THE DETECTION OF METABOLISM

by
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In constructing metabolism experiments to detect extraterrestrial life, various assumptions have to be made. Accordingly, such experiments have less likelihood of returning positive data than would, for example, an experiment seeking to analyze the elemental composition of the surface material. However, these latter results would offer very little possibility for establishing the presence of life. Thus, the immense value of positive data return from experiments seeking to detect metabolism, including growth and reproduction, argues strongly for their inclusion in early planetary landers.

One such experiment (1,2,3) supplies ^{14}C-labeled organic substrates in an aqueous medium to a sample of planetary surface material. The mixture is then allowed to incubate within ambient environmental parameters and the evolution of radioactive gas monitored as evidence for metabolism, growth, or reproduction. Results obtained from a wide variety of terrestrial microorganisms and soils have been reported in the cited references. This experiment is among those selected by NASA to be performed on Mars by the Viking Project in 1976. The experiment makes the following assumptions: (1) if life exists, the planet will contain microorganisms; (2) the microorganisms will be widely distributed over the planet; (3) they will assimilate the labeled substrates supplied in the aqueous medium; (4) They will produce labeled gas; (5) They will metabolize at a rate sufficient to permit their detection during the finite lifetime of the experiment. While these assumptions must be acknowledged as potential limitations on the experiment, no assumption has been made, or is necessary, concerning the metabolic pathways within the organism. The organism is treated as a "black box", whose interaction with its environment is monitored by tracer techniques.

An experiment (4, 5) which does make an assumption concerning the mechanism of the metabolic process is one which seeks to detect the presence and increase of adenosine triphosphate (ATP) within the cell. The firefly bioluminescent assay is specific for ATP which is ubiquitous at the cellular level in all known forms of life. Hence, the presence of ATP in a planetary surface sample, and particularly its increase after incubation of the sample, would constitute evidence for life. However, the experiment assumes that extraterrestrial life will have incorporated the moderately complex ATP molecule into its metabolic process. Despite the rapidity and extreme sensitivity of the method, this important added assumption weighs against the inclusion of the experiment in a first planetary lander. Should life be detected, however, the ATP experiment offers an excellent way to pose a highly important comparative biochemistry question.

In appreciation of the low probability that a single experiment will be based on the correct assumptions required to achieve a positive result if life is present, our group undertook the development of a variety of independent, but compatible and reinforcing life detection experiments based upon metabolism. In addition to the development of appropriate experiments, we sought to demonstrate, through the design and fabrication of a feasibility model, that an instrument could be developed to conduct the fairly complex assay routine. The resulting "Automated Microbial Metabolism Laboratory" (AMML) (6,7) is aimed at simplifying and solving some of the complex instrumentation problems associated with multiple step assays, particularly those based on wet chemistry techniques. This compliment totals six experiments, including the two already described.

In an extension (7) of the labeled substrate experiment, a light is introduced as a means for detecting photosynthesis. The light is turned on and off during the monitoring for radioactive gas producing fluctuations in the rate of evolution corresponding to the light and dark periods. This experiment assumes a heterotrophic-phototrophic capability on the part of the microorganisms such as that demonstrated by algae which utilize labeled glucose and release carbon dioxide in light-dependent fashion.

Another experiment (6) seeks the detection of strict phototrophs. :Radioactive carbon dioxide is supplied to a sample exposed to the light. After an incubation period, remaining unfixed radioactive carbon dioxide is exhausted from the chamber. The light is then excluded and the space above the sample is monitored for evolution of radioactive carbon dioxide as an indication of endogenous respiration. The assumptions in this experiment are that the microbial forms will be phototrophic and will fix and release carbon dioxide. These assumptions are quite compatible with present knowledge of the Martian environment.

In the event that phosphate plays a role in the extraterrestrial life encountered, but that this vital nutrient does not participate as ATP, a phosphate uptake experiment was devised (6). This experiment measures the incorporation of dissolved, inorganic orthophosphate from an aqueous culture medium into which the sample is introduced. The ability of the phosphate trimer to store energy makes its use likely in any life system. It is possible, however, that it could be incorporated via some molecule other than ATP. Thus, phosphate uptake might be detected in the absence of ATP. Furthermore, the phosphate uptake experiment might produce a positive result even in the unlikely event that the life detected is not based upon carbon.

From a chemical standpoint, the sulfate ion is most likely to substitute for the phosphate ion in the latter's energy-storing role in metabolism. Thus, another experiment (7) in the AMML revolves about the uptake of radioactive sulfate. In this case, a medium containing $^{35}SO_4$ is administered to the sample. Aliquots of the sample are removed at intervals, rinsed to wash away nonfixed isotope, and counted for retained radioactivity.

Monitoring for the uptake of radioactive sulfate permits simultaneous monitoring for the uptake of the labeled carbon substrates provided in the experiment seeking the release of radioactive gas. No distinction can be made between the uptake of radioactive sulfur and radioactive carbon, but either would constitute a positive life detection response. This demonstrates the compatibility and reinforcement of the individual experiments.

Since last reported (7), further progress has been made on the AMML. In conducting the phosphate uptake experiment with several species of Chorella, it was discovered that the uptake in the light soon exceeded uptake by the organisms maintained in the dark. Thus, the phosphate uptake test might be used to detect photosynthetic activity. Figure 1 presents the results of such an experiment in which Chorella vannielii was grown in RM9 medium (7) containing 1.0 mg/l of PO_4-P. The figure also includes growth data, as measured by optical density, for correlation with phosphate uptake.

Next, the interrelationship between the phosphate uptake experiment and the labeled carbon substrate experiment was examined. An experiment similar to that shown in Figure 1 was conducted with the addition of one set of replicates containing added glucose. Figure 2 depicts the phosphate uptake obtained in the light and the dark with and without glucose. Growth data are also presented. The positive effect of the glucose in the light and dark is readily apparent. It is also seen that light is effective in enhancing the response with or without glucose.

The next step was to explore the effect of light on the radioactive carbon and radioactive sulfur uptake experiment. The results are shown in Figure 3. The combined ^{14}C and ^{35}S uptake by Chorella sorokiniana in RM9 medium was monitored in the light and the dark. In addition, a control containing Bard-Parker germicide was monitored in the light. The results show the early detection of metabolic activity and an early difference in uptake exhibited between the light and dark exposed cultures.

A combined experiment was next performed on soil obtained from Wheaton Regional Park in suburban Washington, D.C. Aliquots of the soil-RM9 suspension were removed and simultaneously tested for $^{14}C + ^{35}S$ uptake and for PO_4-P uptake. A parallel experiment was also conducted using heat treated soil for control purposes. The results of the combined experiment are shown in Figure 4.

The present AMML can transfer solutions from one chamber to another through a filtration system and present the products to two sensor systems, a geiger tube and a photomultiplier tube. Two factors are limiting. First, only six reagent chambers can be utilized. Secondly, some residual material is held between the syringe pump and the various reagent and reaction chambers and may interfere with succeeding measurements. A new concept envisions a second generation AMML as a turret-like assembly of storage vessels located around the periphery of a disc. A small syringe-type pump rotates under the vessels. The pump is directed by program to advance to a particular vessel and either deliver or pick up a solution. Delivery points other than storage vessels can include functional devices such as filters, heating units, colorimeters, even microcentrifuges, or anyone of a number of laboratory devices that could be miniaturized and incorporated into optional modules. Similarly, a number of sensors could be included.

The instrument could monitor metabolism by performing inorganic or organic assays by command or program. The prepared program is of the same basic design as that of the current AMML. Here a punched tape is generated on a teletypewriter which outlines each discrete step to be performed when this tape is used to initiate the electrical commands. Figures 5 and 6 illustrate the concept. The development of this concept would provide an advanced capability for performing a very wide variety of studies, including comparative biochemistry, on any extraterrestrial organisms detected by Viking. In the absence of a positive response from Viking, the landing of an advanced AMML type of instrument on Mars would permit an extended exploration for life through a wide variety of programmed and command type experiments.

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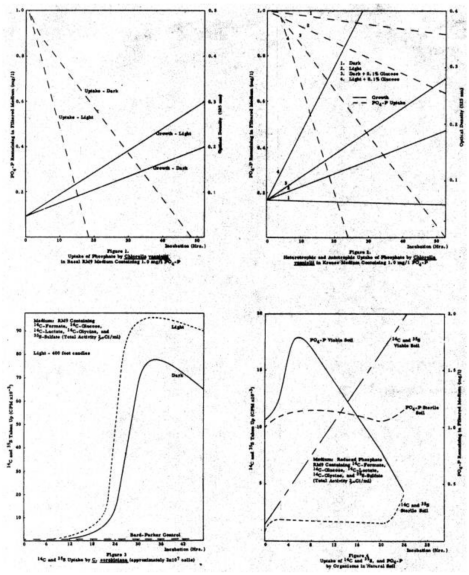

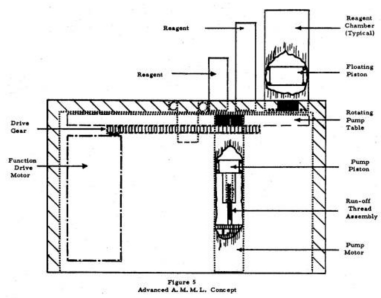

Figure 5
Advanced A. M. M. L. Concept

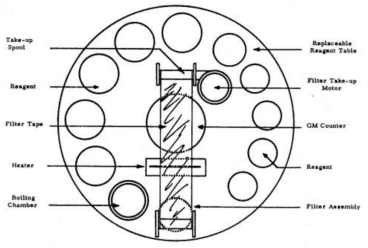

Figure 6
Top View of Advanced A. M. M. L.