Reprinted from:
RADIOISOTOPES FOR AEROSPACE
Part 2: Systems and Applications
(Plenum Press, 1966)

EXTRATERRESTRIAL LIFE DETECTION WITH ISOTOPES AND SOME AEROSPACE APPLICATIONS

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Two of mankind's newest technologies, radiobiology and space travel, are being combined in an attempt to solve the age-old question of whether or not there is life on other planets. A radioisotopic biochemical probe is now under development in the hope one: to will be part of an instrument package to be landed on Mars within the next few years.

The experiment, named "Gulliver," supplies a nutrient medium containing radioactive substrates to a sample of extraterrestrial soil. If any microorganisms are present in the soil, one or more of the labeled compounds may be assimilated, metabolized, and evolved as a gas or gases containing the label. The detection of labeled gas evolved from the soil culture would constitute evidence for life.

The radioisotope tracer method offers several important advantages over conventional microbiological techniques:

1. The sensitivity makes it possible to detect low levels of metabolic activity which might be characteristic of life in the relatively cold, dry environment of Mars.
2. The rapidity of response increases the opportunity for obtaining significant results during the uncertain lifetime of the instrument capsule.
3. The radioactivity counting system is relatively simple, requires little power, and can be made lightweight, small, and rugged.
4. The type of readout permits transmission of the data to Earth without demanding an undue share of the limited bit rate available for the entire spacecraft.

The life detection system has been instrumented and extensively tested in the laboratory and in the field. To date, of more than 100 species of microorganisms tested, none has failed to produce a positive response. Laboratory and field tests with a similar number of natural soils have been equally successful.

A considerable portion of the Gulliver program[1,2,3] has been devoted to development of nutrient media capable of sustaining metabolism and growth with a wide variety of microorganisms including heterotrophs, phototrophs. chemotrophs, aerobes, and anaerobes. The two principal media developed which have demonstrated this capability are shown in Table 1. The M9 medium was designed by gradually adding selected nutrients to a simple inorganic salts medium. The M10 medium is the product of continual simplification of a complex growth medium.

Table 1. Basal Media for Gulliver Experiment.

	M9	M10
NaCl	0.1 g/L	0.0063 g/L
K_2HPO_4	1.0	0.063
$MgSO_4$'$7H_2O$	0.2	0.013
KNO_3	0.5	0.031
Malt extract		0.19
Beef extract		0.19
Yeast extract		0.81
Ascorbic acid		0.13
L-cysteine		0.044
Bacto-casamino acids		0.25
Proteose peptone #3		1.25
Soil extract*	100.0 ml/L	16.0 ml/L
	pH 7.0	

*Soil extract prepared by suspending 500 g. of air-dried soil in 1300 ml. H_2O containing 0.1% Na_2CO_3. The mixture is autoclaved for one hour, filtered, and liquid loss made up to 1000 ml. with water.

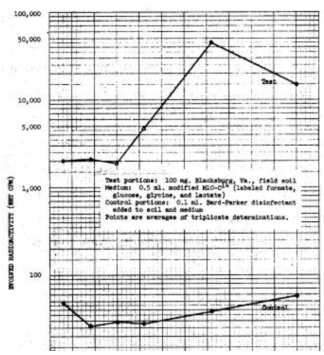
The radioactive substrates selected for incorporation into toe media are listed in Table 2.

Table 2. C^{14} Substrates Used with Gulliver Basal Media.

Substrate	mc/mM	mc/ml	mM/L	%(W/V)
Sodium formate	25.00	6.0	0.24	0.002
D-glucose-U. L.	4.73	1.3	0.28	0.005
DL-sodium lactate-1	5.00	1.3	0.26	0.002
Glycine-1	4.42	1.0	0.22	0.002
Total	-	9.6	1.00	0.011

The life detection principle is illustrated in the following simple experiment. One-hundred-milligram portions of a mixed soil sample taken from a field at Blacksburg, Virginia, were dispensed into two sets of one-inch-diameter planchets. To each planchet was added 0.5 ml. of the labeled medium. To the planchets in one set, 0.1 ml. of an antimetabolite, Bard-Parker disinfectant, was added. The other planchets received 0.1 ml. of distilled water. All planchets were incubated at room temperature. Collections of $C^{14}O_2$ evolved from each planchet were made for 15 minutes at selected hours.

To collect the evolved gas, porous filter pads were fitted into the bottoms of one-inch-diameter planchets identical to those containing the soil and medium. To each pad was added three drops of a saturated solution of barium hydroxide. These planchets were then inverted over the culture planchets for the 15-minute gas collection period. Any $C^{14}O_2$ by the culture planchets was precipitated on the filter pad. The filter pad planchets were removed, dried, and counted for radioactivity. The results, shown in Fig. 1, demonstrate metabolic activity within one hour followed by the onset of the classical growth and declining population curve. Comparison with results from the controls demonstrates a high level of significance.



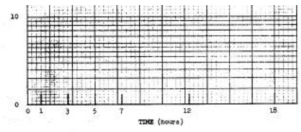


Fig. 1. Detection of microorganisms in soil by radioisotope method.

The second major phase of the Gulliver development program has been devoted to instrumentation. The instrument shown in Fig. 2, about as large as a pint jar and weighing approximately two pounds, has been repeatedly field tested including tests at the 12,000-foot elevation on White Mountain, California; on sand dunes in Death Valley, California; and on saline desert flats at the Salton Sea, California. In each case, life was detected within a maximum of several hours.

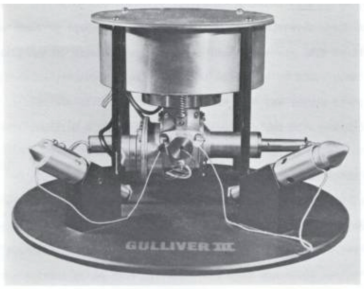


Fig. 2. Gulliver – a radioisotopic, biochemical probe to detect extraterrestrial life.

Upon landing on one planetary surface in an instrument capsule, Gulliver fires two or more string-deploying projectiles. Coated with silicone grease to make them sticky, the strings are wound back into the incubation chamber, collecting particulate matter from the ground surface. A glass ampul is then broken, releasing radioactive medium onto the string and particulates. The medium is then flushed with carrier CO_2 to remove any $C^{14}O_2$ produced by self-degradation of the labeled compounds during the long journey through space. A sea is opened to permit gas to travel from the incubation chamber to the surface of the geiger window which is coated with a "getter" film of lithium hydroxide. A baffle prevents the geiger tube from seeing the radioactive medium. Radioactivity counts of gas captured by the getter are made at selected intervals and the data transmitted back to Earth. Each capsule would contain a minimum of two Gulliver units, one to serve as the test unit and the other as the poisoned control just as in the case of the laboratory experiment described above.

During one of the field tests, it was noted that the rapidity and rate of response of the method was unexpectedly high when a drop of the medium was placed directly on the undisturbed soil. Further tests confirmed the advantages of such an in situ determination. Apparently, the micro-environment is adversely disturbed within the collected sample. Currently, an in situ model of Gulliver, weighing approximately three ounces, is under development as seen in Fig. 3. A number of such units might be fired from the instrument capsule after landing on the planetary surface. Each would be connected to the central capsule by an umbilical cord for power supply and data transmission. The units are self-righting and make contact with the ground surface by means of a bellows. The radioactive medium is introduced directly onto the soil surface. Duplicate units containing the antimetabolite serve as controls.

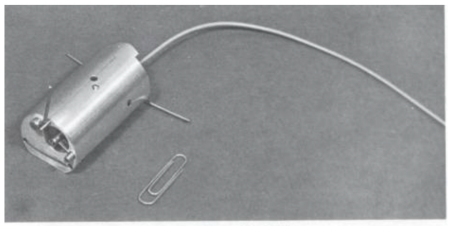


Fig. 3. In situ model of Gulliver.

It is highly likely that photosynthetic organisms play a primary role in any biosphere. An innovation in the Gulliver experiment is designed to detect extraterrestrial photosynthesis. The photosynthesis test developed has an advantage over wet culturing methods in that it does not impose an aqueous environment on the alien organisms.

In operation. a small portion of the ground surface is covered under a cup-like shell. The trapped atmospheric gases are replaced with $C^{14}O_2$ and a light is turned on to induce photosynthesis by any organisms on the covered surface. After a selected interval, the remaining $C^{14}O_2$ is completely replaced with planetary atmosphere. The light is then turned off. If, as on Earth, the organisms must continue to metabolize, they should consume some of the energy compounds just photosynthesized and give back $C^{14}O_2$ in the process. This system has been applied to algae with the results shown in Table 3. The quantity of $C^{14}O_2$ evolved in the dark by the pre-illumined cultures compared to that from the cultures pre-exposed to $C^{14}O_2$ but not light is evidence for photosynthesis. The case can be strengthened by alternate exposure to light and dark following the first simultaneous exposure to $C^{14}O_2$ and light. $C^{14}O_2$ is evolved in the dark, but not in the light because of the renewed onset of photosynthesis.

Table 3. Detection of Photosynthesis by Radioisotope Method.
Test Organism C. Pyrenoidosa.

- TREATMENTS (30 min. each)	NET RADIOACTIVITY - CPM					
	$C^{14}O_2$ EVOLUTION			NET $C^{14}O_2$ FIXATION		
	REPLICATE		MEAN	REPLICATE		MEAN
	1	2		1	2	
Live cells, pre-illuminated	2398	2514	2456	50,773	56,251	53,492
Live cells, continuous darkness	53	53	53	658	644	651
Killed cells, pre-illuminated	10	0	5	152	-	152
Killed cells, continuous darkness	7	3	5	4	3	4

The National Aeronautics and Space Administration plans to take extreme care to avoid contamination of the planets with organisms inadvertently carried from Earth aboard spacecraft. Such contamination could render life detection experiments on the planet ambiguous and could conceivably influence the course of evolution of the planet. Present plans call for terminal heat sterilization of the spacecraft. The very methods developed for the detection of extraterrestrial life might prove useful in a final, prelaunch, determination of one effectiveness of one sterilization procedure.

In the closed ecological system of the cabin, the contamination problem will become increasingly important as the duration of flight is extended. The bacteriological quality of the potable water, treated wastes, and the spacecraft atmosphere could be monitored in a manner that would actuate feedback control for various disinfection processes. Indeed, the original need giving rise to the radioisotope method of bacterial detection was that for a rapid method for the detection of coliform organisms in drinking water to determine sewage pollution. Here, the medium and growth conditions were carefully designed to select E. coli in contrast to the nonspecific response desired in Gulliver. The method requires four to six hours compared to 24 to 96 hours required by standard methods[5]. Table 4 presents confirmed results obtained within four hours over a wide range of E. coli populations.

Table 4. Four-Hour Radioisotope Tests for E. coli in MF MacConkey Broth.

INOCOLUM (Av. no cells from replicate pour plates)	EVOLVED RADIOACTIVITY (Av. net cpm for 5 replicates)	EVOLVED RADIOACTIVITY PER INITIAL CELL (Av. net cpm)
12	57	4.75
28	263	9.40
77	625	8.12

83	807	9.73
85	391	4.60
975	7,120	7.30
1,170	5,540	4.73
2,460	16,600	6.75
9,820	70,600	7.19
41,600	221,000	5.08

After Levin et al. Reproduced courtesy J. Water Pollution Control Federal 33, 1024 (1961).

The method might also be useful in combating the jet fuel contamination problem. At desired intervals, aliquots of the fuel could be drawn through a membrane filter and the presence of any organisms on the filter detected by the radioisotope technique. The method could be automated or a simple, manually-operated, portable kit could be developed.

Man has already endured space flights of up to days. Considerably longer flights are planned for the future. Bacterial infections which might develop in the crew during extended flights could seriously affect the health of the astronauts and imperil the mission. The radioisotope technique can be utilized to select the antibiotic of choice and thus permit appropriate chemotherapy with minimum delay[6]. An effective antibiotic is indicated by a reduction in $C^{14}O_2$ production from aliquots of patient material inoculated into portions of radioactive medium containing various types and concentrations of antibiotics. Table 5 illustrates results obtained in a single antibiotic sensitivity test made directly on patient material. Conventional tube dilution antibiotic sensitivity tests simultaneously made were subsequently found to be in agreement with the radioisotope results.

Table 5. Rapid Determination of Antibiotic Sensitivity of Urinary Infection Made Directly on Patient Material.

ANTIBIOTIC	RADIOISOTOPE TEST (NET CPM EVOLVED AFTER INDICATED LENGTH OF INCUBATION)		CONVENTIONAL 24-HOUR TUBE DILUTION TEST
	1 hr.	3 hr.	
None	2,163		Growth
Chloramphenicol			7
1 mcg/ml	1,459	17,187	Growth
5 mcg/ml	1,084	781	No growth
50 mcg/ml	623	337	No growth

Patient material courtesy of Dr. J.A. Curtin, Washington Hospital Center, Washington, D.C.

Long-term space flights might require the use of algae in a closed ecological system. Should the algal culture fail, or the respiratory quotient change significantly, the consequences would be dire. The method f or monitoring photosynthesis with $C^{14}O_2$ might be used in a pilot cell of a spacecraft algal system. Feedback mechanisms might control light, gasification, and nutrient flow to keep the main algal culture working within prescribed limits.

Other aerospace applications are also possible. The rapidity of the radioisotope technique, its sensitivity in monitoring metabolism and growth, and the ability to narrow or broaden the range of types of microorganisms responding would seem to offer useful applications in many aspects of detection, identification, control, and utilization of extraterrestrial or terrestrial microorganisms.

ACKNOWLEDGMENTS

The Gulliver program is supported under contract with the National Aeronautics and Space Administration. Norman H. Horowitz, Professor of Biology, California Institute of Technology, is co-experimenter with one author.

Research on the rapid method for coliform organism detection was supported by the Atomic Energy Commission and the Public Health Service.

In addition to expressing gratitude to the coauthors of the various papers cited herein, the author acknowledges the assistance of the professional personnel of Hazleton Laboratories who have worked with him on the several projects contributory to this paper. In particular among these he wishes to thank Mr. George Perez for the instrumentation of Gulliver and Dr. John Barnes for executing the work on photosynthesis detection.

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