fixation of CO$_2$. The only reactants employed in the basic experiment are CO$_2$ and solar radiation, both of which are present on Mars. The test can be performed under Martian conditions of temperature, pressure, and moisture. The only alteration of ambient conditions is the addition of a small quantity of $^{14}$CO$_2$.

MATERIALS AND METHODS

$^{14}$CO$_2$ exposure. Approximately 300-mg samples of soils were spread on sterile stainless-steel planchets and moistened with 0.3 ml of sterile distilled water. Four planchets were placed inside a Pyrex chamber having a capacity of approximately 40 ml (Fig. 1). The chamber was evacuated to about 10 mm of Hg and then sealed. A 3.5-ml side-arm vessel containing 0.1 ml of an aqueous solution of NaH$^{14}$CO$_3$ (20 $\mu$g in 0.77 $\mu$moles) was then attached to the chamber. The $^{14}$CO$_2$ was generated by adding 0.2 ml of 2 N perchloric acid through the stopcock on the side-arm vessel. The $^{14}$CO$_2$ was introduced into the evacuated chamber by opening the chamber stopcock allowing the $^{14}$CO$_2$ to equilibrate between the side-arm vessel and exposure chamber and then venting the side-arm vessel stopcock to sweep the $^{14}$CO$_2$ into the exposure chamber. The chamber was then sealed and the side-arm vessel was removed. Thus, the chamber has a pressure of 1 atm of air which is enriched with 0.04% excess CO$_2$. In anaerobic experiments, the chambers and side-arm vessels were thoroughly flushed with argon
the $^{14}\text{CO}_2$ was swept into the evacuated chamber with a stream of argon. For light exposure, the chambers were placed under fluorescent lights at approximately 600 ft-c light intensity. For dark-exposed samples, the chambers were wrapped with opaque cloth before the introduction of $^{14}\text{CO}_2$. After exposure, the samples were gently dried under a heat lamp and saved in a freezer. Unless otherwise indicated, sterile controls were prepared by dry-heat sterilization of soils overnight at 175°C and exposure as described above.

Pyrolysis-gas chromatography (GC) system. Figure 2 illustrates the laboratory apparatus used for testing $^{14}\text{CO}_2$ fixation. The pyrolysis furnace is constructed of stainless-steel tubing [0.25 by 4 inches (0.64 by 10.16 cm)] which is heated by its own electrical resistance. The GC column is made of stainless-steel tubing [3/4 by 20 inches (0.32 by 50.8 cm)] which is packed with 80 to 10 mesh firebrick (Sil-o-cel) coated with CuO (31%, w/w). The column was coated by making a slurry of the firebrick and water saturated with Cu(NO$_3$)$_2$, pumping off the excess water, and then decomposing the Cu(NO$_3$)$_2$ to CuO by heating at 500°C for 48 hr. The GC column is also heated by electrical resistance. The oxidizer column is made of stainless-steel tubing [0.25 by 8 inches (0.64 by 20.32 cm)] packed with granular CuO. A furnace was used to heat the CuO column to 700°C throughout the test. The thermal conductivity detector (Carle Thermistor Detector) permits the measurement of the effluent gases from the apparatus. The CO$_2$ traps are bubblers containing 1 ml of hyamine hydroxide (1 M in methanol). Interchangeable traps were used in order to measure the $^{14}\text{CO}_2$ at different stages of the procedure.

Samples of exposed soils were wrapped in aluminum foil and placed inside the pyrolysis furnace. Before pyrolysis, the GC column was warmed to 120°C and the flow rate of the He carrier gas was adjusted to 18 ml/min. Pyrolysis was performed at 600°C for 1 min. In some experiments, CO$_2$ gas was bled into the carrier gas (one part of CO$_2$ to nine parts of He) 5 min before and during the 1-min pyrolysis. The trapped organics were driven from the GC column by heating to 700°C. When the GC column was heated, the He flow rate was reduced to 7 ml/min. The heated column was next flushed with O$_2$ at 15 ml/min. In some experiments, the pyrolyzer was then heated to 600°C with the O$_2$ as the carrier gas.

Radioactive measurements. The $^{14}\text{CO}_2$ trapped in the bubblers was measured in a liquid scintillation counter (Beckman LS-100) by using 0.2 ml of the hyamine hydroxide solution and 0.8 ml of methanol in 10 ml of toluene containing 0.5% 2,5-diphenyloxazolene (POO). The counting efficiency was 57%. Samples low in radioactivity were counted for 100 min to attain a 2-a error of less than 7%. The soil residue was counted as a suspended solid in 10 ml of dioxane containing 10% naphthalene, 0.5% POO, and 0.5% thixotropic gel powder (Cab-o-sil). All data are corrected for background radioactivity which was approximately 14 counts/min.

Soils. JPL-mixed soil is a sandy clay loam which was collected from the surface 1 to 3 cm at nine sites at the Jet Propulsion Laboratory. The pooled samples were mixed and then passed through a 2-mm sieve. The soil materials ≤ 2 mm were spread and allowed to dry at room temperature before packaging in sample sacks. The soil was then stored at room temperature for about 2.5 years.

The farm soil is an arable, fertile, crumbly, brown soil which was collected near the Santa Anita Race-track, Arcadia, Calif. This soil was also sieved and dried as described above and then stored at room temperature for about 2 years.

The algal crust soil is a thick, compacted, silty soil crust resulting from intertwined blue algal filaments. The 1- to 3-mm surface crust was dried on filter paper at room temperature and then stored at room temperature for 1 to 6 months.

Soils from the Antarctic dry valleys were collected by R. E. Cameron of this Laboratory during an expedition in the austral summer of 1967 to 68 (2). These soils were sieved (≤ 2 mm) and stored aseptically at -25°C until exposure to $^{14}\text{CO}_2$ or inoculation into culture media (1). These cold desert soils are sandy and saline with low levels of organic matter.

Microbiological analyses. The numbers of aerobic, heterotrophic bacteria in JPL-mixed, farm, and algal crust soils were determined by the spread plate technique on Trypticase soy agar. Dilutions were made in distilled water. Plates were incubated at 23°C for 21 days. The number of algae in the algal crust soils could not be accurately determined because the cells were
clumped on the soil particles. It was estimated that the algal soil contained about 10^9 algae cells per g.

The data on the analyses for heterotrophic bacteria and algae in Antarctic desert soils were provided by R. E. Cameron (unpublished data). The values given are the highest counts obtained in any of the growth conditions tested. The media and methods employed have been described (1, 2, 4).

All algal and bacterial counts are calculated for the quantity of soil used for pyrolysis. The counts were made before exposure to ^14CO2, and do not consider any population changes which may have occurred during the exposure period.

RESULTS

Soil pyrolysis. Recorder tracings of the thermal conductivity detector in an experiment with untreated farm soil are shown in Fig. 3. The peak recorded 0.5 min after heating the pyrolyzer represents the volatile fraction which is not retained on the fire-brick-CuO column. The long shoulder on the peak resulted from the water driven from the soil and that produced in the thermal degradation of soil organics. This shoulder is eliminated when the apparatus contains an anhydrous Mg(ClO4)2 trap between the CuO furnace and the thermal conductivity detector. With the water removed, the peak area was equivalent to that given with 10.6 /umoles of CO2.

In other experiments, the materials passing through the firebrick column were trapped on activated charcoal at -195 C. Mass spectrometric analysis showed H2, CO, and CO2 in an approximate molar ratio of 1:1:2. Conceivably, other volatile products present in trace quantities may have been adsorbed to an anhydrous Cs2O4 trap which was used to remove water.

Elution of the trapped organics was observed immediately after heating the firebrick-CuO column to 700 C (Fig. 3). The water in this peak was derived from the CuO combustion of the trapped organics. With the water removed, the peak area was equivalent to that given with 19.1 /umoles of CO2. For a mass spectral analysis of the trapped organic fraction, 80- to 100-mesh firebrick (without CuO) was used as the GC column, and the effluent from the heated column was trapped at -195 C. This fraction contained a complex mixture of organics. About 70 components were detected in the mass/charge range of 10 to 128.

Passing O2 over the heated column should facilitate the combustion of organics which are not combusted by the CuO coating on the firebrick. The shift in recorder base line was caused by the change in carrier gas through the thermal conductivity detector (Fig. 3). The small peak observed in the O2 combustion actually represents an appreciable amount of CO2, since the sensitivity of the thermal conductivity detector to CO2 is low when O2 is used as carrier gas.

Table 1 shows the distribution of radioactivity after pyrolysis and combustion of soils impregnated with ^14C-labeled algae. With the JPL-mixed soil, the pyrolysis CO2 fraction accounted for 25% of the counts. Only a small number of counts leaked from the column in the postpyrolysis flush, indicating that the organic fraction is firmly adsorbed to the firebrick-CuO column. Heating the column released the trapped organics which comprised 24% of the total ^14C. Only 27 counts/min was eluted when the heated column was flushed with O2. This indicates that heating the column in the helium atmosphere removes almost all of the combustible materials in the trapped organic fraction. The O2 combustion of the pyrolysis residue gave the largest yield of ^14C, i.e., 47% of the total. Another 2% of the ^14C was detected in the soil residue after pyrolysis and combustion.

Table 1. Distribution of radioactivity in pyrolysis fractions

| Fraction          | JPL-mixed soil | Nitrate soil no. 285 |
|-------------------|----------------|----------------------|
|                   | counts/min     | counts/min           |
| Pyrolysis CO2     | 2,242          | 5,840                |
| Postpyrolysis flush| 11             | 54                   |
| Trapped organics  | 2,103          | 586                  |
| O2 flush of heated column | 27       | 9                    |
| O2 combustion     | 4,082          | 75                   |
| Soil residue      | 200            | 6                    |

* Corresponds to the fractions indicated in Fig. 3.

Before pyrolysis, suspensions of ^14C-grown algae were added to sterilized JPL-mixed and no. 285 soils and then gently dried under a heat lamp.
A soil from the Chile Atacama Desert (no. 285) was used to assess the effect that a high level of oxidizing agent has on the pyrolysis. This soil contains 13% nitrate (as $^\text{-}\text{NO}_3$). As seen in Table 1, the pyrolysis was converted to a partial combustion. Compared to JPL-mixed soil, in soil 285 the proportion of $^{14}\text{C}$ in the pyrolysate CO$_2$ increased, whereas the $^{13}\text{C}$ in the trapped organics O$_2$ combustion, and soil residue decreased. Nonetheless, even in this poor example, a significant yield of $^{14}\text{C}$ was detected in the trapped organic fraction.

**CO$_2$ fixation in soils.** When either sterilized or untreated soils were exposed to $^{14}\text{CO}_2$ and then pyrolyzed, appreciable amounts of $^{14}\text{C}$ were detected in the pyrolysate CO$_2$ fraction (Table 2). In sterile soils, this represents the release of $^{14}\text{CO}_2$ which is adsorbed to soil or exchanged into soil carbonates. Thus, the detection of radioactivity in this fraction is not necessarily an indication of biological activity. However, a significant level of $^{14}\text{C}$ in the trapped organic fraction was found only when the soils contained viable microorganisms (Table 2). This indicates that the $^{14}\text{CO}_2$ has been incorporated into the cellular constituents of the soil organisms. The highest level of activity in the trapped organic fraction was found after light exposure of an algal crust soil. The CO$_2$ fixation was about two orders of magnitude lower when the algal soil was exposed to $^{14}\text{CO}_2$ in the dark. This activity can be attributed to heterotrophic CO$_2$ fixation by algae and bacteria. In addition, CO$_2$ fixation by chemolithotrophs could contribute to the dark activity. Fixation of CO$_2$ could also be demonstrated with soils which contain no visible indication of algae (JPL-mixed and farm soil).

Even though the data of Table 2 suggest that detection of $^{14}\text{C}$ in the O$_2$-combustible fraction is found only in soils containing viable microorganisms, other experiments have shown that $^{14}\text{C}$ in this fraction is not an unambiguous indication of biological activity. Appreciable levels of $^{14}\text{C}$ have been found in the O$_2$-combustible fraction of sterile alkaline soils after exposure to $^{14}\text{CO}_2$ and pyrolysis in the usual manner. The trapped organic fraction of these soils contained insignificant radioactivity.

Table 3 shows that the highest rate of CO$_2$ assimilation occurred when algal soil was moistened before exposure to $^{14}\text{CO}_2$. A significant incorporation of $^{14}\text{C}$ was observed when air-dried soil was exposed to $^{14}\text{CO}_2$ under conditions of high humidity. However, the superiority of liquid water is shown in the analyses of the samples exposed for 3 and 24 hr. Essentially no $^{14}\text{C}$ was detected in the organic fraction of air-dried soils which were exposed to $^{14}\text{CO}_2$ without the addition
of water vapor or liquid. These data illustrate the problems encountered in testing a life detection experiment which will operate under the arid conditions of the Martian environment. Terrestrial soils must be used to demonstrate that the method will distinguish between biological and nonbiological uptake of $^{14}$CO$_2$. Biological activity with terrestrial organisms can only be demonstrated when water is present. Nonetheless, the data do show that the method does not give a false-positive result when soils are exposed to $^{14}$CO$_2$ with various levels of water.

CO$_2$ fixation in Antarctic soils containing small numbers of microorganisms is shown in Table 4. Biological activity was demonstrable in all samples inasmuch as the radioactivity in the trapped organic fraction from exposed soils containing viable organisms was higher than that of the sterilized, exposed controls. However, our arbitrary criteria for an active sample (see below) require that the radioactivity in the trapped organic fraction be greater than five times background, i.e., > 56 counts/min (net). By this definition, only five of the soils were active. The two most active (615 and 643) had substantial populations of algae. A demonstration that the CO$_2$ fixation in soils 615 and 643 is primarily photosynthetic is seen in the reduced level of activity when these soils were exposed to $^{14}$CO$_2$ in the dark. Soil 613 behaved similarly, although the algal count was low. Apparently, our culture methods were inadequate for detecting the principal photosynthetic species in soil 613. The dark CO$_2$ fixation by soils 609 and 641 was about half that observed when these soils were exposed under illumination.

**Nonbiological sources of interference.** The selection of a sterilization procedure for soil controls is a formidable problem. Ideally, the treatment should selectively destroy the soil microorganisms without otherwise changing the soil properties. However, any procedure which is harsh enough to completely inactivate the metabolic apparatus of the microorganisms will undoubtedly alter the organic and inorganic constituents of the soil.

Table 5 shows tests with farm soil sterilized by three different means. Even though the extent of sterilization was not measured by growth tests, the $^{14}$C-assimilation data show that the soils would be classed as biologically inactive (see below). Comparable amounts of $^{14}$CO$_2$ were absorbed by the dry heat-sterilized and autoclaved samples. The pyrolysis CO$_2$ fraction from the soil treated with ethylene oxide contained considerably more $^{14}$C than the corresponding fractions.

### Table 4. CO$_2$ assimilation by surface soils from the dry valleys of Antarctica

| Soil | Viable microorganisms | $^{14}$CO$_2$ exposure$^a$ | Pyrolysis CO$_2$ | Trapped organics |
|------|-----------------------|--------------------------|----------------|-----------------|
|      | Algae                 | Bacteria                 | counts/min     | counts/min     |
| 609  | 30                    | 300                      | Light$^c$      | 8,354          | 3               |
|      |                       |                          | Light          | 7,023          | 165             |
|      |                       |                          | Dark           | 16,832         | 78              |
| 613  | 9                     | 300                      | Light$^c$      | 10,853         | 7               |
|      |                       |                          | Light          | 14,600         | 773             |
|      |                       |                          | Dark           | 37,841         | 83              |
| 615  | 96,000                | 1,800                    | Light$^c$      | 11,999         | 9               |
|      |                       |                          | Light          | 11,818         | 1,643           |
|      |                       |                          | Dark           | 27,206         | 122             |
| 619  | 1                     | 150                      | Light$^c$      | 4,355          | 8               |
|      |                       |                          | Light          | 4,729          | 43              |
| 641  | 0                     | 750                      | Light$^c$      | 29,391         | 11              |
|      |                       |                          | Light          | 20,206         | 93              |
|      |                       |                          | Dark           | 36,918         | 51              |
| 643  | 17,000                | 75                       | Light$^c$      | 11,272         | 13              |
|      |                       |                          | Light          | 23,072         | 7,212           |
|      |                       |                          | Dark           | 17,016         | 175             |
| 638  | 2                     | 90                       | Light$^c$      | 3,552          | 5               |
|      |                       |                          | Light          | 2,348          | 29              |

$^a$ Soils were exposed to $^{14}$CO$_2$ for 22 hr at room temperature; 15-mg samples were used for pyrolysis.

$^b$ Carrier $^{14}$CO$_2$ was added during pyrolysis.

$^c$ Sterilized before exposure.

### Table 5. Retention of $^{14}$CO$_2$ in farm soil sterilized by various means

| Sterilization$^a$ | Pyrolysis CO$_2$ | Trapped organics |
|-------------------|-----------------|-----------------|
|                   | Relative TC re- | Relative TC re- |
|                   | sponse$^b$      | sponse$^b$      |
| None              | 1.0             | 1.0             |
| Dry heat (175 C, 18 hr) | 0.83 | 14,409 | 0.82 | 7 |
| Autoclaved (21 lb, 125 C, 30 min) | 0.88 | 16,293 | 0.96 | 13 |
| Ethylene oxide (12%, 72 hr) | 1.05 | 60,742 | 1.09 | 65 |

$^a$ Soils were sterilized as indicated, moistened, and then exposed to $^{14}$CO$_2$ for 24 hr under illumination. Exposed soils were dried, and 15-mg samples were pyrolyzed.

$^b$ The response on the thermal conductivity detector (TC) was estimated by measuring peak areas. The peak areas obtained after pyrolysis of 15 mg of nonsterilized, nonexposed soil were set = 1.0.
from the dry heat-sterilized, autoclaved, or non-sterilized soil (Table 2).

An estimation of the gross alteration of the sterilized soils was made by quantitating the size of the pyrolysis CO$_2$ and trapped organic fractions (Table 5). Dry-heat sterilization was the most destructive, resulting in losses of 17 and 18% of the pyrolysis CO$_2$ and trapped organic fractions, respectively. Smaller losses were noted with the autoclaved sample. Slight increases in the thermal conductivity response were seen when the ethylene oxide-treated sample was tested. Presumably, this increase resulted from the alkylation of the soil organic matter.

We recognize that none of these sterilization procedures provides a perfect control for the experiment. Dry heat was used in the remainder of this study, since this will be the only method available on the flight instrument. Hopefully, any difficulties associated with the dry-heat sterilization of Martian soil will be resolved in the tests with terrestrial soils.

Alkaline soils absorb large amounts of 14C$_2$O which is released during pyrolysis. A false-positive result could be indicated if a small fraction of this 14C$_2$O was retained on the firebrick-CuO during pyrolysis and released when the column is heated. Sterilized Chile-Atacama soil 264 absorbed large quantities of 14CO$_2$ (Table 6). However, the 14C in the trapped organic fraction was only 0.02% of the pyrolysis 14CO$_2$. This value increased to 0.08% when algal crust of farm soil (not exposed to 14CO$_2$) was mixed with 14C-soil 264 before pyrolysis. The increase may be caused by exchange of 14C into organic compounds during pyrolysis. Whatever the cause, the effect disappears if 14CO$_2$ is included in the carrier gas during pyrolysis. When carrier CO$_2$ was used, the highest hangup observed was 0.05% (with Antarctic soil 543 plus algal crust).

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**Table 6. Radioactivity trapped on firebrick-CuO column from nonbiological sources**

| Sterilized soil exposed to 14CO$_2$ | 14C-soil added | 14CO$_2$ added during pyrolysis | (A) Pyrolysis CO$_2$ | (B) Trapped organic products | B/A |
|----------------------------------|-----------------|---------------------------------|----------------------|-----------------------------|-----|
| Soil 264 (10 mg)                 | None            | No                              | 97,469               | 16                          | 0.0002 |
| Soil 264 (10 mg)                 | Algal crust (5 mg) | No                              | 89,623               | 72                          | 0.0008 |
| Soil 264 (10 mg)                 | Algal crust (5 mg) | Yes                             | 85,538               | 28                          | 0.0003 |
| Soil 264 (10 mg)                 | Farm soil (10 mg) | No                              | 58,434               | 40                          | 0.0007 |
| Soil 264 (10 mg)                 | Farm soil (10 mg) | Yes                             | 77,673               | 15                          | 0.0002 |
| Soil 543 (10 mg)                 | Algal crust (5 mg) | Yes                             | 141,986              | 78                          | 0.0005 |
| Alkaline JPL soil (15 mg)         | With 1% phenol | None                            | 91,544               | 18                          | 0.0002 |
|                                  | With 10% humic acid | None                          | 31,589               | 3                           | 0.0001 |

* The 14C-soils were mixed with 14C-soils immediately before pyrolysis.

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**Table 7. Effects of ultraviolet irradiation and anaerobic conditions on the abiotic incorporation of 14CO$_2$ into soil organics**

| Sterilized soil | 14CO$_2$ exposure | Pyrolysis CO$_2$ | Trapped organic products |
|-----------------|-------------------|-----------------|--------------------------|
| Algal crust     | Fluorescent, 70 hr |                 | 807                      | -1                        |
| Farm            |                   |                 | 10,656                   | 11                        |
| Algal crust     | Fluorescent plus ultraviolet, 114 hr | | 3,555                   | 6                         |
| Farm            |                   |                 | 25,008                   | 17                        |

* After dry-heat sterilization, soils were equilibrated with water vapor for 1 hr and then placed in exposure chamber.

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We have also investigated the possibility of interference by the Kolbe reaction (9), in which CO$_2$ carboxylates the metal salts of phenols; thus, sodium phenoxide reacts with CO$_2$ at elevated temperatures and pressures to give sodium salicylate. The JPL soil made alkaline by the addition of NaOH was mixed with phenol or with a humic acid rich in phenoxides and was incubated under 14CO$_2$ in the usual way. The hangup after pyrolysis was low (Table 6). This is not surprising, since carboxyl groups are expected to decompose to CO$_2$ during the pyrolysis step.

Additional tests for the abiotic incorporation of 14CO$_2$ into soil organic matter are shown in Table 7. Insignificant levels of 14C were detected in the organic fractions of soils exposed to 14CO$_2$ under
anaerobic conditions with fluorescent or fluorescent and ultraviolet illumination. These are probably the most appropriate controls for the Mars version of the experiment.

**DISCUSSION**

Based on the data with JPL-mixed, farm, and algal crust soils, it appears that $^{14}C$ assimilation can be detected in soils containing $10^5$ to $10^6$ algae or $10^4$ to $10^5$ heterotrophic bacteria after 3 to 24 hr of exposure to $^{14}CO_2$. This expression of sensitivity might be questioned, since only one medium was used for the counts of heterotrophic bacteria and the number of algae was estimated by microscopic examination. A more convincing demonstration of the sensitivity of the method comes from the detection of biological activity in surface soils from the Antarctic dry valleys. The microbial determinations were more extensive on these soils from the hostile environment. The $^{14}C$-assimilation analyses of some of the Antarctic surface soils would indicate an even greater sensitivity for both algae and bacteria. These surface soils give a greater response per culturable organism than the Antarctic subsurface soils (unpublished data), JPL-mixed soil, or farm soil. It is not clear whether the Antarctic surface soils contain abnormally active species or large populations of organisms which are not detected by our culture methods. Measurements of the formation of $^{14}CO_2$ from $^{14}C$-labeled substrates by these Antarctic soils have given similar results, i.e., more $^{14}CO_2$ is produced per culturable organism in the surface soils than in the subsurface soils (Cameron et al., Bacteriol. Proc., 1969, p. 15).

On the basis of studies thus far, it appears highly unlikely that the $CO_2$ assimilation test can yield a false-positive result, an important consideration for a Mars biological test. The detection of significant radioactivity in the trapped organic fraction has been found only in soils containing organisms which are metabolically active. We define "significant" in this context to mean radioactivity in the trapped organic fraction at least five times background and at least 0.5% of the radioactivity of the pyrolysis $CO_2$ fraction. The latter criterion is especially important for soils which absorb large amounts of $^{14}CO_2$ by nonbiological processes. Otherwise, a carryover of a small fraction of the "inorganic $^{14}C" into the trapped organic fraction could give a false-positive result by the other criterion, i.e., by being five times background. We have performed over 80 analyses on a variety of sterilized soils and have never encountered a situation in which both criteria were simultaneously satisfied.

Even though a sterilized control does not seem to be necessary, it could provide supplementary evidence to the two criteria for validity described above. The background radioactivity in the Mars version of the experiment would be determined by pyrolysis of an unexposed soil sample.

Although the experiment is designed for measuring $CO_2$ fixation, the assimilation of other volatile substrates could also be detected. One such possibility is suggested by the recent demonstration of carbon monoxide in the Martian atmosphere (L. D. Kaplan, personal communication). Conceivably, Martian life-forms could oxidize and assimilate CO by a mechanism similar to that carried out by the anaerobic methane bacteria (5). Preliminary experiments have shown that the method can detect $^{14}CO_2$ assimilation in algal crust soils. Additional information on the trace constituents of the Martian atmosphere may suggest other possibilities.

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