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EXPERIMENTS AND INSTRUMENTATION FOR EXTRATERRESTRIAL LIFE DETECTION

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I. Introduction

Any treasure hunt should begin with a description of the object sought. Yet, in the search for extraterrestrial life, the greatest treasure hunt in history, this is not possible. Fortunately, a few considerations [largely discussed by Drs. Brown and Vishniac at this symposium] somewhat reduce this handicap in the impending quest:

1. It seems logical to concentrate the search on relatively primitive types of microbial life. It is possible to imagine, especially from what we already know about environmental conditions on the planets in our solar system, that life on Mars, for example, might be limited to such relatively primitive forms. On the other hand, it is very difficult to imagine an ecology in which only highly organized macroorganisms exist. Such a situation could not provide for the necessary degradation of complex biochemicals and recycling of the components. Regardless of the level of biological development in any ecosphere, it would seem that microorganisms would be essential if the life process were to be sustained.

2. Chemical considerations make carbon the most likely candidate element on which extraterrestrial life would be based.

3. Chemical considerations imply that all biochemical reactions probably take place in aqueous solution.

4. Because the prime source of the energy required to sustain life for evolutionary periods is the sun, photosynthetic forms seem essential in any extraterrestrial population. They may or may not be accompanied by other forms of autotrophs or by heterotrophs.

5. Finally, it is likely that the microorganisms would be widespread over the planet.

II. Sampling

It is fortuitous that microorganisms are the principal life form of interest. This considerably reduces the complexity of obtaining a sample for testing in an automated instrument landed on the planet. However, this is not to imply that obtaining a sample of microorganisms will be simple. The samples should be taken from an area unaffected by the landing of the. spacecraft. If retrorockets are used during landing, contamination may be widespread. Samples should include vertical profiles from the surface to considerable depths. The mere taking of a sample of material, such as loosely conglomerated soil, may produce profound changes in the material. Equilibria and physical arrangements important to biological activity could be disrupted. Above all, great care must be taken against the possibility of contaminating the sample with terrestrial microorganisms. The stringent precautions necessary to prevent planetary contamination are discussed elsewhere by Mr. Hall.

III. Life Detection Methods

Once an extraterrestrial sample is obtained, how is it to be examined for evidence of life? Obviously, this is the most difficult part of the problem. An unknown life form, operating on unknown biological processes in an unknown environment presents an unprecedented challenge to detection. Six general categories of experiments are being developed for the National Aeronautics and Space Administration: (a) physical and chemical assays for simple organic compounds of biological interest, (b) morphological evidence of living forms, (c) assays for intermediate or complex biochemicals, (d) evidence of metabolism, (e) evidence of growth, and (f) evidence of reproduction. These attributes of life are cited in the increasing order of significance I would ascribe to them. Experiments seeking physical and chemical determinations of relatively simple compounds would be those most likely to yield positive results. However, the evidence thus obtained could not establish the presence of life. Morphological distinctions between living and nonliving forms at the microscopic level are frequently difficult on Earth. Interpretation of micrographs may be impossible when unfamiliar forms of life are encountered in a strange inorganic matrix. Organic compounds, including moderately complex ones, are known to be generated abiogenically. On the other hand, while those experiments seeking evidence of reproduction would yield the most conclusive results regarding the existence of life, they are the least likely to be successful. This is because they must be based on assumptions, such as those discussed earlier, concerning the general nature and function of the living systems sought. However, if successful, not only could these experiments establish the presence of life, they might determine metabolic rates, metabolic pathways, the mode of growth, and the generation period. Through such experiments, ultimately, the nature of the life encountered could be compared with terrestrial life for the paramount determination of whether the two forms of life are similar. It is in the categories of metabolism growth, and reproduction that I would like to describe some experiments that my co-workers and I have been developing over the past several years.

A. GULLIVER

Gulliver (1, 2, 3) offers radioactive substrates containing ^{14}C and ^{35}S in aqueous solution to the sample. If organisms are present, and they can metabolize one or more of the labeled substrates, the production of radioactive gas is likely. A "getter" collects the gas so produced by chemically precipitating it on a surface monitored by a radiation counter. An exponential increase in the output from the counter is indicative of growth or reproduction. In the event metabolism occurs in the absence of growth or reproduction, this will be indicated by any significantly positive slope to the curve generated. The curve produced by the test unit is compared to that obtained from an identical, but inhibited, control unit. Inhibition is induced by the application of heat or a chemical antimetabolite. The object of the control is to differentiate between a metabolic response and an inorganic reaction with the extraterrestrial sample. Extensive laboratory and field tests (3) have supported the general applicability to terrestrial microorganisms of the media developed and the antimetabolite selected.

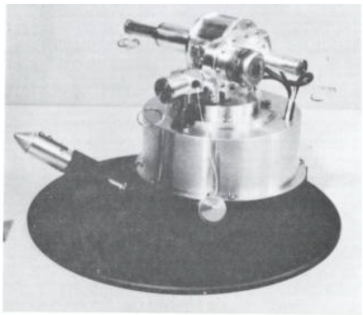

FIG. 1. Mark III model of Gulliver. The projectiles deploy the string over the planetary surface. The string carries particulates back into the incubation chamber where the radioactive broth is applied. The geiger counter and electronics are housed in the cylinder resting on the display base.

The Mark III version of Gulliver, shown in Fig. 1, has been widely tested in the laboratory and field. Placed in a simulated capsule, as shown in Fig. 2, the instrument has performed in extreme terrestrial environments. One test was made at the 12,000-foot elevation of White Mountain, California. The temperature was below freezing at this barren location (Fig. 3) above the timber line. Positive results were obtained within 1 hour. Tests on a sand dune in Death Valley, California, (Fig. 4) produced counts significantly above those of the inhibited control within 2—3 hours. Similar results were obtained on the desert salt fiats near the Salton Sea, California.

During the desert tests, an experiment was performed in which the radioactive substrate was applied directly to the soil. An almost immediate, high level response resulted. After only several minutes of gas collection, the activity exceeded that of the Gulliver III instrument by approximately an order of magnitude. The advantage

seems to accrue from the minimal disturbance imposed on the microenvironment by this *in situ* method. A new model of Gulliver, Mark IV (Fig. 5), was designed and fabricated to take advantage of this finding. As currently visualized, a number of these miniaturized instruments would be ejected from the landing capsule. The units might all be replicates or groups of replicates containing different media or antimetabolites. Each unit is self-righting and contains all necessary components, including a Geiger counter and associated electronics, to conduct the life detection test directly on the "soil." Unlike, Mark III, which samples only the surface material, Mark IV examines the site to the depth penetrated by the medium released. Power is supplied from the spacecraft through an umbilical cord which also serves to relay the data to the central capsule for processing and transmission to Earth.

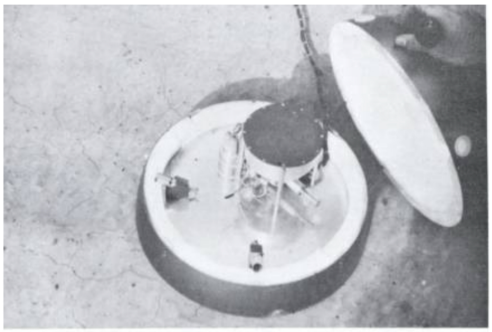

FIG. 2. Mark III Gulliver in simulated planetary lander capsule. Projectiles fire through the assembled housing. Note that opposite end of unit is up compared to Fig. 1. Instrument operates in any position.

B. HETEROTROPHIC PHOTOSYNTHESIS

The probability that any life on Mars must obtain its ultimate energy from the sun is high. Tests for photosynthesis thus assume considerable importance. An experiment has been developed (3) in which the photosynthesis of algae is detected through the dark evolution of radioactive carbon dioxide derived from labeled glucose. The organisms assimilate the glucose heterotrophically. When exposed to light, they retain and fix the carbon dioxide produced. In the dark, however, endogenous respiration releases the recently fixed $^{14}CO_2$ which is then detected in the Gulliver fashion. Data obtained from such an experiment are presented in Fig. 6. Evidence for photosynthetic activity is revealed by the correlation of the evolution of $^{14}CO_2$ with the light and dark periods.

FIG. 3. Mark III Gulliver in test position on bleak, 12,000-foot elevation of White Mountain, California. Reproduced from Levin, G. V., and A. H. Heim (5).

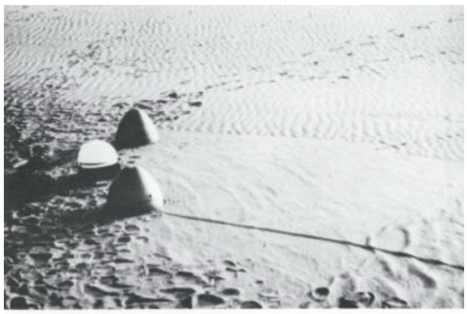

FIG. 4. Mark III Gulliver in test position on sand dune in Death Valley, California.

C. AUTOTROPHIC PHOTOSYNTHESIS

This experiment (3) seeks evidence for strict phototrophs. It is probably the least geocentric of any of the experiments described in this report. Its only assumption is that carbon dioxide will participate in a gas exchange step of the photosynthetic process. The detection of large quantities of carbon dioxide in the Martian atmosphere supports this possibility. In essence, a small portion of the planetary surface material and overlying atmosphere are enclosed. A trace quantity of $^{14}CO_2$ is then introduced into the trapped atmosphere in the presence of light. Time is allowed for any photosynthetic organisms present to fix carbon dioxide, including some of that labeled. The atmosphere is then replaced with Martian atmosphere and the light excluded. The evolution of $^{14}CO_2$ through endogenous respiration by the photosynthetic organisms present is then monitored in the Gulliver fashion. Data obtained from a laboratory experiment with algae are given in Table I.

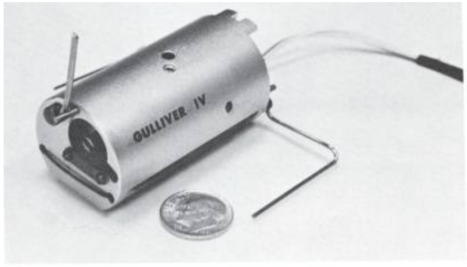

FIG. 5. Mark IV Gulliver, *in situ* model. Fired from the landed capsule, this unit is self-righting and contains the radioactive broth, the Geiger counter and associated electronics, and all the electromechanical components needed for automatic operation. Low voltage power is supplied through the umbilical cord, right, through which the data are also returned to the spacecraft.

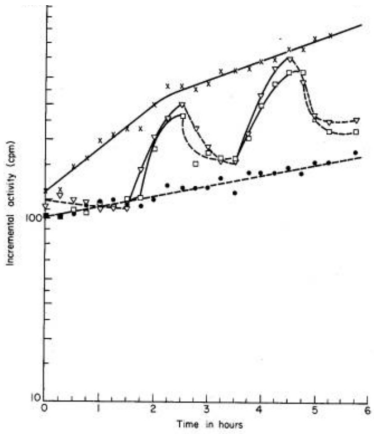

FIG. 6. Heterotrophic photosynthesis experiment, laboratory test data. Medium, urea agar with sodium lactate-1-^{14}C (2×10^{-3} *M*, 10 μcuries/ml.) Activity includes sterile control and background. Activity is $^{14}CO_2$ evolved by *Chlorella pyrenoidosa* in response to light and dark growth cycles. - · · · · - Light control. -×-×- Dark control. ∇−∇−∇ Tests. -□-□- Tests: — — — In light —— in dark. Reproduced from Levin *et al.* (5) p. 130.

D. DIOGENES

The intermediate compound adenosinetriphosphate (ATP) is present in all living terrestrial cells. A very sensitive assay for ATP can be performed using the luciferase enzyme system present in the lantern of the firefly. These two established facts have been combined to produce a highly sensitive, general life detection test (4, 5, 6). A sample of the material to be tested for microorganisms is treated in a manner to release microbial ATP, for example, by extraction with dimethylsulfoxide. An aliquot of this extract is then injected into a solution containing the luciferase system extracted from the firefly lantern (luciferase, luciferin, and magnesium ion in the presence of dissolved oxygen). Any ATP present will result in the production of light. The peak intensity of the light produced is directly proportional to the amount of ATP present. The reaction is monitored in an instrument containing a photomultiplier tube and the result may be displayed on an oscilloscope or recorded on a strip chart. The laboratory instrument built for this purpose is shown in Fig. 7. A typical response recorded by a Polaroid photograph of an oscilloscope is seen in Fig. 8. Developments in the biochemistry and instrumentation of this experiment make it possible to detect approximately 200 *Escherischia coli* or one yeast cell in a total elapsed time of less than 2 minutes. Figure 9 shows a feasibility model of an instrument developed for the Goddard Space Flight Center of NASA. Flight models of this instrument would be carried aboard rockets to make real-time determinations of microbial ATP collected in the upper atmosphere. The instrument can make four assays in a 2-minute period. The results would be transmitted to a ground station by radio.

TABLE 1
LABORATORY DETERMINATIONS OF AUTOTROPHIC PHOTOSYNTHESIS DETECTION EXPERIMENT. TEST ORGANISM: *C. pyrenoidosa* [a]

Treatments (30 minutes each)	Net Radioactivity (C.P.M.)					
	$^{14}CO_2$ Evolution			Net $^{14}CO_2$ Fixation		
	Replicate		Mean	Replicate		Mean
	1	2		1	2	
Live cells, preilluminated	2398	2514	2456	50,733	56,251	53,492
Live cells, continuous darkness	53	53	53	658	644	651
Killed cells, preilluminated	10	0	5	152	–	152
Killed cells, continuous darkness	7	3	5	4	3	4

[a]Reproduced courtesy of: *Proc. 12th Ann. Am. Astron. Soc., Meeting, Anaheim, California, May, 1966.*

ATP has been abiogenically synthesized under supposed primitive Earth conditions (7). Thus, while its presence on another planet would be of great biological interest, it would not establish the existence of life. However, the incorporation of the "delta time" concept into the experiment does convert it into a life detection test. Thus, the determination of an increase in ATP content with time in a culture of the sample material would constitute almost unimpeachable evidence for life.

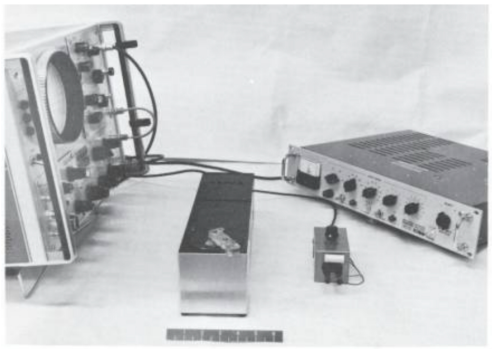

FIG. 7. Laboratory model of instrument developed for assay of microbial ATP by firefly bioluminescent method. Left to right: oscilloscope for readout; instrument proper, housing reaction chamber and photomultiplier tube; nulling circuit (foreground); power supply. Reproduced from Levin, G. V. *et al.* (6a).

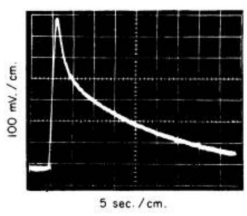

FIG. 8. Typical response of ATP assay, Polaroid photograph of oscilloscope tube.

Off-scale response produced by ATP from approximately 1000 yeast cells. Full scale deflection = 10 cm.

E. PHOSPHATE UPTAKE

It is believed that all terrestrial organisms require inorganic orthophosphate for the production of ATP and nucleic acids. Accordingly, the uptake of orthophosphate from solution can be indicative of metabolism. Such uptake can take place even in the absence of growth or reproduction (8). A life detection test has been developed to the point where approximately 200 *E. coli* per milliliter of medium can be detected within 3−5 hours by following the disappearance of dissolved phosphate from the culture medium. As in the case with Gulliver, an inhibited control is used in the experiment.

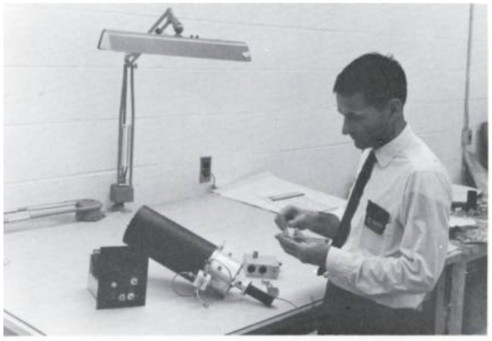

FIG. 9. Feasibility model of rocket-borne, ATP assay instrument. Instrument can extract ATP from particulates and conduct quadruplicate assays within total elapsed time of 2 minutes.

Experimental data obtained with this technique are presented in Fig. 10. The antimetabolite used in the control was 2,4-dinitrophenol which is known to uncouple oxidative phosphorylation. At the indicated intervals, aliquots of the cultures were removed and filtered. They were then assayed for orthophosphate by the stannous chloride-ammonium molybdate method. Radioactive phosphorus was not used because its short half-life precludes its use in a Mars probe. It is interesting to note that the control culture took up some orthophosphate, as would be expected in that 2,4-dinitrophenol uncouples oxidative phosphorylation only, permitting substrate phosphorylation to continue. When a general poison is used, no uptake is observed.

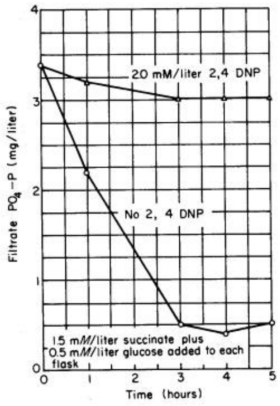

FIG. 10. Phosphate uptake experiment with wild sewage microorganisms. Note effect of 2,4-dinitrophenol (DNP) in uncoupling oxidative phosphorylation. Reproduced from Levin, G. V. and J. Shapiro (8a).

The use of phosphate uptake as a technique for seeking to detect extraterrestrial life brings into play another element essential for terrestrial life. Thus, the phosphate uptake test provides an opportunity to detect noncarbon-based life as well as carbon-based life. The possibility of the evolutionary incorporation of phosphate into any living system seems strongly directed by the high-energy capacity associated with the phosphate timer.

F. SULFUR UPTAKE

This technique (9) seeks to detect the metabolic uptake of inorganic sulfur as an index for life. Of particular interest is the uptake of the sulfate ion. On the basis of chemical considerations, high-energy bonding associated with sulfate polymers is a good candidate to substitute for the role of phosphate polymers in biological energy transfer. This consideration, together with the fact that sulfur is an essential element for all forms of terrestrial life, provides another independent means for seeking extraterrestrial life. The half-life of sulfur is sufficiently long to permit radioisotopic techniques to be used in a Mars probe. Inorganic forms of sulfur other than sulfate may also be included in the test medium. The suspected organisms can be filtered and examined directly for incorporation of the isotope, or the medium can be dried and assayed for radioactivity as evidence of uptake by the microorganisms. A control is also incorporated as part of this experiment.

IV. Automated Microbial Metabolism Laboratory

A major development in the preparations to search for extraterrestrial life has occurred over the past 2 or 3 years. This has been the realization by NASA and the biological community that the expense and importance of the planetary exploration program requires the integration of a number of individual experiments into a single instrument package (10, 11). This package would constitute an automated laboratory to be landed on the surface of the planet. In agreement with this philosophy, my co-workers and I have been developing a relatively simple version of such an automated biological laboratory which we have designated as the Automated Microbial Metabolism Laboratory. Development of the biological experiments and conceptual engineering of the AMML is underway (9, 12). The AMML is anticipated to weigh less than 25 pounds and could serve as the biological laboratory on a relatively small planetary lander. If, on the other hand, the first planetary lander will have a very large payload capacity, the AMML could serve as a subsystem of the total laboratory.

In essence, the six metabolic experiments just described and six associated physical determinations of biological interest are incorporated into the AMML in a manner to make common use of various subsystems and to use standardized modules for others. The physical measurements serve two purposes. They are required for interpretation of the biological results and, with relatively minor modifications, they can serve to make measurements of the environment. Specifically, the parameters to be determined are: (a) temperature, (b) atmospheric oxygen, (c) pH of the surface material, (d) ambient light intensity, (e) background radiation, and (f) soluble phosphate content of the surface material. Temperature measurements would be made by means of a thermister and are required in the metabolic experiments for an assessment of the influence of temperature on the metabolic rates monitored. Oxygen will be determined by an oxygen electrode. This measurement is very important to the photosynthetic experiments to determine whether any photosynthesis detected is of the plant or bacterial type, i.e., whether oxygen is produced or not. A pH electrode would be used in culture experiments to help interpret the data obtained. The ambient light intensity incident to the planetary surface would be measured by superimposing neutral density filters over the photomultiplier tube which serves as a central sensor for the metabolic experiments. Background radiation can be determined in conjunction with the radioisotope experiments. Soluble phosphate content of the surface material can be obtained by a “zero-time” measurement of the culture in the phosphate uptake experiment.

An attempt is being made to convert all of the metabolic readouts to light pulses and thereby utilize a common sensor system, a photo-multiplier tube circuit, for the six metabolic experiments. The isotopic experiments would use scintillators to transduce the beta particles into photons. The output of the ATP experiment is already in the form of light. Attempts are being made to convert the phosphate assay output into light. One possibility is to complex the phosphate with triethylamine containing labeled carbon. Triethylamine quantitatively precipitates orthophosphate (13). Through the use of carbon-labeled triethylamine, the orthophosphate can be determined by measurement of the radioactivity of the precipitated complex. As in the case of the other radioactive tests, the beta emissions would be converted to light pulses.

A schematic of the proposed AMML is shown in Fig. 11. Figure 12 shows a conceptual layout of the instrument. In summary, this instrument would examine an extraterrestrial sample for metabolism, growth, or reproduction through monitoring the biological interface with the environment for the involvement of carbon, sulfur, oxygen, phosphate, and light. It would seek these interactions in both heterotrophic and autotrophic systems. Further, it would look for the production of the intermediate compound ATP. Each of these "windows" into the living process could possibly answer the question of whether life exists. However, incorporated in this fashion, the experiments create a sum greater than its parts. This is because, although separate and diverse, the experiments reinforce and extend each other. The results of one may permit an otherwise impossible interpretation of another which, by itself, might yield doubtful results. For example, the phosphate and sulfur tests might indicate the presence of life which, yielding negative results in the ATP test, would thereby be shown to possess an intermediary metabolism considerably different from terrestrial forms.

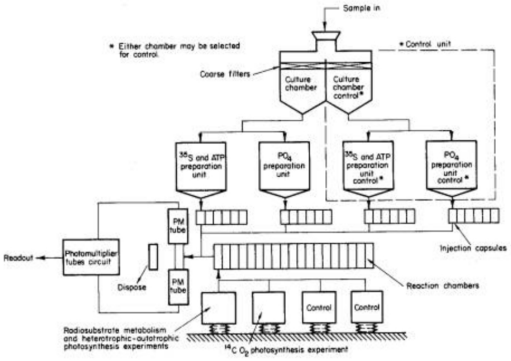

FIG. 11. Automated microbial metabolism laboratory, schematic. Reproduced from: Levin, G. V. and G. R. Perez (12).

The next step in the development of the AMML is the detailed design and construction of an operable breadboard. Then it will be possible to examine a number of microbial cultures and soil samples to test the integrated experiment concept. The data obtained will be used to refine the experiment further in preparation for the exciting biological opportunity opening to us.

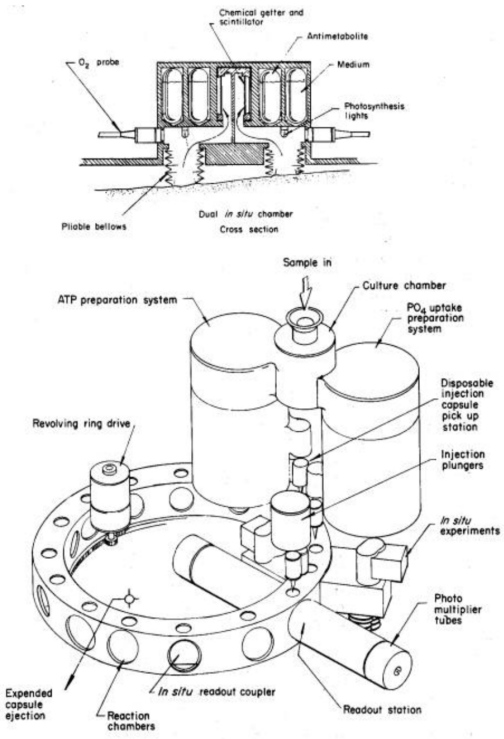

FIG. 12. Automated microbial metabolism laboratory, conceptual design. Reproduced from Levin, G. V. and G. R. Perez (12).

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Dr. Norman H. Horowitz, Division of Biology, California Institute of Technology, is co-experimenter on the Gulliver program and, as such, devised the autotrophic photosynthesis experiment.

In addition, the author wishes to express thanks for the scientific and technical assistance of his co-authors named on the various papers cited herein.

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