Extraterrestrial Life Detection Based on Oxygen Isotope Exchange Reactions

Abstract. *A method is described for detecting extraterrestrial life, based on catalysis of isotopic oxygen exchange between water and oxygen-containing anions such as phosphate, nitrate, or sulfate. This catalytic activity appears to be unique to living systems. Its applicability requires very few assumptions concerning the chemical nature of "life." Data obtained so far indicate that the experiment is sound and technically feasible.*

There is no assurance that life on other planets (*1, 2*), if it exists, has evolved along a path similar to life on Earth, or that its functional chemistry is identical. A search for extraterrestrial life should, therefore, not be restricted to tests based closely upon analogy with Earth life. Experiments for detection of extraterrestrial life should make use of a property that is characteristic of all known or rationally possible living systems. Such a property, which underlies the method described here, is the enzymatic catalysis of oxygen exchange between water and certain common oxyanions.

The following characteristics seem to be required of all conceivable forms of life: (i) The living system is a chemical one and is based upon an aqueous medium. This is a result of the unique solvent, ionic, and thermal properties of water. (ii) The chemical processes involved are catalyzed by some special means. Maintenance and growth of living organisms involve a flux of energy, maintained by the synthesis and utilization of high-energy (metastable) compounds. Efficient functioning of such systems requires that the various reactions be catalyzed in order to achieve a useful steady-state level of such metastable compounds and to provide opportunities for control of reaction rates. (iii) The high-energy compounds contain or use some forms of oxyanions such as nitrate, sulfate, or phosphate. The ubiquity of the elements of low atomic number in these oxyanions, their different levels of oxidation, the change in energy involved in transitions between these states, and their high solubility in water, make this assumption reasonable. (iv) A life-induced transfer or exchange between the oxygen present in the anion and in the water will accompany the reactions which the oxyanions undergo. Any reduction of the anion implies a release of its oxygen, which, in terrestrial life, is generally found in water [see Eq. 1, in which (H) indicates metabolic reducing power]. On Earth a very important role is played by high-energy phosphor-oxygen and sulfur-oxygen linkages (such as in adenosine diphosphate, adenosine triphosphate, phospho-adenosine phosphosulfate, and adenosine phosphosulfate).

The formation and breakage of these bonds is accompanied by the exchange of oxygen from the anion into water (Eq. 2). The equations describe only the overall balance of possibly complex mechanisms in which much more oxygen might be exchanged than would correspond to the net rate of the process (*3*). According to the above assumption, similar reactions will occur in other life systems.

Net transfer:

$$AO^{18} + (H) \rightarrow A(H) + H_2O^{18} \quad (1)$$

Exchange:

$$AO^{18} + H_2O^{16} \rightleftharpoons AO^{16} + H_2O^{18} \quad (2)$$

In the actual experiment one uses anions enriched with an isotope of oxygen (O^{17} or O^{18}) and measures the appearance of this isotope in the initially unlabeled water, due to enzymatic catalysis according to Eqs. 1 and 2. If exchange or transfer occurs the presence of an enzymatic (living) system is indicated.

The basic feature of the method is the generality of its principle. In addition, the method should be unambiguous (it has to discriminate positively between living and nonliving systems) and be technically feasible and sufficiently sensitive. We report the results of preliminary experimentation which has shown the method to be highly promising.

A mass spectrometer has been used to measure the concentration of the stable oxygen isotope O^{18} in water, or rather the *change* of this concentration due to O^{18} exchange. For a simple and light-weight inlet system, we used a principle developed earlier in our laboratory (*4*). A thin Teflon membrane separates the mass spectrometer vacuum from a liquid or gaseous sample, which need be only a few microliters in volume. Water and (dissolved) gases diffuse through the membrane into the mass spectrometer at a rate which allows continuous and rapid detection (< 1 minute). In most experiments we analyzed O_2 gas evolved electrolytically from the water sample, determining the ratio R between mass numbers 32 and 34. We are more interested in the change, ΔR, which might have occurred in the experiment, and the more sensitive measurement is a comparison of two water samples, one designed as a control, the other recovered after an incubation which might have enriched its O^{18} content. A duplex inlet arrangement was used which allowed alternate measurements of the mass ratio of two samples. Sample switching was done at atmospheric pressure, the two gas streams being alternately passed over the membrane leak. Manipulation of this system proved simple; minimal size sample is about 50 μl, time required for analysis is 20 to 30 minutes, and the present limit of detection is $\Delta R \pm 0.05$ percent (see *5*), when one uses a conventional double collector instrument including a Consolidated isatron source and Cary vibrating reed electrometers.

Instead of O_2 one could sample the water itself. This has the drawback of the large "memory" for water shown by conventional mass spectrometers. One can also measure the O^{18} content of CO_2 after equilibration of the water with added bicarbonate and carbon dioxide. We have used this method, employing carbonic anhydrase to accelerate equilibration. This enzyme, however, cannot withstand the heat sterilization required by space probes. Actually, the use of O^{17} and nuclear magnetic resonance may prove a superior and simpler method. This technique allows *in situ* measurement of the O^{17} in the water simultaneously with that in the anions (*6*).

In most reported experiments we used 200 μl of 0.1*M* solutions of O^{18}-labeled phosphate or nitrate to moisten 500-mg samples of homogenized soil or to dissolve or disperse reagents or biological materials. In the majority of experiments the *p*H was around neutrality and incubation was carried out in sealed 5-ml, air-containing vessels at room temperature. After incubation the water was recovered by reduced-pressure distillation and its relative O^{18} content was compared with that of the original water used for the experiment.

An essential requirement for the method is that the spontaneous (nonenzymatic) rate of isotope exchange between the oxyanion and water be sufficiently low so that no appreciable

uncatalyzed exchange will occur during the time interval of the measurement. A literature survey revealed sufficient data to calculate rates of spontaneous exchange for a number of inorganic and organic anions with a fair degree of reliability.

The rate constants for several oxyanions are low enough to be compatible with our scheme, for example, for nitrate the half-time of exchange is 1.45×10^{17} hours at *p*H 7 and 70°C (*7*). For sulfate (*8*), phosphate (*9*), and acetate (*10*) the respective half-times are: 2×10^5 (*p*H 2, 100°C), 5×10^3 (*p*H 8.6, 100°C), and 3.5×10^6 (*p*H 7, 25°C). With phosphate, wet sterilization yields a small amount of O^{18} exchange during the heating of the phosphate solution; therefore, the anion should be sterilized in the dry state. Entirely suitable besides nitrate, sulfate, phosphate, and organic (including amino) acids, are chlorate, bromate, and phosphite. Nitrite and sulfite might be useful under some conditions of incubation. Unsuitable, because of their rapid spontaneous exchange with water, are carbonate, silicate, borate, and arsenate.

The spontaneous exchange of O^{18} between water and silicates as well as carbonates, both abundant in soils, interferes with our measurement: part of the labeled oxygen, after being enzymatically transferred from the anion to water, might go into silicate. Such a "dilution" of the label by oxygen from the silicate will lower the excess abundance of the H_2O^{18} and thus the signal. We estimated the magnitude of this dilution by equilibrating a soil sample of known water content with O^{18} enriched water and observing the decrease of the excess abundance. A random sample yielded a "water equivalent" of exchangeable oxygen of 18 percent of the dry weight of the soil. Note that this dilution is identical to that caused by water in moist samples. It introduces a quantitative error but does not reduce the validity of a definite answer regarding life.

While the last statement holds true under terrestrial conditions, it will be valid on Mars only if the isotope abundance (in water, silicate, and so forth) approximates that on Earth (0.2 percent for O^{18}, 0.04 percent for O^{17}) or is otherwise accounted for.

The relative abundance of the oxygen isotopes on Mars is unknown. Our method requires knowledge of this abundance, which it actually might determine in control experiments. To cope with an isotopic abundance different from the terrestrial value, one can include parallel experiments in which the soil samples are inactivated (sterilized), or the anions are not labeled. If earlier probes into the Martian atmosphere provide a reliable value for the abundance, such measures may be unnecessary.

In order for the method to be unambiguous, catalysis of exchange by "nonliving" materials should be negligible. The limited literature revealed no reports of significant catalysis by inorganic compounds. Our observation (Table 1, Fig. 1) that sterilized soil samples did not yield significant exchange with phosphate or nitrate further indicates that inorganic catalysis is negligible.

We have tested a possible catalysis of O^{18} exchange between phosphate and water by: chloride, nitrate, bromate, bromide, sulfate, silicate, and calcium phosphate (all in 0.1*M* solution). No significant effect was found after 2 weeks of incubation at *p*H 4, 7, and 10.

A more fundamental concern is catalysis by "nonliving" organic materials which might have accumulated on the Martian surface. Ideally, the method should respond exclusively to "enzymes" (catalysts having a degree of effectiveness and specificity that is incompatible with random accumulation). Therefore, a number of organic or denaturated biological materials have been tested for capacity to catalyze exchange between phosphate or nitrate and water. These included a variety of amino acids, purines and pyrimidines, acetate, and glycine. No significant catalysis by these compounds was found. As seen in Table 1, nutrient broth and yeast extract also failed to catalyze O^{18} exchange.

The ideal life detection method, while fastidiously unresponsive to nonliving materials, should detect all conceivable living systems. As indicated earlier, one would expect all terrestrial organisms to catalyze oxygen exchange. We tested a variety of organisms, cell-free extracts, tissue macerates, and a number of soil samples with varying bacterial content. Table 2 presents some observations of exchange between

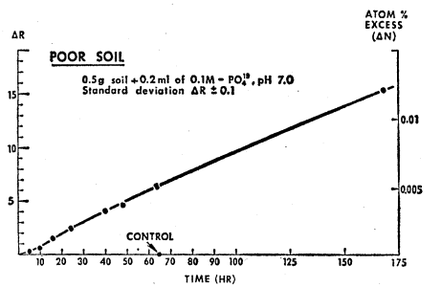

Fig. 1. Time course of soil-catalyzed exchange between phosphate and water. In the control experiment sterilized phosphate (95 atom percent excess O^{18}) was added to an autoclaved mixture of soil and water. Plate counts (see *11* for method) yielded 7.5×10^5 organisms per gram of soil.

Table 1. Exchange between labeled phosphate (52 atom percent excess O^{18}) or nitrate (80 atom percent excess O^{18}) and water, catalyzed by randomly collected soil samples. Moisture content of the samples varied between 2 and 6 percent. Incubation was at *p*H 7 and room temperature during the indicated time. No exchange could be detected with labeled phosphate and autoclaved parallel samples of Nos. 1 to 4. Limit of detection: $\Delta N \pm 2 \times 10^{-3}$, except in the experiment with phosphate and 1 percent nutrient broth: $\Delta N \pm 2 \times 10^{-4}$.

Sample	Hours	ΔN	Percent exchange
Anion: Nitrate			
Soil 1	168	0.022	10.4
Soil 2	168	.017	8.7
Soil 3*	90	.000	0.0
Soil 3†	88	.202	47.7
Soil 3*†	88	.000	0.0
Anion: Phosphate			
Soil 1	48	0.034	.5
Soil 2	48	.021	3.1
Soil 3	48	.020	2.9
Soil 4	48	.38	5.6
1 percent nutrient broth*	300	.0007	0.1

* Autoclaved. † 0.3 percent glucose + 0.3 percent yeast extract added.

Table 2. Rates of oxygen exchange between phosphate and water. Incubation at room temperature and neutral *p*H. Whole cells and extracts were suspended in media which contained (beside 95 atom percent excess O^{18} labeled 0.1*M* phosphate) ingredients that allowed metabolic activity, though not necessarily optimum rates. Values given are rates observed during the first few hours of incubation, uncorrected for occasional lag phases. Protein content of soils was estimated from bacterial counts.

Organism or preparation	Rate of exchange*
1. *Saccharomyces cerevisiae*, whole cells	5
2. *Saccharomyces cerevisiae*, cell-free extract	15
3. *Chlorella*, illuminated whole cells	2.5
4. *Thiobacillus novellus*, cell-free extract	30
5. *Hydrogenomonas*, whole or broken	12
6. "Rich soil" (2×10^7 organisms per gram)	14
7. "Poor soil" (7.5×10^5 organisms per gram)	35

* Microatoms O/mg protein hr^{-1}.

phosphate and water, expressed as microatoms of oxygen transferred per milligram of protein per hour. The exchange rates proved to be the same order of magnitude. In several instances we simultaneously measured the rate of oxygen metabolism and observed comparable values. Whole as well as broken cells of the bacterium *Hydrogenomonas* showed a correlation between the rate of exchange and metabolic activity, that is, the availability of metabolic substrate. Disruption of the cells by sonic treatment prior to incubation tended to stimulate activity, which could be retained during storage of the preparations in the lyophilized form.

Table 1 lists exchange between nitrate or phosphate and water, catalyzed by randomly collected soil samples. With one exception, the data in this table were collected with a simple, single-collector mass spectrometer instrument; nevertheless the exchange was clearly measurable. Relatively few experiments have been made with nitrate: these indicated a seasonal variation in soil activity, and generally gave positive results, often exceeding those obtained with phosphate. Figure 1 shows the time course of exchange catalyzed by a soil sample of low bacterial count. These data were obtained with the more sensitive double-collector duplex inlet instrument. In this case an unambiguous indication was obtained in less than 10 hours. The time course shows the more-or-less expected response of microbial activity upon moistening this soil with a 0.1*M* solution of phosphate. So far our experiments have been exploratory and restricted to the two anions discussed. As it stands (see Table 2), we can assume the limit of detection (for $\Delta R = 0.05$ percent) to be $\sim 10^4$ organisms per gram of soil or 10^3 organisms per 0.1-gram sample in 24-hour incubation.

Tremendous amplification of the exchange rate is obtained by growth which can be induced by adding appropriate nutrients. Since the advantage of the proposed method is that it does *not* require growth or depend upon close similarity to terrestrial biochemistry, its uniqueness would be defeated by such additions, unless they could be extracted from Martian soil or were selected to provide specific answers concerning the metabolic activity detected.

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References and Notes

1. *Life Sciences and Space Research (COSPAR)* (Wiley, New York, 1963–65): vol. 1, R. B. Livingston, A. A. Imshenetsky, G. A. Derbyshire (1963); vol. 2, M. Florkin and A. Dollfus, Eds. (1964); vol. 3, M. Florkin, Ed. (1965).
2. W. Vishniac, *Extraterrestrial Life*, E. A. Shneour and E. A. Ottesen, Eds., Natl. Acad. Sci.–Natl. Res. Council, Publ. 1296A (NAS–NRC, Washington, D.C., 1966), p. 282.
3. M. Cohn, *J. Biol. Chem.* **230**, 369 (1958).
4. G. Hoch and B. Kok, *Arch. Biochem. Biophys.* **101**, 160 (1963).
5. H. C. Urey and C. R. McKinney, *Rev. Sci. Instr.* **21**, 724 (1950).
6. D. Kahn, *Research Institute for Advanced Studies Techn. Rep. 67-1* (RIAS, Baltimore, 1967).
7. M. Anbar and S. Guttman, *J. Amer. Chem. Soc.* **83**, 4741 (1961).
8. T. C. Hoering and J. W. Kennedy, *ibid.* **79**, 56 (1957).
9. C. A. Bunton, D. R. Llewellyn, C. A. Vernon, V. A. Welch, *J. Chem. Soc.* **1961**, 1636 (1961).
10. C. A. Bunton and G. Stedman, *ibid.* **1959**, 3466 (1959).
11. D. Pramer and E. L. Schmidt, Eds., *Experimental Soil Microbiology* (Burgess, Minneapolis, 1964).
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Splashing of a Water Drop

Abstract. *Measurements have been made of the number of spray droplets produced by the impact of a water drop on water, and of the charge to mass ratio for these droplets. For a drop 3 millimeters in diameter, the number of spray droplets increases linearly with the fall-distance of the drop over the range 10 to 200 centimeters. When the drop falls 100 centimeters, about 25 spray droplets are produced. The majority of the droplets carry a negative charge, and the ratio of the charge to the mass varies from 4 to 28 electrostatic units per gram.*

If a drop, several millimeters in diameter, falls from a height of about 30 cm into a liquid, the following sequence of events takes place (*1*). When the drop collides with the surface (Fig. 1a) a crater of liquid is thrown up which increases in height as the drop penetrates the surface. Small jets are shot out from the rim of this crater to form a "crown" of liquid (Fig. 1b; 0.003 second after Fig. 1a) and these break up to give numerous spray droplets (Fig. 1c; 0.003 second after Fig. 1b). As the walls of the crater thicken they begin to subside (Fig. 1d; 0.008 second after Fig. 1c) and the downward flow of liquid results in the ejection of a large column of liquid from the center of the crater (Fig. 1e; 0.026 second after Fig. 1d) which is known as the Rayleigh jet. The Rayleigh jet may pinch off to form one or more large drops (Fig. 1f, 0.030 second after Fig. 1e; and Fig. 1g, 0.002 second after Fig. 1f). Rayleigh (*2*) has explained this phenomenon in terms of the amplification of an unstable wave on the surface of the jet (Fig. 2).

The photographs also show that the drops thrown off from the jet oscillate as they fall through the air. The liquid used was a mixture of milk and water. Measurements from the photographs show that the period of vibration τ of a drop 3.6 mm in diameter is about 2.2×10^{-2} second. Rayleigh (*2*) has shown that in the case of small vibrations of a liquid drop

$$\tau = \sqrt{\left(\frac{3\pi\rho V}{8T}\right)}$$

where ρ is the density of the liquid, T the surface tension, and V the volume of the drop. The surface tension and the density of the mixture of water and milk were 50.5 dyne/cm and 1.014 g/cm^3. Hence, for a drop of diameter 3.6 mm, the theoretical value of τ is 2.4×10^{-2} second, which is in good agreement with the experimental result.

The spray droplets that are thrown out from the crown, and the drops from the Rayleigh jet, carry an electric charge. Lenard (*3*) observed that the air in the neighborhood of a waterfall, or a shower bath, has a negative space charge, and he attributed this to the splashing of the water drops. More recently, Pierce and Whitson (*4*) have measured the changes in the electric field in the vicinity of waterfalls in the Yosemite Valley and have verified Lenard's observations. However, few measurements have been made of the total number of spray droplets, or of the charge on individual spray droplets produced by the splashing of water drops on water. We here describe recent measurements of these two quantities.

Water drops were produced by passing distilled water under slight pressure through a 26-gauge stainless steel hypodermic needle. The pressure was adjusted so that the drops breaking away from the tip of the needle were 3 mm in diameter. After falling freely through a measured distance in air, the drops collided with distilled water contained in a vessel 10 cm in height