

A Unified Procedure for the Detection of Life on Mars

A mass spectrometer can be used to perform a variety of remote biologically oriented experiments.

Richard Radmer and Bessel Kok

By means of automated spacecrafts the Viking program will explore Mars to conduct scientific investigations from orbit, during entry, and on the surface. The first mission in 1975 will obtain data relevant to the past, present, and future existence of life on Mars (1). As now planned the mission will perform four different experiments with soil samples to detect active biology. These are designed to measure (i) exchange of gases resulting from utilization of terrestrial organic substrates, (ii) increase in the aqueous population of bacteria as an increase in turbidity, (iii) release of $^{14}\text{CO}_2$ from organic compounds labeled with ^{14}C , and (iv) incorporation of $^{14}\text{CO}_2$ and ^{14}CO into organic matter (1, 2).

We suggest here a unified approach for subsequent Viking missions in which a single instrument can be used to make many measurements related to life and the environment. The approach appears to be consistent with the severe technical constraints of the missions, and, because of the wide range of observations possible, the danger that no useful data would be obtained is minimized. The technical and scientific aspects of this approach

are not new; currently available spaceborne equipment would be adequate for the purpose. The biological principles have been well documented, and the use of many of them has been previously proposed for methods to detect life.

Proposed Strategy and Instrumentation

The constraints of remote detection of life are scientific and technical. The fundamental scientific problem is that there is no real understanding of what constitutes living matter. Consequently, it may be difficult to recognize Martian life, stimulate its metabolism, or predict which of many possible stimuli will provoke measurable responses. In addition, the abundance of life on Mars is expected to be low; at best the signals to be detected will be small. These considerations underlie our aim to attempt a graded approach which monitors both general and specific indicators of living matter.

In a general approach, such as an "in situ" observation, one makes few assumptions and imposes few perturbations on the system; such an approach could respond to a wide range of life activities, but its sensitivity is apt to be limited. In a specific approach, such as the search for a certain enzyme activity, one makes specific assumptions and

disturbs the environment, but such an approach might be quite sensitive provided of course that the sought after activity is present. It should be emphasized that all experiments to detect life are quantitative (and therefore ambiguous); they do not yield yes or no answers. Consequently, the cross-correlation of many observations will yield a higher confidence level than that obtained from the sum of the individual measurements.

Equally important are the severe technical limitations imposed on spaceborne equipment with respect to power, weight, and complexity. These limitations make it imperative that each instrument be used to its fullest capacity. Since any type of manipulation will be difficult, the experimental procedure can be greatly simplified if gases are measured, since they diffuse freely. Thus we arrived at a procedure in which a single instrument can detect a variety of metabolic reactions and environmental factors by measuring the appearance or change in concentration of one or more gases. The mass spectrometer, versatile and simple to operate, appears to be uniquely qualified for this task. Flight models are available which have the capability of measuring a wide range of gases and their isotopes with high sensitivity and a dynamic range of $\sim 10^6$. In the proposed procedure a mass spectrometer communicates with a carousel of a dozen ampoules (~ 1 milliliter each) which are filled with Martian soil samples and incubated as described below. The gas phase contained in these ampoules is repeatedly and nondestructively analyzed during the prolonged period (months) of incubation on Mars to detect biologically induced changes in concentration.

Detection of Metabolism

Of all the recognized manifestations of living systems (reproduction, growth, and so forth), metabolism (3) appears to be the most promising attribute on which to base life-detection experiments (4). We might expect that on Mars metabolism is based on carbon

Dr. Radmer is a research scientist at the Research Institute for Advanced Studies (RIAS), a division of Martin Marietta Corporation, 1450 South Rolling Road, Baltimore, Maryland 21227. Dr. Kok is associate director and head of the biosciences department at RIAS.

and uses an aqueous transport and solvent system (5). However, departures from present-day terrestrial biochemistry may be significant. Therefore, single, specific experiments (for example, the detection of adenosine triphosphate) might result in false negative conclusions.

Clearly, the next best thing to photographing obvious manifestations of life is the observation of complex endergonic biochemical conversions such as growth, photosynthetic evolution of O_2 , or CO_2 fixation. However, such processes require biological energy-coupling systems, and therefore a high degree of cellular or at least structural integrity. It will probably be quite difficult to provide Martian organisms with an adequate environment and substrate for such activities.

Isoenergetic processes (such as the hydrogen isotope scrambling reaction $H_2 + D_2 \rightarrow DH$) and exergonic reactions (such as the oxidation of organic compounds) require only catalysis to occur at appreciable rates. These conversions therefore can reflect the activity of isolated enzymes inasmuch as they do not require structural integrity. However, such conversions are also more apt to occur spontaneously and might be catalyzed by nonliving soil components, which would introduce ambiguity. One could consider the inclusion of control experiments designed to prevent all metabolism without affecting nonbiological reactions. However, attempts to sterilize a sample (for example, by heat) often result in alterations that prevent it from serving as an adequate control. Alternatively, it might be possible to dispense with control measurements and rely instead on the cross-correlation of many observations to arrive at firm conclusions.

Knowledge of the kinetics of an observed reaction may also aid in its interpretation. For instance, autocatalytic kinetics such as those of the evolution of CO_2 and CH_4 (Fig. 1) or transients (Figs. 1, 2, and 5) would be presumptive evidence for a biological process.

In the following sections we discuss a series of assays ranging from in situ observations, which attempt to detect metabolic activity in an unperturbed system, to complex experiments which attempt to detect specific biochemical reactions. Although the desirability of terrestriocentric biochemical experiments with aqueous solutions is a matter of some debate, inclusion of such experiments does not overly complicate the

Table 1. Rate of O_2 consumption in situ at three different temperatures. The tabulated rates of O_2 consumption were determined during the early phase of the reaction (<50 percent of completion). In this region the reaction was zero order with respect to O_2 . The soil contained 3×10^7 bacteria per gram as determined by plate count. See text for other details.

Temperature (°C)	Rate (atm/hour)
31	0.0060
80	.044
90	.106

experiment, and, if successful, they might be quite informative.

1) *In situ experiments.* Living systems perturb the environment and move a planet's surface and atmosphere away from thermochemical equilibrium. For example, the coexistence of methane and oxygen in the earth's atmosphere is presumptive evidence for the presence of life. Therefore, a detailed atmospheric analysis is by itself a general experiment to detect life (6) and should be a prime goal of the exploration of Mars.

The interface between atmosphere and soil is the most likely site for the metabolic processes which maintain the thermochemical disequilibrium. Consequently, at this interface one expects to find life-induced fluxes of gases. The ideal in situ experiment would not in any way disturb the environment while it monitored the natural life cycle. Without any disturbance the interaction of metabolism in the soil with the gas phase of the environment would be difficult to detect because of the rapid exchange between this gas phase and the large atmospheric buffer. However, if communication with this buffer were somehow removed, metabolic activity could be determined by monitoring the small isolated gas phase enclosed with the soil.

Isolation might be achieved by placing a translucent dome over the planetary surface or by inserting an inlet probe at some depth into the soil. However, for increased sensitivity, even at the price of further deviation from a true in situ observation, this experiment would preferably be done by transferring the Martian soil (plus atmosphere) to a sealed vessel onboard the lander which is thermostated (at $10^\circ \pm 5^\circ C$). Under these conditions one can observe with good sensitivity the sequence of events as the free energy of the system decreases.

The severe fluctuation in daily tem-

perature on Mars causes a variation in the position of chemical equilibrium so that an initial adjustment of this equilibrium could occur in the incubation chambers. To minimize this the samples should be acquired at the warmest part of the Martian day when the temperature of the soil approximates that of the lander and all chemical systems are closest to equilibrium.

As an illustration of an in situ incubation, Fig. 1, A and B, shows the changes in gas phase observed in an enclosed sample of forest soil. Oxygen disappeared with a concomitant evolution of CO_2 , and, as the system became anaerobic, a transient production of NO (or N_2O) occurred. Hydrogen and methane were evolved after 4 and 14 days of incubation, respectively; the onset of CH_4 evolution was accompanied by H_2 consumption, apparently as a result of the conversion

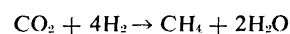


Figure 1B illustrates the drastic changes in gas composition during a 50-day in situ incubation. Although these data are too complex to be analyzed into a set of unique constituents, the overall picture is a striking example of the anaerobic synthesis of many organic compounds. This rich soil sample did not present a very challenging exercise in detecting life; however, the data do illustrate the versatility of this type of measurement.

With poor soil far less dramatic changes are observed, but after sufficiently long incubations we have never failed to measure substantial changes in the composition of the gas phase. In general, the low pressure of the Martian atmosphere will enhance the sensitivity of such an experiment because of the smaller concentrations of "diluting molecules."

A control procedure which avoids the difficulties of sterilization and at the same time yields useful information on disequilibrium of the Martian system of soil and atmosphere is the following. Three parallel samples are incubated and monitored in situ, one at 10° to $30^\circ C$ and the others at 80° and $90^\circ C$ (beyond the range of most biological activity). All samples consume O_2 and evolve CO_2 . However, they do so by different mechanisms; the gas exchange at $30^\circ C$ is mainly due to respiration, whereas that at 80° and $90^\circ C$ is due to the nonbiological oxidation of soil organics. From the rates at the two high temperatures one can calculate a

gross nonbiological activation energy; in the experiment described in Table 1 this value is ~ 22 kilocalories per mole. This indicates that the background rate of nonbiological oxidation at 80°C is ~ 190 times the rate at 30°C and that 96 percent of the O_2 uptake at 30°C is indeed biological. It should be emphasized that since these nonbiological reactions reveal that the earth surface is far from equilibrium, an experiment of this type on Mars would be highly instructive.

In this control procedure it is assumed that an increase of temperature increases the reaction rates without affecting the position of equilibria. Although valid for the case presented above, significant deviations might occur in processes with equilibrium constants close to unity (such as solubility and adsorption). This fact does not invalidate this control procedure, but one should be aware of these phenomena.

2) *Addition of water.* Since water is presumably a rate-limiting factor for any recognizable biota on Mars, its addition might stimulate their metabolism. (It should be noted that the addition of liquid water at 15°C will more than double the total atmospheric pressure above the soil.) Stimulation of growth by wetting is very common in terrestrial soil samples. However, a high concentration of water might lyse Martian organisms which are accustomed to extremely arid conditions; although the local presence of liquid water on Mars is not excluded, there appears to be about 100 times less precipitable H_2O in the atmosphere of Mars than there is in that of the earth (7). Consequently, in an experiment based on the addition of water it may be preferable to add the water as a vapor or as a gradient by introducing a small amount of liquid at the bottom of the ampoule. Provided that the added H_2O does not destroy the Mar-

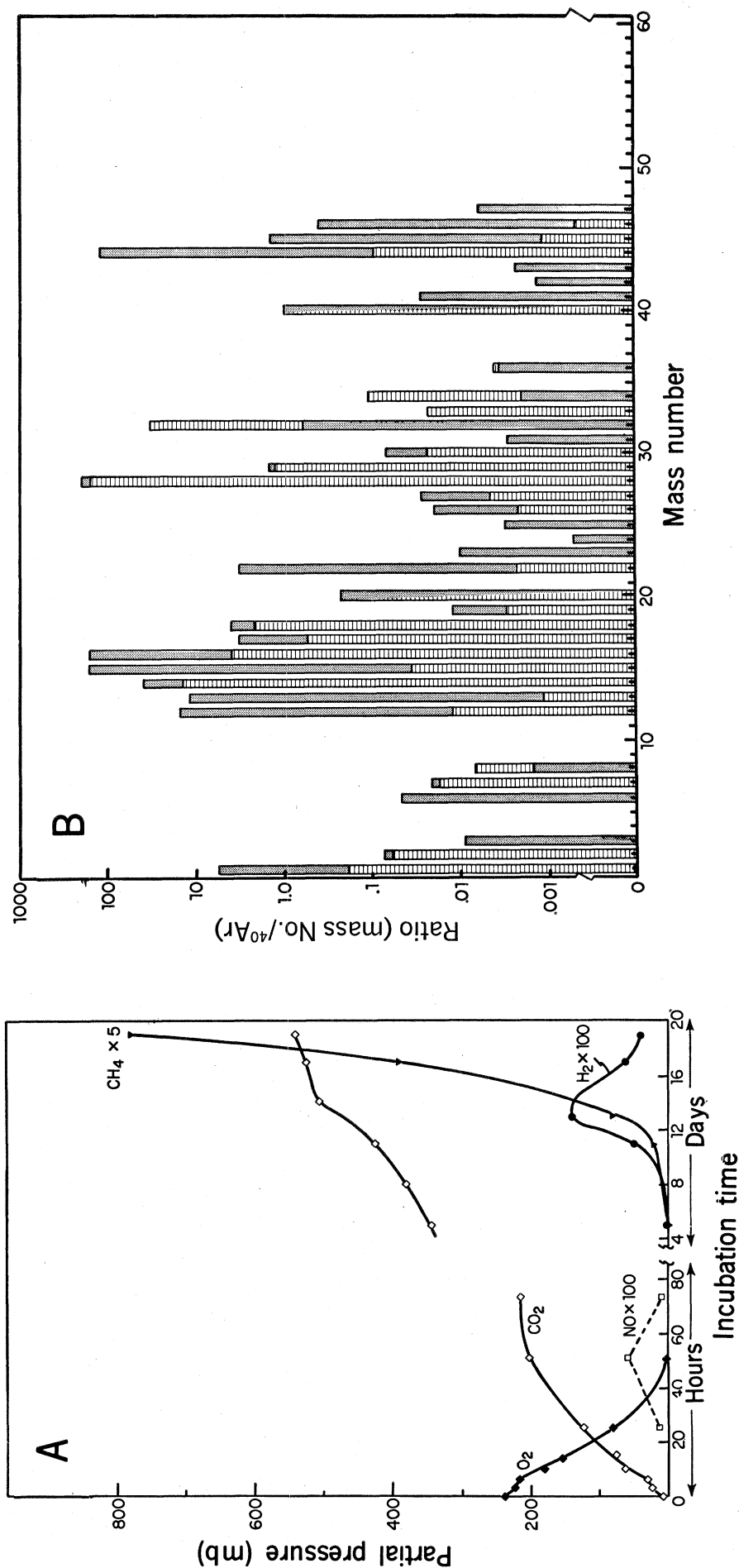
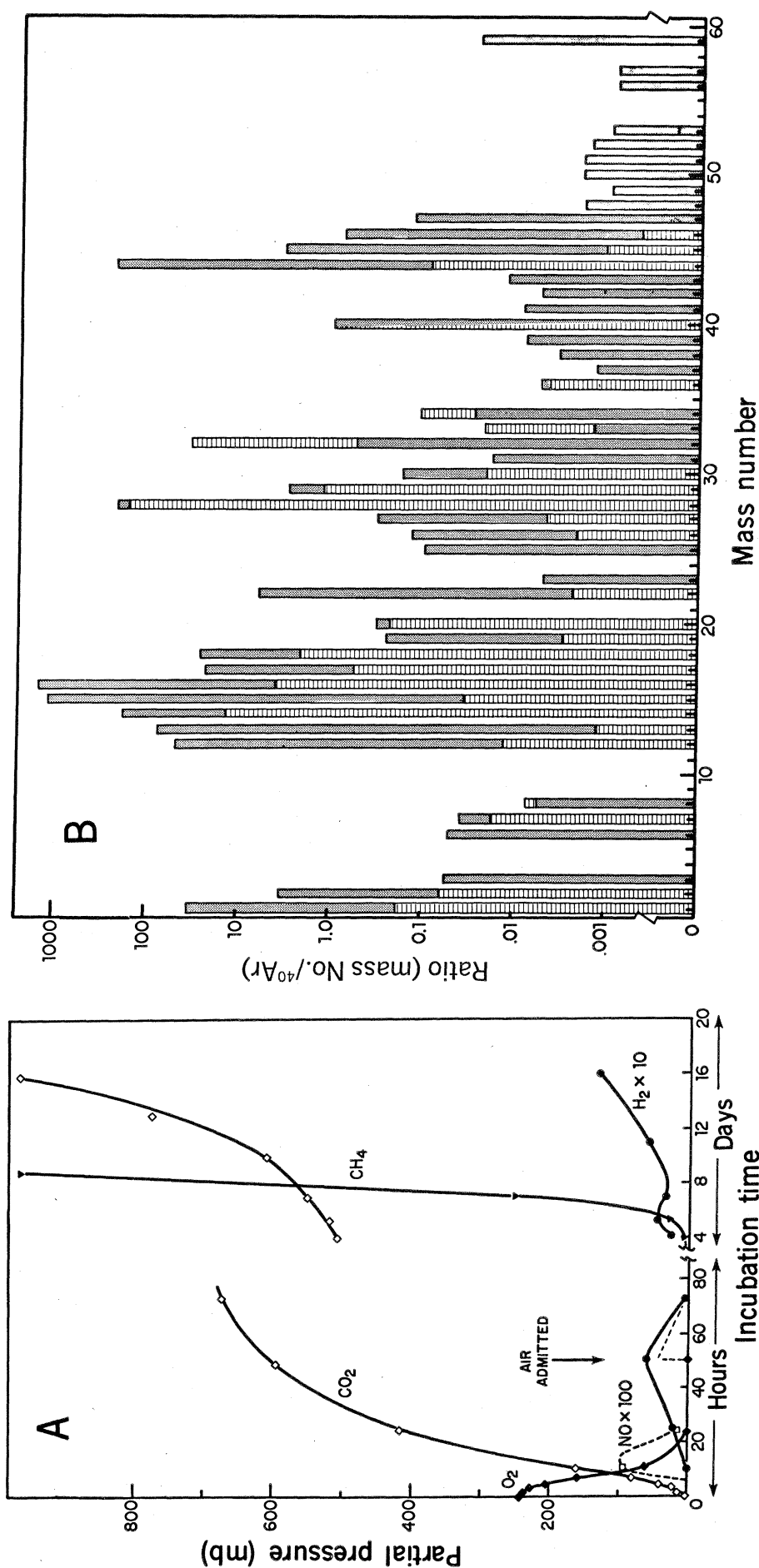


Fig. 1. Changes in the gas phase above an in situ incubation of forest soil at 30°C . A total of 250 milliliters enclosed 220 grams of soil, having a plate count of 3×10^7 organisms per gram of soil and a water content of 16 percent by weight. (A) Time course; (B) comparison of the composition of the gas phase at T_0 and after 50 days incubation. Before incubation, horizontal shading; after incubation, gray shading. In this and all succeeding experiments each observation is a measurement of mass ratio; the peak height of the monitored gas is compared to the height of the peak of ^{40}Ar used as an internal standard.



tian biota, this experiment might have a greater biological sensitivity than the in situ observation. Incubation at high temperatures after the addition of H₂O might serve as a control.

3) *Addition of substrates.* On Earth, the most effective way to promote biological activity in soils is by the addition of an aqueous solution of nutrients. Figure 2, A and B, shows the results of an incubation of forest soil with nutrient broth. The rapid changes in gas composition are indicative of intense and varied biological activities. Incubation of a soil having a plate count of bacteria 100 times lower showed a fivefold decrease in the rate of O₂ consumption, but after a few weeks the atmospheric analysis closely resembled that shown in Fig. 2B. Thus, on Earth, the results of this assay show little relation to the density of organisms originally present in the soil.

In contrast to the in situ experiment, which attempts to measure the indigenous metabolism with minimum perturbation, this experiment deliberately disturbs the environment in an attempt to enhance metabolism and induce growth. Consequently, the success of this experiment depends less on technical performance than on our ability (or good fortune) in choosing a medium which is utilized by Martian biota. Even if growth like that on Earth does not occur, Martian biological catalysts (subcellular particles or enzymes) might carry out partial conversions of the substrates and thus yield detectable signals (for example, ¹³CO₂ evolution).

The principle ambiguities in this experiment are caused by nonbiological reactions in which the added substrates might participate. A sample which is sterilized by heat before the introduction of substrate can provide an adequate although not ideal control for this experiment and for all others discussed in this report.

4) *Specific metabolic probes with stable isotopes.* Many biologists have stressed the importance of probes for specific metabolic processes which

Fig. 2. Changes in the gas phase above a sample of forest soil (see Fig. 1) incubated in the presence of yeast extract and KNO₃. Soil (300 grams) plus 60 milliliters of a solution of 1 percent yeast extract and 10 mM KNO₃ was incubated in a 250-milliliter glass vessel. (A) Time course; (B) comparison of the composition of the gas phase at T₀ and after 50 days incubation. Before incubation, horizontal shading; after incubation, gray shading.

would help to define the Martian biological system. Such assays could also serve as sensitive detectors of life, particularly in the case of exergonic and isoenergetic reactions catalyzed by biological systems; these can often proceed

at high rates and thus be detectable, despite a very low net biological activity.

A common method for improving the specificity and sensitivity of a biological assay is through the use of rare

isotopes. In mass spectrometry, use of a rare isotope can move the signal from a point on the spectrum which has a high background to a point which has a low background. In addition, some important processes, such as exchange

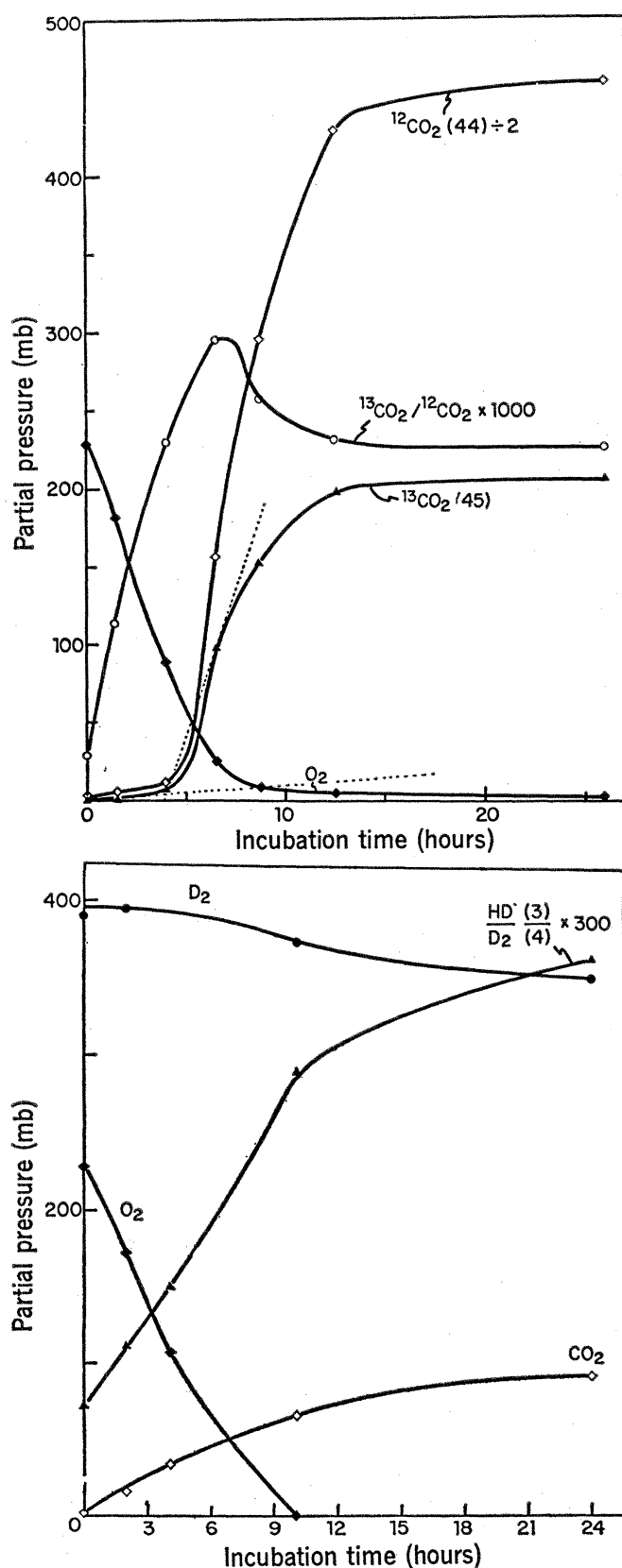
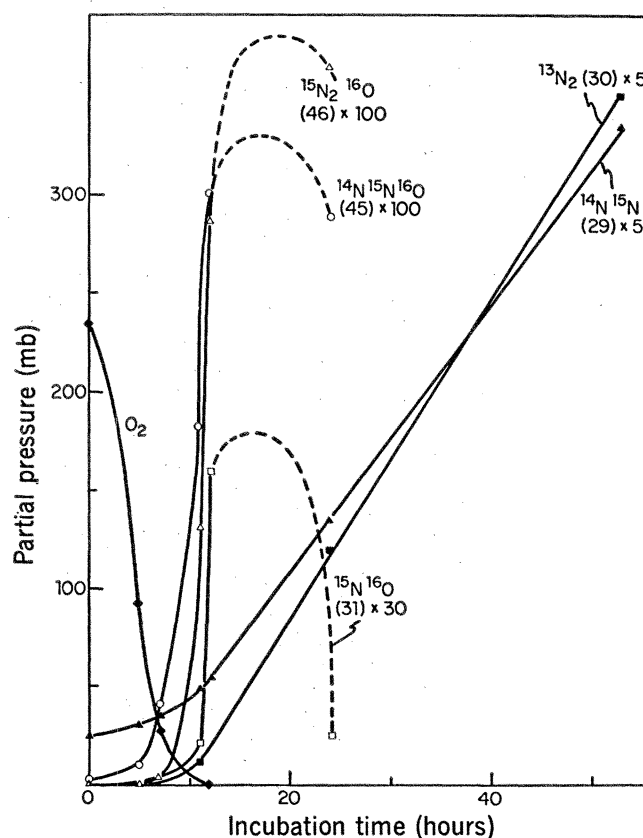


Fig. 3 (left). The evolution of CO_2 by a sample of forest soil (see Fig. 1) incubated with sodium [^{13}C]acetate. Soil (100 grams) plus 20 milliliters of a solution of 10 mM sodium acetate uniformly labeled with ^{13}C (61.2 atom percent ^{13}C) was incubated in a total volume of 60 milliliters. Fig. 4 (bottom left). The time course of hydrogen uptake and H_2 and D_2 exchange in a sample of forest soil incubated in an atmosphere enriched in H_2 and D_2 . Soil (100 grams) plus 20 milliliters of a solution of 10 mM sodium acetate was incubated in a total volume of 60 milliliters. The initial gas phase above the soil in this experiment was approximately as follows: D_2 , 40 percent; H_2 , 30 percent; O_2 , 20 percent; Ar, 10 percent. Fig. 5 (bottom right). The evolution of various labeled nitrogen compounds from a sample of forest soil incubated with $^{15}\text{NO}_3^-$. Soil (100 grams) plus 20 milliliters of a solution of 10 mM sodium acetate and 10 mM K^{15}NO_3 (99 atom percent ^{15}N) was incubated in a total of 60 milliliters.



reactions, can be only detected by the use of isotopes. The following paragraphs describe examples of specific assays of metabolism in which stable isotopes are used.

The biological oxidation of organic compounds to CO_2 is an important terrestrial process. An experiment intended for the 1975 mission monitors the release of $^{14}\text{CO}_2$ from organic substrates labeled with ^{14}C (8). With the replacement of ^{14}C by ^{13}C , this experiment can be performed with a mass spectrometer instead of a detector for radioisotopes with equal or better sensitivity (9). Use of a stable isotope prevents radiodecomposition of the labeled substrate. This permits complete labeling and restricts the background signal to that of the thermal degradation of the substrate. Figure 3 presents data obtained in an experiment in which soil was incubated with ^{13}C acetate. The extreme initial slope of the ratio of $^{13}\text{CO}_2$ to $^{12}\text{CO}_2$ illustrates the high sensitivity of this measurement as a result of the low initial concentration of $^{13}\text{CO}_2$.

As in the experiment described above, the labeled substrate must be selected without prior knowledge of the Martian biology. Since labeling with ^{13}C is an innocuous alteration, the two experiments can be combined in a single ampoule. Then, in addition to $^{13}\text{CO}_2$, other organic volatiles produced from the labeled substrate can be detected; in plots like that in Fig. 2B new mass peaks will appear which are displaced by one mass unit.

Many terrestrial organisms metabolize molecular hydrogen, either releasing it from reduced compounds or using it for the reduction of oxidized compounds. In addition, many of these organisms and their cell-free extracts catalyze the exchange reactions $\text{D}_2 + \text{H}_2 \rightarrow 2\text{DH}$ and $\text{D}_2 + \text{H}_2\text{O} \rightarrow \text{DH} + \text{DHO}$, often at a greater rate than the net throughput. This exchange or scrambling reaction is isoenergetic and does not require growth or cellular integrity.

Figure 4 presents data obtained in an experiment in which soil was incubated in an atmosphere containing H_2 and D_2 . Both net hydrogen uptake (as measured by the decrease of D_2) and the formation of DH were detectable after an incubation of a few hours. (Under the conditions of this experiment, the two reactions $\text{H}_2 + \text{D}_2 \rightarrow 2\text{DH}$ and $\text{D}_2 + \text{H}_2\text{O} \rightarrow \text{DH} + \text{DHO}$ will both yield DH.)

Under anaerobic conditions many

terrestrial organisms are able to use oxyanions such as NO_3^- , SO_4^{2-} , and PO_4^{3-} instead of O_2 as terminal electron acceptors. On Mars, where the availability of O_2 is at best limited, this metabolic pattern might be prevalent, and an experiment aimed at detecting these reactions would be very informative.

Two different approaches are available for detecting the reduction of the oxyanions by mass spectrometry. In the first, the oxyanion is labeled with ^{18}O . Upon reduction, the ^{18}O will appear in H_2O , which in turn spontaneously exchanges with CO_2 ; thus, this process can be monitored by the increase in $\text{C}^{18}\text{O}^{16}\text{O}$ (an increase in the ratio of mass 46 to mass 44) (10).

In the second approach, the central atom of the oxyanion is labeled (^{15}N in nitrate), and the reaction is monitored by the appearance of labeled gaseous products such as $^{15}\text{N}_2$ (mass 30), ^{15}NO (mass 31), and $^{15}\text{N}_2\text{O}$ (mass 46). Because of the very low natural abundance of compounds at mass numbers 30, 31, and 46, this approach is more sensitive than the first method which, because of the high concentration of H_2O , must cope with a large initial concentration of ^{18}O .

Figure 5 illustrates the results of an experiment in which $^{15}\text{NO}_3^-$ was added to a soil sample. The formation of labeled nitrogen and the NO and N_2O transients present striking evidence for the process of nitrate reduction. The use of $^{15}\text{NO}_3^-$ greatly facilitates the detection of N_2O , which now appears at masses 45 and 46 a region of low background (CO_2 isotopic peaks), instead of mass 44, the base peak of CO_2 . Since the added NO_3^- contains almost pure ^{15}N , the amounts of $^{14,15}\text{N}_2\text{O}$ (and $^{14,15}\text{N}_2$) will be a measure of the amount of NO_3^- originally present in the soil.

Similar reactions occur with other oxyanions as electron acceptors. In the case of SO_4^{2-} , the gaseous product H_2S appears at mass 34, a position in the spectrum with a relatively low background (O_2 isotopic peak). The use of rare isotopes (^{33}S or ^{34}S) would move the H_2S signal to a region of very low background. In the case of PO_4^{3-} , the gaseous product PH_3 would appear at mass 34, a region of very low background.

In addition to the more obvious experiments listed above, several others concerning metabolism and environment might be considered. In terrestrial processes of phosphorylation, oxygen is

exchanged between water and phosphate. Although the transfer of the ^{18}O label from phosphate into water requires an enzyme, the transfer from water to CO_2 occurs spontaneously. Thus, life can be detected by observing the transfer of ^{18}O from PO_4^{3-} into CO_2 (10). As in the observation of nitrate reduction in which ^{18}O is used, the oxygen label must be measured in the presence of a large initial concentration of ^{18}O which requires rather sophisticated mass spectrometry.

The nitrogen-fixing complex of earth organisms is nonspecific for N_2 ; for example, it reduces acetylene (mass 26) to ethylene (masses 27 and 28). The use of ^{13}C acetylene brings the reduction product $^{13}\text{C}_2\text{H}_4$ to an empty spot in the mass spectrum (mass 30) and results in increased sensitivity.

In aerobic terrestrial photosynthesis light causes the evolution of oxygen from H_2O . The use of H_2O labeled with ^{18}O enhances the sensitivity of this experiment, since the O_2 appears at masses 34 and 36, both of which have a very low background.

Ideally, a search for life on Mars should be preceded or at least accompanied by a thorough evaluation of the habitat. For example, the instrumentation is readily adaptable for detailed atmospheric analyses. Since the ampoules can be used as reaction chambers for any assay which induces a compositional change of a gas phase, a number of soil parameters might also be determined; for example, the addition of acid causes the evolution of CO_2 from carbonates, pH might be estimated by the rates of acid-catalyzed hydrolyses, and a standard determination of amino nitrogen rests upon the liberation of N_2 and NO .

Biological Sensitivity

For detection of life on Mars, where one expects biota to be sparse, sensitivity is a goal second only to unambiguity. If the method relies on significant and rapid growth, the limit of detection can be one organism, whatever its size. However, for methods that do not rely upon growth, procedural sensitivity will be of crucial importance up to the point that interference from nonbiological factors sets the limit.

In observations of the type described in Fig. 1B the challenge is the detection (rather than the measurement) of minute amounts of newly formed products in the presence of high concentra-

tions of N_2 and O_2 (CO_2 on Mars). Hence the sensitivity of this assay depends largely upon the cleanliness (lack of background), and the dynamic range of the mass spectrometer. In the experiment described in Fig. 1B this range is six orders of magnitude. In all other assays, we measure a change in the concentration of a gas already present. The sensitivity now can be defined as $\Delta S_{\min} = pS$, where S is the magnitude of the signal and p is the precision. (Note that for very small signals, $S \rightarrow 0$, p approaches infinity, and as defined we lose precision; but, since $\Delta S_{\min}$ is finite, we actually gain sensitivity to change.)

One factor governing sensitivity is the ratio of the volumes of soil and atmosphere in the incubation ampule. The higher this ratio the greater the number of organisms per diluting molecule in the atmosphere. However, the most important factor in the atmosphere in which the measurements are made. As a general rule, it can be stated that a low initial concentration at a mass number affords a high sensitivity to change. As an example, consider respiration ($O_2 + \text{organic carbon} \rightarrow CO_2$); on earth, the partial pressures of O_2 and CO_2 are ~ 0.2 and $\sim 3 \times 10^{-4}$ atmosphere, respectively. If a precision of ~ 1 percent and adequate dynamic range are assumed, the minimum detectable change in the pressures of CO_2 and O_2 would be 3×10^{-6} and 2×10^{-8} atmosphere, respectively. Thus, because of its low concentration, CO_2 is about three orders of magnitude more sensitive an indicator of respiration than O_2 is. Similarly, the fixation of N_2 is difficult to detect because of its high background (~ 0.8 atmosphere), but the formation of a few parts of NO per million (mass 30, a position of very low background) can be readily observed (Fig. 1).

It is difficult to arrive at a universal definition of biological sensitivity. Tech-

niques to determine the census of soil organisms yield answers which vary by at least an order of magnitude (11). The metabolic rate of a soil depends on many parameters in addition to the soil census, such as the available organics and water (11, 12). Consequently, it is difficult to correlate metabolic rate in moles per hour per gram of soil, the amount of living matter (gram per gram of soil), and the number of organisms per gram of soil. Thus, the two often-used criteria for sensitivity of detection, the minimum change of a metabolic variable and the minimum number of organisms, are not easily convertible.

To permit some basis of comparison, assume that we are dealing with a homogeneous population of standard bacteria having a dry weight of 1 gram per 10^{13} cells and a metabolic rate of 100 milliliters (CO_2 or O_2) per gram per hour at standard temperature and pressure. If we were to monitor with 1 percent precision a 90-day in situ incubation of 1 gram of soil with 1 milliliter of air, then the minimum detectable bacterial density would be 10^5 and 300 organisms per gram of soil for O_2 and CO_2 , respectively. The actual plate count and the half-times observed in the experiment described in Fig. 1 extrapolate to ultimate sensitivities of 5000 for O_2 and 8 for CO_2 .

For the experiment of Fig. 3, in which ^{13}C was used, we predict a sensitivity of 50 standard bacteria per gram of soil. [Because of the low concentration of $^{13}CO_2$ (3 parts per million), p is low initially (~ 30 percent)]. The plate count and actual data of Fig. 3 (dotted slopes) extrapolate to sensitivities of 15 organisms per gram of soil for the low initial slope and < 1 organism per gram of soil for the slope during logarithmic growth.

Considering that plate counts tend to underestimate the population and the minimum size and the high metabolic rate of our standard bacteria, the agree-

ment is reasonable. Even if we allow an additional order of magnitude for shorter incubation and less precision than assumed above, these calculations suggest that the ultimate limit of detection will be set by nonbiological interferences rather than by limitations of measurement.

References and Notes

1. NASA Viking 75 Project Mission Definition, September 1970. Prepared by the National Aeronautics and Space Administration, Langley Research Center, Viking Project Office.
2. G. A. Soffen and A. T. Young, *Icarus*, in press.
3. Metabolism may be defined as the totality of the chemical processes that a cell or organism is able to perform [H. R. Mahler and E. H. Cordes, *Biological Chemistry* (Harper & Row, New York, 1966), p. 404].
4. R. S. Young, R. B. Painter, R. D. Johnson, *An Analysis of the Extraterrestrial Life Detection Problem*, NASA SP-75 (National Aeronautics and Space Administration, Washington, D.C., 1968), p. 11.
5. R. D. Johnson and H. P. Klein, *Amer. Inst. Aeronautics Astronautics Mtg.* AIAA Paper No. 68-1122 (1968).
6. J. E. Lovelock, *Nature* **207**, 568 (1965); D. R. Hitchcock and J. E. Lovelock, *Icarus* **7**, 149 (1967).
7. T. Owen and H. P. Mason, *Science* **165**, 893 (1969); S. Glasstone, *The Book of Mars* (National Aeronautics and Space Administration, Washington, D.C., 1968), p. 80.
8. G. V. Levin, A. H. Heim, M. F. Thompson, D. R. Beem, N. H. Horowitz, in *Life Science and Space Research*, M. Florkin and A. Dollfus, Eds. (North-Holland, Amsterdam, 1964), vol. 2, p. 124.
9. For the detection of $^{13}CO_2$ on the Mars lander, assume that on Mars the pressure of CO_2 approximates 10^{-2} atmosphere and the ratio of ^{12}C to ^{13}C is approximately 100 (that is, the distribution of isotopes on Mars is not grossly different from that on Earth). Assume that 1 gram of soil and 1 milliliter of gas are incubated, the precision of measurement is 1 percent and 100 percent of C is ^{13}C . Then $\Delta[^{13}CO_2]_{\min} \sim 4 \times 10^{-11}$ mole. For the detection of $^{14}CO_2$ on the Mars lander, assume that the minimum detectable activity is 150 disintegrations per minute (7) (due to the gamma field from radiothermal generators), and approximately 5 percent of C is ^{14}C ; then since 1 mole of ^{14}C emits 1.4×10^{14} disintegrations per minute, $\Delta[^{14}CO_2]_{\min} \sim 5 \times 10^{-11}$ mole.
10. B. Kok and J. E. Varner, *Science* **155**, 1110 (1967); in *Life Science and Space Research*, A. H. Brown and F. G. Favorite, Eds. (North-Holland, Amsterdam, 1967), vol. 5, p. 206.
11. D. Pramer and E. L. Schmidt, *Experimental Soil Microbiology* (Burgess, Minneapolis, 1966).
12. M. Alexander, *Introduction to Soil Microbiology* (Wiley, New York, 1964), p. 161.
13. We thank Otto Ollinger and George Johnson, Jr., for technical assistance, and George Cheniae for helpful discussions. Supported by grant NAS W2109 from the National Aeronautics and Space Administration.