"GULLIVER", AN EXPERIMENT FOR EXTRATERRESTRIAL LIFE DETECTION AND ANALYSIS*

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Abstract: Based on the probability that extraterrestrial life biochemically somewhat similar to life on Earth, a life detection experiment is being prepared to explore Mars. The experiment will be performed by an automated device which will carry a microbiological medium being developed to support a wide range of earth microorganisms. Selected ingredients of the medium will be labeled with radioactive isotopes. A sticky string, shot out from and reeled back into the device, will gather a sample of the Martian soil. It is hoped the radioactive atoms in the compounds will be metabolized by the unknown organisms in the soil and evolved in a labelled gas. The gas will be collected by a chemical "getter" and the radioactivity measured for transmission to Earth. A positive response from the test unit and a negative, or lesser, response from a poisoned control unit would constitute evidence of life. The device can also differentiate between photosynthetic and nonphotosynthetic metabolic activity. Data from field tests on Earth are presented.

"Gulliver" [1] is an experiment designed to detect extraterrestrial life and to begin a study of its metabolism. Any first attempt to find extraterrestrial life must be based upon certain assumptions. As will be discussed by Horowitz [2], the Gulliver experiment assumes that:

A. Extraterrestrial life will be of an aqueous, carbonaceous nature.
B. Its biochemistry at the cellular level will be similar to that on earth.
C. If any life exists on an alien planet, the widespread existence of microorganisms is likely.

These hopefully reasonable assumptions provide the basis for the design of an experiment which can be flown and performed in the near future.

Having decided on a probable form of life, the next question is where to seek it. There seems to be general agreement that the planet in our solar system most likely to support extraterrestrial life is Mars. Venus has been mentioned as a possibility, but recent temperature information has been adverse from the standpoint of supporting life as we know it. Lack of knowledge concerning planets beyond our solar system and the inability to send instruments there preclude such exploration from immediate consideration.

Having chosen the type of life to look for and the place to look, the problem of how to implement the search for it remains. This paper offers one approach to that problem.

Because of technical considerations, early experiments with instruments to be landed on other planets may be of relatively short duration. Environmental considerations [3] make it likely that, if life similar to ours does exist on Mars, the number of organisms per unit of surface area is less than on earth. Both of these considerations make it imperative that a life detection test be highly sensitive. Radiosotopes fulfill the requirement for sensitivity. Moreover, they can probe metabolic reactions at the molecular level. These advantages combine to offer a technique that can provide very rapid detection of fundamental metabolic processes. Furthermore, radioisotope techniques can be employed with relatively simple instrumentation which can readily be miniaturized. Power requirements for operation are small.

Essential to the radioisotope approach is the development of a medium, or media, containing appropriately labeled compounds. The ease with which microorganisms can be detected by collecting and counting $C^{14}O_2$ evolved by them from substrates containing C^{14} has been demonstrated [4]. The production of gases is common among earth microorganisms, and probably all species produce carbon dioxide. Other metabolic gases which could be readily labeled in one or more elements are methane, ammonia, hydrogen sulfide and molecular hydrogen. At present, only C^{14} is being incorporated into experimental media because it has served successfully with a wide range of microorganisms.

Two types of media are currently being developed. The first is a complex medium incorporating essential inorganic salts and organic extracts and compounds [1]. This medium is being designed to support as wide a variety of microbial species as possible. Aerobes, anaerobes, facultative anaerobes, thermophiles, mesophiles, psychrophiles, heterotrophs, autotrophs (including phototrophs and chemotrophs), spore formers and nonspore formers have been successfully detected with it. However, some species of microorganisms are known to be inhibited by various organic compounds present in complex media. Therefore, a parallel effort is underway to develop a simple medium which, except for the labeled compounds, contains no organic constituents.

The selection of radioactive compounds for incorporation into the media is based upon their ready metabolic conversion to radioactive gas. The widespread importance of the Krebs cycle in aerobic metabolism and of the Embden-Meyerhof pathway in anaerobic metabolism makes the use of C^{14}-metabolites related to them highly attractive since both sequences of reactions produce carbon dioxide. To date, best results have been obtained with a combination of C^{14}-sodium formate and uniformly labeled C^{14}-glucose. However, various C^{14}-labeled amino acids, microbial extracts and other compounds are being tried. The choice of radioactive substrates for Martian experiments must also involve a decision as to the probable nature of Martian biochemistry. This will be discussed in the paper by Horowitz [2].

When the final media are developed, racemic mixtures of the labeled and nonlabeled optically active compounds will be incorporated where possible since optical specificity may be exhibited by life on Mars and it might be different from that on Earth.

The current model* of Gulliver is shown in fig. 1. The experiment will function in the following manner. At least two instruments, one a test and the other a control, will be incorporated into a capsule destined to land on Mars. Sealed ampoules contain the radioactive medium. The entire instrument is being designed to withstand heat sterilization. When the capsule lands on Mars, squibs will fire the projectiles. Each will deploy a 25 foot length of silicone grease-impregnated retrieval line over the surface of the planet. The motor will then reel the line, together with adhering particulate matter, into the incubation chamber. During this period, the culture chamber will achieve equilibrium with the Mars atmosphere. After the line is retrieved, the incubation chamber will be sealed and the ampoule will be broken, releasing the radioactive medium onto the line. Attached to the outside of the chamber is a thermostatically controlled heater to prevent freezing. If organisms are present on the soil particles and are able to metabolize any of the labeled substrates, radioactive gas will be produced.

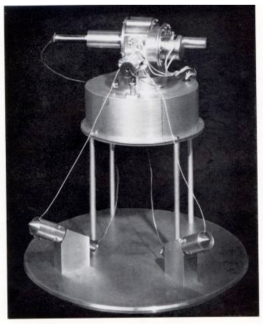


Fig. 1. Gulliver, Mark III.
Size: 158 cubic inches, weight: 1.3 pounds. The instrument is designed to function independent of its orientation. A hydrophobic solid foam baffle prevents the liquid broth from contacting the detector.

A thin window Geiger tube is mounted directly above the culture chamber. It is prevented from "seeing" the radioactive medium by metal and solid foam baffles. However, gas produced by the culture is free to travel through the baffles. The window of the Geiger tube is thinly coated with barium hydroxide which traps carbon dioxide. As $C^{14}O_2$ collects on the Geiger tube window, it is detected by a rise in measured radioactivity. The Geiger tube counting the C^{14} is surrounded by a ring of Geiger tubes connected in anti-coincidence circuitry. Should other radioactive elements be used in the experiment, appropriate "getters" will be applied to the face of the radiation detector.

Simultaneously with the inoculation of the test instrument as just described, the control instrument will also be inoculated. However, shortly after inoculation, its culture chamber will be injected with a broad spectrum anti-metabolite (also under development). All data will be transmitted to Earth by radio. The production of a typical biological growth curve from the test instrument and a negative, or materially reduced, response from the control instrument will constitute evidence for microbial life on Mars. In the event reproduction does not occur during the course of the experiment, respiration of the metabolizing culture could still be detected.

While the present model of Gulliver utilizes Geiger tubes for the detection of evolved radioactive gas, the use of solid state detectors has been explored [1] and internal flow counting is now under study. The latter offers increased sensitivity through better geometry for gas collection and radioactivity counting.

Gulliver has been tested in the laboratory and in the field a number of times. Fig. 2 presents field test data obtained from two of the instruments, one serving as a test and the other as a sterile, uninoculated control. The experiment was conducted near Washington, D.C., this past winter. The 650 CPM evolved by the sterile control in 24 hours is caused by self -degradation of the radioactive compounds. While of a minor magnitude compared to the response from the test instrument, the sterile control does require attention. The current instrument is designed to provide carrier carbon dioxide to flush out radioactive carbon dioxide accumulated during the eight month flight.

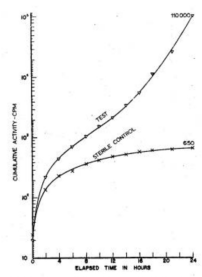
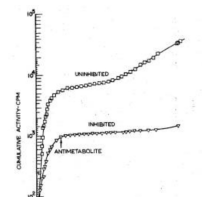


Fig. 2. Field test of Gulliver; November 27, 1962.

The results from a field test in which an antimetabolite was injected into the control instrument are shown in fig. 3. Unfortunately, the timing of the antimetabolite injection coincided with the initial plateau demonstrated by the uninhibited culture. Even so, the difference in the two responses becomes apparent. Future field tests of this nature will determine the most advantageous time to inject the antimetabolite.



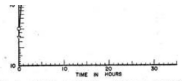


Fig. 3. Field test of Gulliver; February 7, 1963.

While the above curves demonstrate the capability of the instrument, the sensitivity possible with the radioactive method is not yet fully realized. This is demonstrated by table 1 which presents results obtained from small quantities of soil inoculated into radioactive medium in one inch diameter planchets (fig. 4). A planchet containing the soil and medium is merely covered with a second planchet containing a porous pad moistened with a solution of barium hydroxide. Carbon dioxide produced by the culture is trapped on the barium hydroxide moistened pad in the planchet immediately above it. The gas collection planchets are periodically removed, dried and counted for radioactivity in an internal flow counter. With this simple method, responses from soils have been obtained routinely in less than one hour. Efforts continue to modify the Gulliver instrument to achieve this potential sensitivity. However, the problems imposed by the requirements for remote operation make this difficult.

Fig. 4. Planchets used for culturing and gas collections. Left to right: culture planchet being covered with gas-trapping planchet, barium hydroxide moistened pad being inserted into gas-trapping planchet, view of one inch diameter planchet used in test.

TABLE 1

Evolution of $C^{14}O_2$ from various soils incubated in planchets

Test sample	Quantity (mg)	Time elapsed after inoculation (min)	Radioactivity less sterile control (cpm)
*Desert soils**			
#1	25	45	4083
#75	25	45	1478
#1	50	45	3450
#1	50	180	9100
#74	50	45	336
#74	50	180	1140
#75	50	45	4760
#75	50	180	11800
#1	100	210	5725
#74	100	210	2799
#75	100	210	5099
Garden soils			
A	100	45	6747
B	100	45	7132
C	100	45	1040
Field soils			
	10	30	3802
	25	45	4000
		180	8750
	50	45	5000
		180	8150
	100	45	5550
	100	180	10300

Data are taken from many experiments carried out under various conditions and in different media.

*Desert soil samples, courtesy of Jet Propulsion Laboratory, California Institute of Technology.

While not shown on the current model, modifications are planned to enable the experiment to distinguish photosynthetic activity. This is possible with relatively simply modification as demonstrated in fig. 5. The curves show the responses of *Chlorella pyrenoidosa* cultures grown in simulated Gulliver chambers altered to permit light from fluorescent tubes to be admitted or excluded by the movement of a slide. The cultures contained equal inocula. One was maintained continually in the light and another continually in the dark as references. It is seen that more $C^{14}O_2$ was evolved from the labeled medium in the dark phase than in the light phase. This is because most of the $C^{14}O_2$ expired by the culture in the light was fixed by photosynthetic activity while the $C^{14}O_2$ expired in the dark was available for collection and counting. The two curves oscillating between the light and dark phases demonstrate the rapidity with which photosynthetic activity can be detected. Grown in the light for less than two hours, the cultures released little $C^{14}O_2$. When light was excluded, the rate of $C^{14}O_2$ evolved from the culture increased approximately five-fold within 45 minutes. When light was again admitted, $C^{14}O_2$ evolution promptly fell. An almost identical response occurred through another complete dark-light cycle. Thus the possibility of detecting photosynthetic activity on Mars by using natural or artificial light to modulate $C^{14}O_2$ production by the photosynthetic component of the culture is apparent.

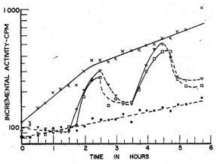


Fig. 5. $C^{14}O_2$ evolved by *Chlorella Pyrenoidosa* in response to light and dark growth cycles. ——— dark cycle, – – – light cycle. Medium: Urea agar with 1-C^{14} sodium lactate (2×10^{-3}M).

What is the significance of the data that Gulliver might transmit to Earth? A negative result, if instrument failure is precluded, means that life similar to that on Earth was not found at the time and place the Martian surface was sampled. Speculative exploration would probably continue until manned landings on Mars had been achieved, or, possibly, until the earlier date when a sample of Martian soil had been returned to Earth by instrument. A positive result from Gulliver will do more than establish the existence of extraterrestrial life although that objective in itself seems adequate to justify an interplanetary experiment. In the event reproduction occurs, more than one exponential growth phase, such as is seen in fig. 2, would indicate the presence of more than one type of organism. Gross characterization of the organism may begin with the determination of the lag phase which the curve may reveal. This characterization would be augmented by the generation period which can be obtained readily from the slope of the exponential portion of the curve. If the curve were synchronously modulated by the admission and exclusion of light to the culture chamber, the presence of photosynthetic organisms would be strongly indicated.

If life is found on Mars, Gulliver can be modified to perform more refined biochemical experiments on subsequent missions. The use of multiple chambers will permit the study of the organisms under controlled environments with selected substrates, cofactors or inhibitors. These may be present initially or added during the course of the experiment. The aim of such studies, and of the initial phase of exobiology, would be to determine if life forms on Earth and Mars arose independently, or from a common origin. In the case of the former, this would clearly imply that, under the appropriate environmental conditions, the evolution of life might be expected to be widespread throughout the universe. If life on Mars and Earth shared a common ancestry, investigations would seek to determine whether one planet in some way inoculated the other or whether both were inoculated from a third source.

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