

General Disclaimer

One or more of the Following Statements may affect this Document

- This document has been reproduced from the best copy furnished by the organizational source. It is being released in the interest of making available as much information as possible.
- This document may contain data, which exceeds the sheet parameters. It was furnished in this condition by the organizational source and is the best copy available.
- This document may contain tone-on-tone or color graphs, charts and/or pictures, which have been reproduced in black and white.
- This document is paginated as submitted by the original source.
- Portions of this document are not fully legible due to the historical nature of some of the material. However, it is the best reproduction available from the original submission.

EXTRATERRESTRIAL LIFE DETECTION BY ENZYMATICALLY

INDUCED EXCHANGE OF O¹⁸

Annual Report

Period Covered: May 15, 1968 - May 15, 1969

under
Contract NASW-1735



National Aeronautics and Space Administration
Washington, D. C.

Principal Investigator: Bessel Kok
Research Investigator: Richard Radmer

Research Institute for Advanced Studies
(RIAS)

Martin Marietta Corporation
1450 South Rolling Road
Baltimore, Maryland 21227

N69-41322

FACILITY FORM 922

(ACCESSION NUMBER)	(THRU)
10	1
(PAGES)	(CODE)
CR-106454	04
(NASA CR OR TMX OR AD NUMBER)	(CATEGORY)

INTRODUCTION

There is no assurance that life on other planets, 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 solely upon analogy with Earth life and should make use of a property that is characteristic of all known or reasonably conceivable living systems. Such a property is the enzymatic catalysis of oxygen exchange between water and certain common oxyanions.

There are two pathways by which this may occur:

1. Exchange $AO^{18} + H_2O^{16} \rightleftharpoons AO^{16} + H_2O^{18}$
2. Net Transfer $AO^{18} + [H] \rightarrow AH + H_2O^{18}$

Previous studies at RIAS have been concentrated on evaluation of $PO_4^{=}$ as a suitable substrate for this experiment. These experiments have demonstrated that O^{18} is transferred from "tagged" $PO_4^{=}$ to H_2O by a wide variety of biological material⁽¹⁾. Furthermore, it has been shown that the non-biological exchange rate of $PO_4^{=}$ is very low and is not affected by any of the many minerals and organic compounds tested. Thus there is very little "background" exchange which might lead to ambiguous results.

RESEARCH DURING PAST YEAR

1. $\text{SO}_4^{=}$ and NO_3^- As Exchange Substrates

Many terrestrial organisms metabolize NO_3^- and $\text{SO}_4^{=}$ ions only to assimilate nitrogen and sulfur. Consequently, reduction of these compounds (and the consequent oxygen transfer) is coupled only to cell growth and maintenance, and not to general energy transformations of the cell as is $\text{PO}_4^{=}$. However, when NO_3^- and $\text{SO}_4^{=}$ are used as terminal electron acceptors in place of O_2 these oxyanions are coupled to the general energy economy of the cell. In an anaerobic environment such as on Mars one could reasonably expect NO_3^- and $\text{SO}_4^{=}$ reduction to be the major pathway of "respiration".

We have tested this by using E. coli, a facultative anaerobe capable of utilizing NO_3^- as a terminal electron acceptor. These studies indicate that under anaerobic conditions the oxygen of NO_3^- is rapidly transferred to H_2O . We also have found using soil samples that the rate of oxygen transfer from NO_3^- to H_2O can be enhanced more than fifty fold by incubating the samples under N_2 rather than air. Thus, under such conditions NO_3^- might be the preferred substrate for this life detection system.

Studies with soil samples and bacterial cultures made during the past year indicate that the exchange of oxygen between $\text{SO}_4^{=}$ and H_2O and between NO_3^- and H_2O are valid criteria for establishing the presence of life.

However, as may be expected in an aerobic terrestrial environment, $\text{SO}_4^{=}$ and NO_3^- metabolism are not as sensitive an indicator of life as is $\text{PO}_4^{=}$ metabolism. For example, a soil sample which gave 0.14% oxygen exchange when incubated aerobically at room temperature for 28 days with $\text{SO}_4^{=}$ and a .36% exchange using NO_3^- gave a 2.9% oxygen equilibration when incubated 2 days with $\text{PO}_4^{=}$. Although such data indicate that $\text{PO}_4^{=}$ is probably the best substrate to use in an aerobic terrestrial system, in different environments (and different life systems) NO_3^- and $\text{SO}_4^{=}$ might be much better substrates.

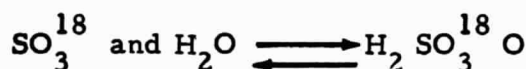
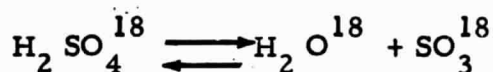
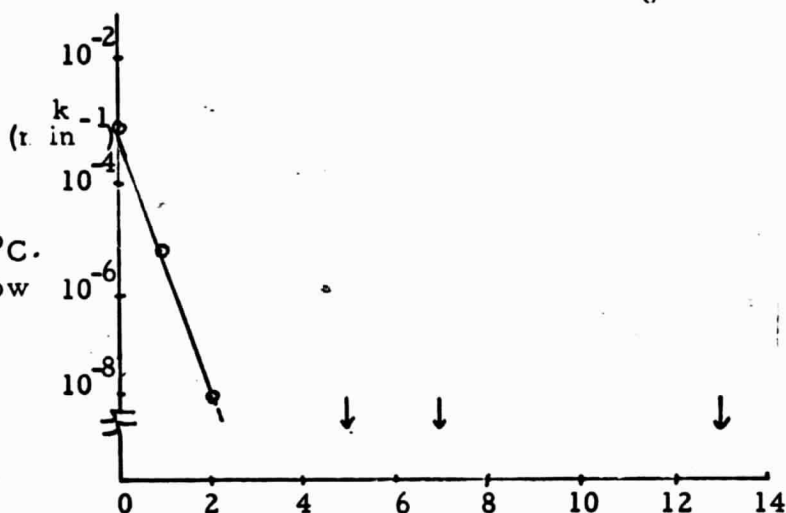
2. Non-Enzymatic Exchange As Functions Of pH

Another project started during the past year is the determination of the rate of non-enzymatic oxygen exchange between NO_3^- or $\text{SO}_4^{=}$ with H_2O as a function of pH and temperature. These studies indicate that the rate of exchange of oxygen from both NO_3^- and $\text{SO}_4^{=}$ is very low at high or neutral pH and is acid catalyzed.

Figure one shows representative data for non-life mediated exchange of oxygen between $\text{SO}_4^{=}$ and H_2O . Other O^{18} exchange data for H_2SO_4 with H_2O has suggested that, at low pH, the exchange occurs via the acid anhydride⁽²⁾:

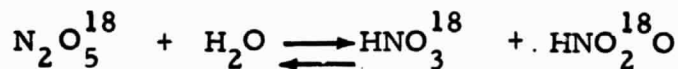
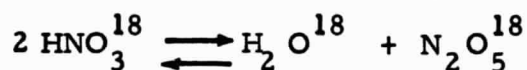
pH Vs. Non-Biological Oxygen Exchange Rate Constant (k) For $\text{SO}_4^{=}$ - Temp 100°C .
 (↓) Measurements Below Detector Sensivity -
 About $k = 10^{-8}$

Figure 1



Our data is not inconsistent with this mechanism.

While more accurate data must be collected for NO_3^- , the relationship between k and pH is generally the same as for $\text{SO}_4^{=}$. There is data⁽³⁾ elsewhere which suggests that this exchange also occurs via the acid anhydride, i.e.



At the temperatures and pH reasonably expected on an extraterrestrial body like Mars both $\text{SO}_4^{=}$ and NO_3^- should have non-biological exchange rates sufficiently low to be essentially undetectable in a life detection experiment.

3. CO_2 vs O_2 Isotopic Mass Ratio Measurements

A complication of our previous technique was the need to measure the $\text{O}^{16}/\text{O}^{18}$ ratio in a separate standard H_2O sample before each reading of an unknown sample. This calibration step was required to correct for drift of the mass spectrometer. Since a non-negligible equilibration time was required before each reading, measurements were time consuming and of inherently compromised accuracy. A new technique has been adopted during the last year; after distilling the water from the sample the distillate is equilibrated with bicarbonate. The exchange of oxygen between H_2O and HCO_3^- is spontaneous. Acidification then releases CO_2 which is fed into the mass spectrometer. The natural ratio of $\text{C}^{12}/\text{C}^{13}$ may be used for calibration by measuring the 44/45 mass ratio. The $\text{O}^{16}/\text{O}^{18}$ ratio is determined from the 44/46 ($\text{C}^{12}\text{O}^{16}\text{O}^{16}/\text{C}^{12}\text{O}^{16}\text{O}^{18}$) ratio.

The second equilibration step, between HCO_3^- and H_2O , requires approximately four hours at room temperature. However there is evidence this time may be reduced to only a few minutes if HOBr is used as a catalyst⁽⁴⁾. The mechanism of this catalysis is unclear.

4. Mechanism of Enzymatically Induced Exchange

A more detailed investigation of the mechanism of biologically-induced exchange is also being undertaken. Yeast inorganic pyrophosphatase

has been shown to promote extremely rapid exchange of oxygen between phosphate and H_2O ⁽⁵⁾. We are now trying to identify the true substrate for this enzyme in a buffered $\text{Mg}^{++}/\text{P}_2\text{O}_7^{-4}(\text{PP}^{-4})$ system. A computer program is nearly completed which will calculate the various equilibrium concentrations of MgPP^{-2} , Mg_2PP , PP^{-4} , HPP^{-3} , H_2PP^{-2} and MgHPP^{-1} from the known equilibrium constants pH and Mg^{++} concentration. We are now measuring the equilibrium constants for each of the dissociation steps at various ionic strengths and temperatures.

PROPOSED WORK

We hope to complete the measurements of non-biological oxygen exchange between $\text{SO}_4^{=}$ or NO_3^- and H_2O as functions of pH and temperature. In addition, this inorganic exchange will be studied in the presence of a variety of organic and inorganic compounds to determine whether any of these substances might stimulate non-enzymatic oxygen exchange and thus render our results ambiguous.

We expect to complete shortly the computer program for calculating equilibrium concentrations of species in a buffered solution of Mg^{++} and PP^{-4} . The next step is to alter the relative concentrations of the equilibrium species and measure the effect of each specie on the initial velocity of pyrophosphatase hydrolysis. From these measurements we hope to identify those species which act as substrate(s) and/or inhibitor(s).

The laboratory techniques have been studied sufficiently to look at ways of simplifying or improving the experimental apparatus for potential development as a functional extraterrestrial life detection experiment. Membrane and mechanical leak inlet systems to the mass spectrometer and collector designs using both cups and slotted plates have been used; close comparison of their relative effectivenesses is required.

REFERENCES

1. Kok, B. and Varner, J., Science 155 1110 (1967).
2. Hoering, T. C. and Kennedy, J. W., J. A. C.S. 79 56 (1957).
3. Tereshkevich, M. O., Pozhidaeva, E. Y. and Dlugoch, R. E., Zh. Obshch. Khim. 37 (1) 17 (1967).
4. Kiese, M. and Hastings, A. B., J. Biol. Chem. 132 267 (1940).
5. Cohn, M. J. Biol. Chem. 230 369 (1958).