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an analysis of the EXTRATERRESTRIAL LIFE DETECTION PROBLEM

Richard S. Young, Robert B. Painter,
and Richard D. Johnson

Ames Research Center
Moffett Field, Calif.

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PREFACE

In 1959, NASA appointed a Bioscience Advisory Committee (the Kety Committee) to assess and prescribe the role of NASA in the life sciences. The Committee wrote:

"The basic study of extraterrestrial environments is ultimately likely to be most productive in furthering an understanding of the fundamental laws of nature. Among the most perplexing questions which have challenged men's minds are the nature and origin of life and the possibility of its presence in the Universe other than on the Earth alone. For the first time in history, partial answers to these questions are within reach. Limited knowledge acquired over the past century concerning atmospheric and climatic conditions on other planets, the topographical and seasonal variety in color of the surface of Mars, the spectroscopic similarities between scattered sunlight from portions of that planet and those demonstrable from algae and lichens on Earth have suggested the presence of extraterrestrial environments suitable for life, and permitted the formulation of hypotheses for the existence there of some forms of life at present or in the past. These hypotheses may, within the foreseeable future, be tested, at first indirectly by astronomical observations made beyond the interference of the Earth's atmosphere and by samplings taken mechanically from various celestial bodies, and finally, by direct human exploration. The discovery of extraterrestrial life and a description of its various forms, knowledge of the presence and types of complex molecules based on carbon or other elements, or conversely, the absence of living organisms or of their traces in environments conducive to life will have important implications toward an ultimate understanding of biological phenomena."

The Space Science Board of the National Academy of Science has issued a statement in which the search for, and subsequent study of, extraterrestrial life is put forth as the prime goal of Space Biology. Some of the nation's leading scientists have expressed the opinion that this is one of the most important scientific objectives of NASA.

In the past few years NASA has been supporting individual scientists and laboratories in the development of certain techniques, and in some cases prototype instruments, for the detection of extraterrestrial life. Guidelines and ground rules for a cohesive study of the solar system and beyond for evidences of life - past, present, or future - are needed. Criteria must be evolved for the development of instrumented payloads designed for specific planets, so that investigators may work from a common foundation.

This study by the NASA-Ames Research Center's Exobiology Division was prompted by these considerations.

INTRODUCTION

One of man's oldest and most fundamental interests has been an attempt to understand the Universe. With the ability to explore space growing at a rapid rate, we will soon be able to explore the nearby planets. We will be able to determine if life exists on Mars or elsewhere and what types of chemistry are prevalent on these and other planets. Biology, which until now has been an Earth-bound science, may well join the physical sciences as a contributor to and beneficiary from the study of the cosmos, which has yielded so much to chemistry and physics.

Until recently, mankind has generally considered life to exist nowhere but on Earth. However, when we look at the Earth as being nothing more than one small part of a vast Universe containing billions of stars and potentially more billions of planets, this is a limited point of view. There seems to be nothing particularly special about the Earth when one considers the chemistry and physics of the Universe. Spectroscopic evidence from distant stars indicates that the same elements that form the building blocks from which living and nonliving materials are formed on Earth, exist in the same relative amounts on stars many millions of miles away.

Biology, unlike most of the physical sciences, is lacking in universal principles because of its observational restriction to the planet Earth. Although the Earth-bound biologists can only speculate about the possibility of the universal rules of life, there are things we can do in the laboratory to improve our knowledge of the phenomenon of terrestrial life and the possibilities for extraterrestrial life. Obviously, we must wait for the actual opportunity to study a life form, the origin of which is not the Earth, as it is made available through space travel. We will be able to compare its biological, physical, and chemical properties to those of life on Earth, and determine whether the properties of the elements and compounds which make up living things are such that life has arisen elsewhere as a natural product of planetary evolution or whether the particular arrangement of molecules which we call life is confined to Earth. It is also conceivable that a form of life exists on other planets which is very different, both functionally and chemically, from ours. Should this be the case we have the prospect of what amounts to a new science of biology based on principles other than those we now understand. In exploring other planets we must not ignore the capricious nature of evolution. We are confident that with the proper physical environment and conditions of temperature, atmosphere, and water, life would unquestionably have been able to arise elsewhere in the Universe. Once this has taken place, some sort of evolutionary development will occur. However, we cannot predict what forms of life will arise under a completely different set of physical conditions. We have never seen evolution operating under sets of planetary conditions which are atypical to the Earth, but can only speculate about the possibilities for nature in other parts of the Universe. Philosophers, for hundreds of years, have speculated on the origin, or origins, of life. It is entirely possible that life may have had more than one origin, in both location and time, but it is only through space exploration that any light will be cast on this question. It should also be recognized that the detection of life on any planet requires a study of the complete evolutionary history of that planet, through the use of many scientific disciplines.

In order to accomplish these objectives, we must design and build devices to be flown in space vehicles which have the ability to survive the long trip in space and yet

remain capable of functioning on arrival on the surface of a given planet. The experiments will be performed by automated devices since it will be possible to reach planets such as Mars with an unmanned mission before technology permits man to travel for such distances and for such long periods of time. Because the technological problems and cost of flying to planets are enormous, we must be certain that the experiments that have been planned are well designed and are sure to give results which can be transmitted by radio back to Earth in such a fashion that they will be clearly understood.

Concerning the timing for the mission, one limitation is that of launching the spacecraft on favorable trajectories during the Martian opposition. As the launch opportunity becomes less favorable, more and more of the payload weight must be devoted to booster weight.

A second point concerning timing has to do with the problem of obtaining significant biological data before contamination occurs. There is no way of predicting when some unsterilized space probe (American or Russian) may hit Mars. There is no precise method for determining the likelihood of contaminating Mars, but the laboratory data indicate that Earth organisms do present a potential biological hazard to Mars. Therefore, in order to maximize the chances of studying an intact Martian biota, the time scheduling of critical experiments may be of the utmost importance.

We must consider the relative merits of landing a small payload as early as possible, with the concurrent reduced scientific capability and chance of experimental success, versus waiting until the capability of larger payloads exists with increased chances of success. It might seem advisable to do an experiment at any and all opportunities, but in this case each vehicle landed on Mars increases the chance of contamination. Therefore, every chance for success should be available in the first landing mission. Because of the very large uncertainties involved in experimentation on Mars, the single, limited, light-weight experiment must be considered a "long-shot."

The comparatively large capabilities of the Voyager systems should not be treated as merely a means to perform a larger number of individual experiments, but instead as a chance to wipe the slate clean and to conceive of experiments which are not borne under severe weight restrictions. While the principles underlying many of the early life detection experiments will certainly be worth consideration in any planetary exploration program, it seems more advisable to review the problem as developed from basic principles in an attempt to provide an integrated experimental package possessing the greatest possible assurance of obtaining meaningful data.

First, however, it must be decided what environmental information is going to be essential for the detection of life and what criteria are acceptable as being indicative of life on Mars. Not all scientists are agreed as to what is life or what is nonlife. Then, it must be determined how those characteristics considered to be most typical of life will be detected. Ways must be found to assure that the life found there is not simply that which is carried along from earth by the vehicle; thus, there is the necessity of finding ways of sterilizing the huge spacecraft and instruments to be used for the trip. The sterilization procedure must be effective, yet it must not be detrimental to the vehicle or instruments so as to decrease their reliability. Obviously, years of preparation and work by many people are required just to insure that the experiments get to Mars in operating condition. The next problem is to determine exactly what

should be done after landing. What must we know about the planet itself before we can hope to study its biology? Many environmental parameters must be measured first. Then, a number of possible stages of chemical and biological evolution must be considered. Life has apparently been present for more than half of the four to five billion year history of the Earth. The highest form of life on Earth, man, has been present for only a small fraction of the biological history of the Earth. The evolution of life on any planet must occur at a rate that is determined by the particular characteristics of the planet. Since it seems probable that no two planets are exactly alike, it must be assumed to be very unlikely that life on an extraterrestrial body is at the same stage of evolution as our own. We must be prepared to investigate a large number of possibilities.

Any information obtained from an extraterrestrial body, concerning life on that body, would cast at least some light on the origin and evolution of life. It would surely be one of the most important scientific discoveries of this century. Lack of evidence for life on any given planet would not resolve the question of extraterrestrial life or the origin of life, but would simply mean that we must look further.

THE ATTRIBUTES OF LIFE

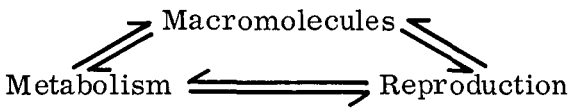
The difficulties associated with assigning an unequivocal definition to the phenomenon of life lead one to utilize various approaches to a better understanding of the living state. From the standpoint of the problem of the detection of life on extraterrestrial bodies, it may be pertinent to list and scrutinize closely the criteria most commonly attributed to living systems. Thus the initial task of the exobiologist is to describe life in such a manner that tests can be devised that can demonstrate, unequivocally, the existence of extraterrestrial life.

The manifestations of life most often listed are: (1) growth, (2) movement, (3) irritability, (4) reproduction, (5) metabolism. Taken collectively, they indeed are indicative of life, but which are fundamental and which are not? To the exobiologist, it becomes obvious after delving into the problems of detecting life, that certain of these criteria will probably be of little value in the search for living forms on other planets, especially if those forms are exclusively microbes, as is suspected by some to be the case for Mars. Instead, there emerges from this effort a concept of life based on modern information, and especially on the wealth of experimental and theoretical developments that have occurred in biology in the last decade.

Perhaps the most important development in biology that contributes to a modern enumeration of the fundamental attributes of life is the evolution of the concept of information storage and transfer. Of course, this idea has been inherent in biology since the advent of modern genetics, but only within the last decade has a truly mechanistic elaboration been possible. The contemporary concept of the living system, the cell, is one of an information storing and transferring system of macromolecules under tight endogenous control. Jacob and Monod (1963) have stated it succinctly: "A cell may be visualized as a society of macromolecules, bound together by a complex system of communication regulating both their synthesis and their activity."

What then of the other attributes of life? If the cell is a unit consisting of macromolecules capable of information storage and transfer, then one can immediately perceive that two other attributes of life are necessary for the maintenance of this unit. These are, of course, reproduction and metabolism. Reproduction can be visualized as the means by which the information is maintained by its constant renewal. As for metabolism, it has been beautifully characterized by H. J. Muller (1960), "The turnover or metabolism occurring in the other material of a cell represents, we might say, the fire that the genetic material keeps going outside itself, to get the other material to work for it, in the service of its own distinctive goal: its own survival and replication. Thus, metabolism is not the essence of life, but a kind of upper level expression of life after the genetic material has succeeded in making for itself a workshop of protoplasm."

The minimum requirements for life then can be summarized as a kind of circular equation:



which illustrates the interdependence of these three fundamental attributes of life. A fourth criterion for life, as we perceive it as a constantly evolving phenomenon, is mutability. There can be no doubt that mutations are necessary for life to persevere in the face of the continual changes in environment that it encounters. However, to the scientist searching for life it is difficult to imagine how this attribute could be used in a detection scheme, simply because so much would have to be known about the system before tests for mutation could be begun. In other words, mutability is an attribute that depends so heavily on the other three, that it can never be measured prior to knowing a great deal about at least one of them.

With these three attributes as the bases of life, it is necessary to examine each one of them in relation to its relevancy to the problem of the detection of life. Such an examination, of course, can only be accomplished in terms of our present day knowledge and technology, and it might be necessary to alter the evaluations as future work reveals more details of life processes and as more sophisticated technology develops.

Many scientists might assign top priority among the manifestations of life to reproduction. There is certainly no argument that life cannot exist very long without it; yet to the exobiologist it probably presents the most formidable problems as the basis for a life detection experiment. The reasons are both technological and inherent in the phenomenon. Only the former will be touched upon here, and the latter will be more thoroughly examined later.

Reproduction is a difficult attribute for which to test, primarily because it is a discontinuous process. The reproductive rate varies enormously from species to species and, depending on environmental conditions, often within the species. Even reproduction at the macromolecular level (preferably termed replication) is discontinuous in many life forms. Extremely complex reproduction schemes are relatively common, as in the case of host-parasite situations that require intermediate hosts, and the reproduction rate often is influenced by environmental factors (e.g., seasonal changes). All of these factors, and others, which are known to complicate

observations of reproduction on this planet, indicate that the detection of reproduction in an exotic situation could be extremely difficult.

Metabolism appears to be a more promising attribute than reproduction on which to base life detection experiments, mainly because it is essentially a continuous process. Even forms of life that are considered to be in a highly inactive state (e.g., bacterial spores and plant seeds) carry on measurable, albeit extremely low, rates of metabolism. Of course, a great number of parameters can be measured to indicate metabolic activity (e.g., changes in pH, Eh, temperature, gas evolution, and others), and this possibility of diversity of approach strengthens the case for using this attribute as a basis for life detection experiments.

To attempt to use the presence of informational macromolecules as a basis of life detection experiments appears, at present, to be practically impossible. Comparison of unknown DNA molecules with known ones, using the hybridization technique, is now feasible, but this approach appears to be much too limited to be used for detection of exotic forms of life. The identification of macromolecules, in general, appears somewhat more possible but still very difficult. However, this attribute can be broadened to include the total chemical constitution of the cell. Under such a widened interpretation, many kinds of chemical experiments can be performed, from a simple elemental analysis, through identification of functional groups, small molecules, and optical activity to the macromolecular components. It is evident that the more kinds of information of a chemical nature that can be gained the better, and that the more complicated the molecule identified, the more pertinent it will be.

The other manifestations of life - growth, irritability, motility - are obviously components or consequences of one or more of the three prime attributes. Growth could be used in a life detection system only if it could be differentiated from accretion of nonliving material. Irritability, an outgrowth of metabolism, would be difficult to use in a biosphere composed exclusively of microorganisms. Motility could conceivably be a very valuable attribute, either macroscopically or microscopically, and it obviously will be used as soon as the relatively large power requirements for image transmission can be accommodated.

Other hints to the presence of life may be possible. For instance, some scientists have proposed that a simple microphone be included. However, such "long-shot" approaches have comparatively little chance to obtain relevant information about the presence of life. The result of lengthy discussions and deep deliberation on this problem is to make manifest the concept that a truly meaningful life detection program must be based on the fundamental attributes of life. Only by expanding upon them can a sound set of experiments capable of obtaining the maximum amount of information with a minimum of trivia be generated.

Thus, for the purposes of life detection, chemistry, metabolism, and reproduction seem to be the best of the attributes of life upon which to build an experimental program, and, indeed, even among these characteristics one can derive priorities on the basis of practicality.

Chemistry

Many studies of living organisms involve the use of physical or chemical properties. The following discussion, however, deals with those techniques which are not merely tools for measuring reproduction or metabolism, but instead have certain properties which in themselves are sufficiently characteristic to be used in the detection and classification of living systems.

Any discussion of the structure and composition of extraterrestrial life is immediately faced with the futile consideration of exotic forms of life based on a molecular chemistry far different from that on Earth. A proposal for a molecular backbone other than carbon-carbon bonds, or for a solvent system other than water, to mention the two most popular suggestions, is met with arguments on two fronts. First, exotic possibilities are discounted for reasons of cosmic abundance and reactivity. Silicon polymers are not found in Earth biology in spite of the high abundance of silicon, because the silicon-silicon bond strength is not as great as the carbon-carbon systems, and the silicon-based molecules are more subject to chemical attack. Second, it is often argued that the chemical and biological processes have evolved as they have on Earth, because of the inherent superiority of these molecular arrangements in forming a functional, complex, biological system. This superiority would be expected to be applicable elsewhere as well.

On the other hand, the totally unknown factors surrounding extraterrestrial life do not allow other possibilities to be dismissed with any degree of certainty whatsoever. Molecular systems which seem entirely unsuited to biology on Earth may be very reasonable possibilities under vastly different conditions, for example, at lower temperatures, or in the absence of hydrolytic or oxidative surroundings. Present chemical knowledge is so overwhelmed with carbon-based molecules, all derived from living systems, that experimenters have not had the time, interest, or raw materials with which to study other systems in equal depth. Certainly, organic chemistry would be a very primitive science if the biological stockroom (including coal and petroleum) were not available.

The impasse reached in these arguments will only be resolved by experimental data from other planets. To be realistic, however, proposed planetary experiments must not be based on completely hypothetical systems which are totally unknown, but rather on those characteristics of Earth biology which are the most likely to be found elsewhere. It is the physical and chemical manifestations of a particular molecular structure which are of primary interest and not necessarily the molecules themselves. For example, it is probable that extraterrestrial biological systems will contain polymeric material, possibly, but not necessarily including protein, nucleic acid, and carbohydrates. Likewise, the amide linkage, the helical structure, and the capacity to hydrogen bond are all more universal than a certain set of 18 amino acids. Wherever possible, these more general characteristics must be sought out in preference to, or at least in conjunction with, a limited specific feature so that certain possible systems, with only minor deviations from that known on Earth, will not be needlessly eliminated. A list of these general characteristics might include the following:

a. Gross morphology of larger life forms - more than one cell. High resolution TV.

b. A liquid solvent system, undoubtedly a polar molecule, capable of dissolving ionic species and solvating polar functional groups. Detection would be feasible by gas chromatography, mass spectrometry, and infrared spectroscopy.

c. Polymer formation, particularly organic polymers. Sample preparation and interference from soil particulate matter would interfere strongly with some techniques, but osmometry, ultracentrifugation, and perhaps viscometry merit further investigation.

d. Optical activity is always associated with life, and its presence is highly suggestive of complex biological systems. Polarimetric instrumentation is available, but the methods suffer from a lack of sensitivity and are subject to interference from particulate matter. Gas chromatography now appears to be a promising technique.

e. Carbon-based chemistry should be sought since this system seems the most likely and the one upon which many other experiments will depend. A simple instrument which will detect organic carbon only, with a high degree of sensitivity, is needed. This will prevent the waste of spacecraft time, power, and reagents on tests for specific organic compounds when there is not even evidence of the presence of organic carbon.

Certain instrumental techniques are available for surveying chemical compounds present in the atmosphere or soil. These methods include gas chromatography, mass spectrometry, and spectroscopy in the entire range from far UV through the IR. The gas chromatograph and mass spectrometer, in some instances coupled together, provide an excellent means of performing atmospheric and organic analysis. The compounds of interest in the soil, presumably organic, must in some cases be converted to volatile compounds before analysis. The composition of the original material must then be obtained by inference from these volatile products. Thermal degradation of high molecular weight organic material is already in use with pyrolysis-gas chromatography and pyrolysis-mass spectrometry techniques. These methods are not limited to the search for specific compounds, but are intended to analyze whatever material is present so that by reference to suitable on-board standards, the results can be telemetered to Earth for subsequent identification. In some cases, instruments similar to and functioning under the same conditions as the spacecraft instrument will be needed so that the identification can be verified on Earth. Because of the abundance of exceedingly complex data obtained from biological compounds, and because the gas chromatograph is inherently not a quantitative instrument, care must be taken so that the significance of the data can be recognized on Earth.

It is felt by many investigators that the mere presence of one or two specific compounds common to Earth biology will be highly indicative of extraterrestrial life (e.g., protein or nucleic acid). The problem is not simply which of these Earth biochemicals is necessarily the most significant, but what methods and specificity will be used to detect these compounds. There are many color tests and bioassays, but the dependence on sample preparation is so great that these tests are not as effective with completely unknown samples as more general methods would be. As an example, the gas chromatographic analysis of certain derivatives of amino acids which have been formed from the hydrolysis of potential proteinaceous material is to be preferred to a specific color test for one or more amino acids found in the material. The reduction of specific compounds to known derivatives is apt to provide a more flexible scheme of

analysis than would the search for only one particular compound. In this manner, analyses could be performed on material for protein, nucleic acids, lipids, and carbohydrates. For any analysis, there is a need for the facility to prepare samples with a device which can extract, filter, strip off solvents, and perform simple chemical reactions. Only after rigorous purification separation, and formation of derivatives will any analysis provide results with confidence.

Before the analysis of organic matter in the soil, some background information on the soil itself is necessary to provide an understanding of biological results. For this reason, a capability to do inorganic analysis should be included. The analysis might include tests for the specific ions found in Earth biological systems, such as halide, phosphate, sulfate, sulfide, sodium, potassium, calcium, magnesium, manganese, and iron. In addition, some information should be sought on the basic mineralogy of the sample.

All of the experimental techniques which might be used in the above proposed analyses are based on instrumentation which is common to several analyses. This requires a versatile sample plumbing system in which the samples can be transferred from one instrument to another so that analytical results can be meaningfully correlated.

The last major question of this discussion concerns the worth of chemical analysis in themselves. No single chemical identification will prove beyond doubt the presence of extraterrestrial life. The results will, however, strongly fortify any biological analysis and provide extremely interesting information about the planetary environment. Should negative results be obtained with the preliminary chemical analysis, this could be so highly indicative that any further analysis of a given sample would be fruitless, and another area could be tested.

An additional aspect of chemical analysis which makes it of prime importance in a life detection scheme is that it permits the detection of previously existing life (through an analysis of the "fossil record"). Of course, abiogenically produced compounds will also be detected but their detection may be as important to considerations of the origin of life as the detection of life itself.

Metabolism

The metabolic experiments must be specific enough to eliminate nonbiological changes. For example, while it is rather difficult (but perhaps not impossible) to conceive of a nonmetabolic system that would yield the kinetics of pH change caused by a growing culture of lactic acid-producing bacteria, the time course of heat evolution by the same culture might be duplicated by an illuminated, nonbiological system. This kind of consideration emphasizes the importance of controls and indicates that different experiments will have different numbers, as well as kinds of controls. The possibility that nonbiologic systems can mimic living ones suggests that the inclusion of a "sterilized" control is always necessary. The further possibility that the nonliving system might be affected by the sterilizing agent complicates the picture still more.

The parameters that may be used to indicate the presence of a metabolizing system dictate the complexity of the final experimental device. One parameter that should be of value is gas exchange. If an experiment is designed to measure the change in concentration of just one component of the gaseous environment, it can be very simple; but if all the gases are monitored continuously (which would allow a more general approach), the experiment complexity will, of course, increase accordingly.

Changes in pH and Eh would be relatively easy to monitor. Although controls are essential in these measurements, the number of them would probably be fewer than with many other parameters. Another way of detecting metabolic activity in solutions is by monitoring the changes in concentration of solutes in the medium. Thus, the decrease in concentration of a specific substrate might be an indicator of metabolism. Increases in temperature of a closed system may also possibly be used as an indication of metabolic activity, but controls on this experiment would have to be carefully designed.

A definite advantage of experiments measuring changes in any of the above parameters is that the information received may also secondarily indicate the presence of a reproducing system. The kinetics of CO_2 production of an exponentially growing bacterial culture are peculiar to this kind of system and are not reproducible by non-growing ones. Thus, the monitoring of changes in these parameters could yield kinetics that will indicate whether the living system is growing or not. It should be pointed out, however, that the detection of a single microorganism in a given soil sample is most unlikely, using metabolism as the criterion.

Another kind of metabolic study is the search for a particular enzyme, on the one hand, or the substrate of the enzyme, on the other. There are serious disadvantages to this approach, such as, the instability of most biologicals to the sterilization which must be accomplished on all life detection probes. A second, less important disadvantage is the fact that such experiments only detect parts of living systems and do not determine if life is actually extant. A more serious drawback is that our knowledge of the chemistry of Martian organisms and the environmental conditions in which they exist is insufficient for us to predict the reaction requirements of specific Martian enzymes.

One of the main problems to be considered in the design of many metabolic experiments is the selection of substrates. Even if the metabolism of extraterrestrial organisms is similar to that of known Earth organisms, substrate selection will still be difficult. For instance, the detection of the metabolic generation of CO_2 from radioactive glucose may not be successful. There are Earth organisms that do not produce CO_2 from glucose. Many such organisms metabolize anaerobically, and it is pertinent to point out that, since there is no detectable molecular oxygen on Mars, any organisms growing there may be doing so anaerobically. One could also include radioactive formate which can be cleaved by terrestrial anaerobes to form CO_2 and hydrogen. However, there is certainly no assurance that Martian organisms could form the enzyme, hydrogenlyase, responsible for this reaction. Thus our total ignorance of possible life forms on Mars allows only three alternatives: (1) Assume that all metabolic schemes are universal and use common single substrates such as glucose; (2) assume that some metabolic schemes are common and include a whole array of substrates; and (3) assume that the organisms can utilize materials in their immediate environment and attempt to utilize them by extraction and concentration or by in situ experiments.

The first alternative allows the design of relatively simple experiments that should yield straightforward answers to the specific question, "Is there a life system that can metabolize this particular compound?" but, in case of a negative answer, would still leave the question of the presence of life unanswered. The other end of the spectrum, alternative 3, would require relatively complicated experimental design, but would probably have the best chance of obtaining an affirmative answer. If the life detection lander is a relatively large one, all three approaches may be implemented.

A much simpler and more general end product of metabolism may prove to be far more useful as a life detection criterion. This is the production of heat as the result of metabolic activity. Microcalorimetry of a soil sample does not require the provision of an unknown substrate, but is an in situ measurement.

The fact that metabolism is a continuous attribute of life allows the design of life detection experiments a maximum of flexibility. This flexibility is enhanced by the possibility of using one or more of the several parameters that can be used to monitor metabolic activity. It thus appears that experiments using evidences for metabolism rate a very high priority, perhaps the highest, in the ultimate design of a life detection system. However, severe limitation to the use of metabolism as a continuous attribute might be imposed in the case of Mars. On this planet the diurnal temperature extremes are such that it is believed that organisms living there must go through a daily freeze-thaw cycle. Metabolism in the frozen state is essentially nonexistent and, therefore, would not be directly detectable. Thus, in the case of Mars at least, evidences of metabolic activities will have to be sought during the warm periods, or, less preferably, on artificially thawed samples.

Reproduction

The demonstration of reproduction would certainly be a dramatic means of life detection, and indeed, if it could be unequivocally shown, would probably be the most convincing. However, as stated previously, the primary limitation of this attribute is its discontinuous nature. If Martian organisms grow asynchronously, as do most terrestrial ones, this would offer no obstacle. However, the diurnal temperature cycle and the seasonal temperature variations peculiar to Mars are such that temporal fluctuations in reproductive rates might very well be expected. The probability that inopportune observation times might obscure reproduction is, therefore, a distinct possibility. In addition, the substrate requirements for reproduction may well be even more difficult to provide for than for metabolism.

The problems with reproduction as a life detection basis do not however, end with this point. What may be even more important are the problems involved with the technology of detection of reproduction. A list of some direct methods includes:

1. Turbidimetry and nephelometry
2. Conductivity
3. Increases in sedimentable weight and/or volume
4. Electronic determination of size and number of cells
5. Visual - TV-microscope.

The problems involved with turbidimetric and nephelometric methods are mainly concerned with interference by nonliving colloidal materials. The sampling of some kinds of soils often results in the suspension of colloidal materials of a size that make their removal difficult and interfere with the transmission of light in such a way as to make detection of subsequent changes difficult or impossible. Moreover, it is possible that some of this material will undergo slow physical changes in the suspended state that causes swelling and increases in light absorption - such a process might conceivably be misconstrued as a growth process, although serial transfer would help reduce interference from background particles. Such changes of strictly physical-chemical type might also introduce artifacts into conductivity, and weight and volume determinations. The combined determination of the change in size and number of the individual components as a function of time by means of techniques similar to Coulter counting, or by TV-microscopy, appears to be the most clear-cut, unambiguous means for demonstrating reproduction. It is difficult to conceive of a nonliving process that will actually increase the number of similarly sized particles in a closed system, and it appears that instrumentation for measuring changes of this kind would be the most valuable in attempting to demonstrate this attribute. However, technical problems make this a most difficult measurement.

Another facet of reproduction is that occurring subcellularly at the organelle or macromolecular level. This is especially true of genetic material, the replication of which is a prime prerequisite for reproduction. If the exotic genetic material is nucleic acid, the demonstration of temporal increases of this material would be very suggestive of a living process. However, in our total ignorance of the situation, the assumption that nucleic acid is the universal genetic material may be completely groundless. If so, a search for replicating genetic material would be practically impossible, simply because it would be so difficult to identify the material in the first place.

To sum up the use of reproduction as a basis for life detection, the discovery of this attribute would be a very convincing demonstration of the presence of life. A promising (but most difficult) approach would appear to be direct evidence for increases in number of particles or larger components with time. Because of its discontinuous nature and difficulty of implementation, reproduction probably should rate a lower priority than evidence for metabolic activity, but because of its importance to the phenomenon of life, experiments attempting to demonstrate it definitely should be included. The implementation of experimentation to determine the replication of genetic material appears to be so difficult that it should be given a rather low priority as a life detection principle; although, of course, the subsequent study of exotic forms of life will necessarily include this kind of experimentation.

Summary

In any life detection system it appears highly advisable to include experiments based on these three attributes of life (macromolecules, reproduction, and metabolism). It is probable that the most information will be obtained from chemical experiments, but this information will be of limited value in terms of life detection, without additional evidence of either metabolism or reproduction. The presence of organic compounds and even polymers, such as proteins and nucleic acid would not necessarily indicate life, since the research of Miller and Urey, Oro, Ponnamperuma, Fox, and others has shown the abiogenic formation of materials of importance in cell

composition, some of which have highly complex structure. These and other pertinent references are cited in the bibliography. Thus, random accumulations of chemicals of biological importance do not prove the presence of living material; this is especially true for Mars, where the high flux of ultraviolet light at the surface might be the energy source for formation of organic compounds (particularly sugars).

On the other hand, a metabolic or reproductive experiment by itself, that yields a positive answer, even if accompanied by good controls will never be completely conclusive. In addition, our knowledge of Martian environmental conditions and the makeup of Martian organisms is so deficient that the design of specific metabolic experiments requiring the function of specific enzymes is almost certain to be inadequate.

However, if such results are accompanied by chemical analyses that support the presence of life, the doubts would be effectively negated. This is especially true if the results are demonstrated on the same sample, simultaneously or sequentially. Therefore, it appears necessary to:

1. Send experiments based on all three attributes;
2. Attempt to perform all analyses on the same sample, or aliquots therefrom; and,
3. Consider that life exists, only after at least one clear cut result is obtained with a reproduction or metabolic experiment, plus supporting chemical evidence.

MARS

In our earthbound considerations of the possibilities for extraterrestrial life, we naturally think first of those locations where conditions most closely resemble those of Earth. The nearest planets in our solar system, Venus and Mars, receive energy from the Sun in amounts similar to that received on Earth. The mass of both planets, also, is similar to that of Earth, and sufficient to retain some atmosphere. The Mariner fly-by has determined the temperature of Venus to be about 600° K, however, which makes Venus an unlikely candidate for detection of life, and focuses our attention on Mars.

Theoretical studies indicate that all the planets had similar origins, being formed from a nebula or gas cloud. It is assumed that both Mars and Earth had a primitive atmosphere composed of ammonia, methane, water, and hydrogen. This atmosphere, under the influence of energy sources like ultraviolet light, electrical discharge, and heat, gave rise to a large variety of organic compounds. The question of whether the physical environments of Earth and Mars are different enough to result in the formation of different compounds from the available atmosphere cannot be answered until samples from Mars are analyzed.

If we assume that essentially the same compounds were produced on the two planets, then Mars had available not only the common amino acids, sugars, purines, etc., but also great numbers of other compounds which are known to be produced under these conditions and not yet identified. If a living system subsequently developed on Mars, presumably it could have used the same compounds and systems which have been

successful on Earth. On the other hand, it seems possible that differences might arise during the selection of the vast numbers of compounds needed for a living system. These differences could range from minor changes in an amino acid molecule to completely different classes of compounds functioning in place of nucleic acids in information storage and transfer, in place of proteins as catalysts, and others. Between these two extremes - biochemistry identical to that of Earth organisms, or entirely different systems - are all possible gradations of variations in metabolic systems. It has also been suggested that extraterrestrial life might be based on silicon chemistry instead of carbon, but this seems unlikely within our solar system. If living forms are found on Mars, it will be essential to determine as much of their biochemistry as possible. By comparison with the properties of terrestrial organisms we may be able to answer one of the most fundamental and exciting questions of Exobiology - If life arises independently in two separate locations, will the course of chemical evolution be the same?

For practical considerations in the design of life detection systems for Mars, it is obvious that most of the experiments will of necessity be based on the properties of Earth organisms. Thus it would be logical, for example, to test for the growth of Martian organisms in media containing some of our most common metabolites. It also seems wise, however, to include some experiments which are less dependent on Earth-type conditions. These might include physical measurements such as heat production, pH changes, and attempted growth of organisms on materials extracted from Martian soil.

It is evident that some differences should be expected between terrestrial and Martian metabolism, in view of the virtual absence of oxygen from the Martian atmosphere, and the very low water content. These and other environmental parameters are examined in more detail in the appendix.

Before any accurate assessment of potential microenvironments can be made, however, we must have considerably more detailed information concerning certain features of Mars. Little can be done from the Earth to improve our current knowledge of surface characteristics, since visual surface resolution better than 40 or 50 km is not possible without fundamental improvements in technological capabilities. Thermal mapping is possible only with topographical resolutions of approximately 500 km. Spectrophotography is possible only with topographical resolution of approximately 1000 km. To design meaningful experiments and select optimum sites for experimentation, more data are needed. Fly-by missions can improve current measurements by placing instrumentation much closer to the surface of the planet, if only for short periods of time. Observations of the surface of Mars indicate the existence of both seasonal and secular changes which have been attributed to proliferation and growth of Martian organisms. The fly-by has a distinct disadvantage in that it is within range for no more than a few hours, and thus is quite incapable of observing seasonal changes. Therefore, a Martian orbiting vehicle is of much more potential significance to biology and the exploration of the planet.

A variety of experiments can be performed from such a vehicle. Sagan has suggested that a Martian orbitor with a 10-inch reflecting telescope at an average distance from the Martian surface of 10,000 km will permit a surface resolution of 25 m at visual wavelengths near 5000 Å. A TV camera in such a system should be able to follow the wave of darkening. The sudden appearance of dark areas in what was previously noted as desert should also be visible with much better resolution. Thermal

mapping of Mars from an orbiter should be possible with 1-km topographical resolution, and would permit a determination of the minimum nighttime temperature, which at present is unattainable. It should permit the identification of localized concentrations of heat or water, which are also unobservable at the present time. Differences in chemistry in localized areas such as the dark regions should be discernible. Such a vehicle should also permit the identification of geologically suitable sites for landing craft. An ultraviolet spectrometer in such an orbiting vehicle will permit a determination of ultraviolet intensity on the surface of Mars as a function of time and geographical location, which may be of great biological significance if the flux is very high.

Since the existing evidence for life on Mars suggests a strong seasonal dependence for Martian biological activity, all launch opportunities are not promising for biological experimentation on Mars. The best opportunity for in situ studies of the wave of darkening exists in the 1969 opportunity. Equally good site selection will be available in 1971 only if the payload is reduced. In the 1973 arrival window, the presumed seasonal biological activity will be well past maximum. The post-1975 opportunities remain very unpromising until 1984. On the basis of their scientific desirability and engineering feasibility, a variety of Martian landing sites can be selected for the four biologically most interesting opportunities, 1969 to 1975.

For our purposes in consideration of larger payloads and larger vehicles up to the Saturn V booster, we need not restrict ourselves to a 180-day effective lifetime for the scientific package, and our considerations of Martian landing windows are not quite so critical. However, it must be remembered that less favorable oppositions of Mars require significant reductions in the size of the payload and more weight in the booster and power capability of the vehicle.

From the evidence available to us now, it is clear that certain areas on Mars are definitely more suggestive of life than others, and must be considered as preferred areas for the earlier investigations. Certainly the dark areas of Mars fall into this category. These dark areas are fairly extensive, and since the bulk of them are in the southern hemisphere, it should probably be most fruitful to attempt a landing in this area. Since the wave of darkening is a visible seasonal change, we must attempt to investigate the changes in the surface occurring at the time of the wave of darkening. Therefore, it has been suggested that the areas of Solis Lacus and Syrtis Major be considered as sites for early investigation. These are major areas of darkening very close to the equator which have been observed steadily for a hundred or more years. It would be desirable to land in the Martian spring at the time the wave of darkening is expected to pass, and during the early life of the vehicle when operational difficulty should not be expected. It would also be desirable, if possible, to land simultaneously in a light region.

On the other hand, if orbital vehicle observations indicate areas of localized geothermal activity or areas of high water concentration, these should very definitely be explored as areas of preference. One other region of great potential interest is the region of the dark band at the receding pole cap in the Martian spring. It is conceivable that liquid water may be present at this time; however, a better resolution of temperatures in high latitudes may provide another interpretation of the phenomenon of surface darkening at the receding pole cap.

Landing accuracies within a diameter of some 50 km are theoretically possible and would be very desirable. A mobile capability after landing improves the capabilities of the detection system. It would be highly desirable, for example, to land near the edge of Syrtis Major so that the vehicle would be able to explore both the dark area and the light region bordering it with the same set of experiments. Clearly, however, we must remain as flexible as possible in our selection of landing sites until fly-by and orbital missions supply much more information which will enable us to be more selective in our choice of specific regions on the surface of the planet for study.

ON MARS

Among the many problems to be encountered on the surface of an unexplored planet is that of obtaining and processing a sample of the surface for analysis. The analytical techniques themselves are completely dependent on proper treatment of the sample. Once the sample is prepared, the most efficient sequence of experimental determinations must be programmed to function automatically.

Sample Acquisition and Preparation

Sampling is probably the most important single feature of an extraterrestrial life detection device. The importance of sampling cannot be denied, but, to the uninitiated, the mere scooping up of a few samples of dirt may not seem to be that formidable.

The proposed early life detection devices had to provide a means of sampling from a totally unknown surface without the luxury of heavy equipment. To this end, the proposed devices for the most part were designed for dust collection via sticky strings, charged tapes, vacuum cleaners, or aerosol collectors. The sampling efficiencies were often so low as to severely strain the sensitivity of the various detection devices. When the large payload mission is considered, however, the problem of sampling is reduced, because of the greater capability and versatility.

Assuming, for this discussion, that a spacecraft will be landed in the desired Martian locality, Syrtis Major, for example, the following paragraphs indicates some of the problems in sampling.

1. How far from the point of touchdown is sampling necessary or desirable? It is possible that the thinness of the Martian atmosphere may necessitate the use of retrorockets for the landing on Mars. Obviously the burn area under the retrorocket is a most undesirable location to perform biological research; thus some degree of mobility is required. If long range mobility is feasible, then limited mobility is not a pertinent consideration. Should the main unit not be capable of mobility, sampling away from the burn area could be made possible by mobility of the sampler alone, the sampler and some of the experiments, or the whole experimental package.

2. Within a given limited area surrounding the spacecraft, how will one site be chosen over another? It is generally agreed that within the same ecosystems the microbiology will be uniform. Therefore, no particular advantage will be had by

sampling at all points of the compass at some arbitrary distance (several hundred meters) from the spacecraft. A few samples should be representative of all found within the area. Should the geology be inhomogeneous, however, a wider range of samples might be required. A mobile system with television transmission to Earth would afford the opportunity to pick the most likely sampling sites within the general area. Any design for mobility must, of course, be able to cope with any ruggedness in the terrain.

3. For what contingencies in the surface must the sampler allow? Dry or wet, rough or smooth, hard or soft, dusty or coarse? The sampling system must provide for all of these and any other reasonable conditions. Much should be learned during lunar exploration. In addition, care must be taken that one surface condition does not completely foul the sampling device for other conditions, for example, a dust collector sucking up a moist sample. With this in mind, it is possible to conceive of a complex programmed mobile device containing several collection devices.

4. How deep is it reasonable to attempt to take samples? Microbial soil biology is normally such that a population found near the surface will decrease with depth. If Mars has a permafrost layer there may be moist areas at some greater depth where microorganisms exist at populations greater than on the surface. The depth to which one might sample, then, would be limited by the feasibility of drilling to any extent beyond several feet.

5. How gently must the sample be treated? It is relatively simple to specify devices which will obtain samples even under the most drastic conditions. It seems wise, however, to apply energy to the sample only as needed. For most chemical analysis, the treatment may be severe as long as the sample is not appreciably heated. For microbiological investigations, however, the extent of permissible mechanical action is unclear. The additional surface area produced by grinding or crushing may increase the available population, while grinding too finely, or the heat produced by grinding, would kill the population.

6. How might the sample be prepared for analysis? First, it would seem wise to fractionate the material according to its natural size so that any biological material would not be unnecessarily diluted with inert minerals. The various sized fractions could then be analyzed. Size could be reduced by crushing or grinding as required with the resulting material also fractionated and subjected to analysis as appropriate. Particle separation on the basis of density (through flotation) should also be done, since organic matter and inorganic material differ significantly in density.

Although it may not be necessary to perform all experiments on all samples, sample reserves should be established to cross check results from different experiments. For example, one might not initially perform metabolism or reproduction experiments on samples from large rocks, but if a gas chromatogram from the rock sample should show substantial concentrations of organic materials, then excess portions of the same could be programmed into growth experiments. The sampling train, from acquisition through transport, subsequent preparation, and final transport to the analyzing device will involve a complex mechanism which must be extremely versatile and reliable.

Until the experiments are defined in themselves, it is impossible to estimate the size, shape, and form of samples that will be required. It is best to design a system which can provide a variety of sample preparations in quantities which do not limit the detection sensitivities of the various analytical techniques.

Optimum Experimental Configurations

When faced with the increased weight capabilities of larger boosters, many biologists are moved to propose experimentation which will provide detailed characterization of life, assuming that the detection can be accomplished quite simply within the increased capability. This tendency must be avoided until the confidence in the detection devices is so great that instrumentation can be diverted to such detailed description of life. In view of the uncertainties in the many possible forms of life, no detection device can be designed with absolute confidence. To accommodate these uncertainties, however, the instrumentation package must be made larger and more complex to eliminate as many unknowns as possible. This increased capability applies to uncertainties in instrument reliability and planetary conditions as well as the biological features. Even the smallest of the proposed early mission devices is capable of success under the right circumstances. What then is the increased confidence that can be had with increased capacity, and at what point does this capacity cover the more reasonable contingencies?

While the capabilities of larger payloads may provide reasonable assurance of success, this confidence cannot be had simply by the random inclusion of several limited, unrelated devices. Instead, the increased capability must be utilized by the integration of as many of the more promising measures of life's attributes as possible in a manner which the instrumentation and the resulting data reinforce.

The exact determination of what should be included in this "minimum" package cannot be defined at this time because the formulation of the instrumentation and corresponding weights is only an educated guess. Such a package might include the following: (1) A reproduction and detection device that will give a measure of the number and size of particles as a function of time, chosen on the basis of being the least susceptible to interference from soil. A device will also be needed for providing a growth medium prepared from extracts of the soil; (2) a combination of metabolic devices measuring changes in concentration of substrate and metabolic products, utilizing wherever possible instrumentation also required for chemical analysis (e.g., mass spectrographic analysis of gaseous metabolic products, or the change in optical activity of a medium as a result of substrate depletion). A battery of specific enzyme-substrate systems might also be included; (3) analysis for the presence of organic carbon, polymeric materials, optically active materials, and the more significant of the biological compounds (e.g., amino acids). A general survey organic analysis should be included by use of a gas chromatograph and a mass spectrometer; (4) aside from the detection devices per se, provision must be made for an adequate sampling system. It is likely that this sampling probe will not be capable of extensive drilling or digging, but will have to rely primarily on material easily obtained from the surface.

With careful selection of instrumentation and the deliberate elimination of extraneous nonbiological devices, it is felt that all of the above experiments could be

carried out within a payload weight of 200 pounds. Since both the metabolic and reproductive attributes of life are included along with chemical analyses and since the experiments do not rely solely on one medium, substrate, or enzyme, but on a multiplicity of such substances, both local and terrestrial, it is felt that such an integrated combination of devices has a very reasonable chance of success.

A still greater increase in mission capability offers additional advantages including the following:

1. Much larger flexibility with launch windows, landing site selection, and extension of experimentation into unfavorable oppositions.
2. Greater allowance for landing contingencies, in the form of retrorockets.
3. Opportunity for mobility of the package on the planetary surface.
4. Redundancy, not only of components, but of the complete experimental package, possibly at different locations on the planet.
5. Increased facility for nonbiological experiments.
6. Greatly increased operational lifetime for the experiments, perhaps allowing data collection throughout an entire Martian year.
7. Computer control of payload and data processing devices.
8. Enlarged biological experimentation and sampling capability.

The last item would allow additional devices for measuring reproduction and metabolism and performing chemical analyses, but might also include a microscope with high magnification. The increased payload could effectively be used to provide sampling systems capable of penetrating more deeply into the planetary soil and coping with various particle sizes and surface hardnesses.

A great variety of experiments could be contemplated for inclusion in an extremely large payload. In fact, with careful miniaturization, a more or less complete biological and chemical laboratory might be assembled. The pertinent question is whether this broad approach will assure success any more than the expanded "minimum" concept or whether the weight might be more effectively spent elsewhere. Completely redundant packages, with limited mobility, might be more effective insurance for success. The mobility provides for redundancy in sample site selection, but not over a large area of the planetary surface. A multiplicity of packages (of two or four times the weight of the "minimum" package) spread around the more interesting Martian areas, with long lifetime and limited mobility, will provide component, package, sample and site redundancy with maximum effectiveness. Those components which are essential to several or all of the instruments in a given package should have high reliability and whatever redundancy is necessary. Those single components or devices, however, might most effectively be utilized by allowing redundancy from package to package. The large package configuration on several landers is felt to allow the widest possible approach to life detection and will give the greatest chance for success.

Mission Profile After Landing

The following description of a potential life detection mission, together with the accompanying diagrams, is not intended to reflect any degree of design finality or definitive instrument selection, but is simply intended to illustrate what sequence and combination of operations might be performed in a larger scale mission.

Phase I - Lander deployment and preliminary observations. - Upon landing, a complete system check is made on all equipment, the communication antennas are deployed and a fix is attained for telemetry to Earth. A panoramic video is deployed, and the 360° horizon is scanned. Based on this image after transmission to Earth, a nearby site away from the burn area is selected and the mobile subsystem is detached from the main craft and moved to the selected area.

In this position, the mobile package undertakes measurements of the surrounding environment: atmospheric composition, meteorological conditions, light flux, audible sounds, surface hardness, soil pH, and close-up pictures of the ground. This monitoring process could be continued periodically throughout a complete Martian day and night. In the interim, the panoramic video system continues to scan the surroundings, so that on Earth, topographical maps of that area can be constructed. Based on this assimilation of the local conditions, a more refined site selection is made. The spacecraft mobile unit moves to this site and repeats the above measurements of the surroundings. The resulting information is then programmed into the spacecraft computing system, and the proper sequence of sampling and analysis is chosen.

Phase II - Sampling. - Within a selected area of several square meters, the sampling devices are deployed, depending upon the previous measurements of the surface. Suggested sampling devices include scoops, scrapers, brushes, suction apparatus, drills,or covers. The material from the collector is conveyed separately to the sample preparation apparatus.

Phase III - Sample preparation. - The material from the sampling system is sized on a stacked sieve which separates out all material larger than 4 cm, passes through all finer than 1 mm, and subjects intermediate samples to a mild grinding process for subsequent resizing. The grinding operation is monitored by the mass spectrometer for gases released in the grinding and by a thermocouple to prevent overheating of the soil sample. A portion of the fine soil is conveyed to an extraction apparatus where the material is treated with a battery of solvents, acidic and basic, aqueous and organic, polar and nonpolar. The acid and base fractions are then treated with ion exchange resins as necessary, and all the fractions are concentrated by solvent evaporation (with solvent recovery, if possible). All waste materials are transferred to a container which can subsequently be sealed and dropped after completion of sampling at that site.

Phase IV - Chemical experimentation. - A sample of soil is oxidized, and a sensitive carbon detection device analyzes for the presence of carbon dioxide produced from organic molecules present in the soil. Hopefully, the carbon detection apparatus is of such sensitivity and reliability that negative results at this point eliminate from subsequent analysis any test involving organic compounds. Should organic carbon be found, then volatile materials in the soil could be sought via analysis by gas chromatography after heating of the sample to 100°C. Subsequently, the samples are pyrolized at higher temperatures to detect the presence of higher molecular weight compounds. The mass spectrometer may be used to confirm and supplement the analysis of the gas chromatographic fractions. Again it should be pointed out that careful sample preparation is required for such analyses.

The extracts previously prepared are subjected to optical rotatory dispersion measurements (ORD) to examine for the presence of optically active species and to

obtain the ultraviolet spectrum of the sample as well. In certain cases, infrared spectroscopy is used for functional group analysis.

The aqueous extracts are then tested for specific inorganic ions which are usually associated with living materials, using primarily colorimetric techniques. The determination of inorganic materials is completed by spectrographic analysis of soil samples.

Analysis for specific biochemical compounds is undertaken on the extracts, using colorimetric tests and spectroscopic analysis in some cases, while in other resorting to hydrolysis, derivative formation, and gas chromatographic analysis.

Phase V - Biological experiments. - Media are prepared either from materials transported from Earth or from natural soil extracts, and are inoculated with dilute soil suspensions. These suspensions should be made from fractions separated from soil samples on the basis of density, organic materials being less dense than inorganic. Another similarly treated sample is sterilized by either heat, chemicals, or radiation. For measurements of reproduction, the difference between the sample and reference growth chambers is monitored with respect to turbidity and/or nephelometry, particle counting, and video-microscopy. In addition, various plating techniques will be attempted with video monitoring.

In an analogous fashion, the sample and reference growth media are subjected to analysis for metabolic changes in the form of changes in pH and Eh, optical activity, conductivity, and other solution properties. Heat production is measured in a dual chamber calorimeter (again referenced to a sterile control). The gases above the growth media are monitored for evidence of respiration, and certain specially radioactively labeled substrates are used for detecting metabolism in the form of production of labeled gases from this substrate.

It is, or course, desirable to do as much of the above as possible in as many locales as is feasible. Some of the analyses should be repeatable as a function of time of day and season.

Figures 1 through 4 schematically illustrate some of the measurements which might contribute to the detection of life with an automated payload.

STERILIZATION

As has been pointed out many times, we must have confidence that the life detected on an extraterrestrial body is not that which has been carried there by the spacecraft from Earth. The potential danger to extraterrestrial life from Earth microorganisms has been stressed repeatedly.

One of the most recent positions taken by the scientific community on the sterilization question has been voiced by the Space Probe Sterilization Standards Study Group of COSPAR. This group met in Florence, Italy, in May 1964, and produced the following recommendations:

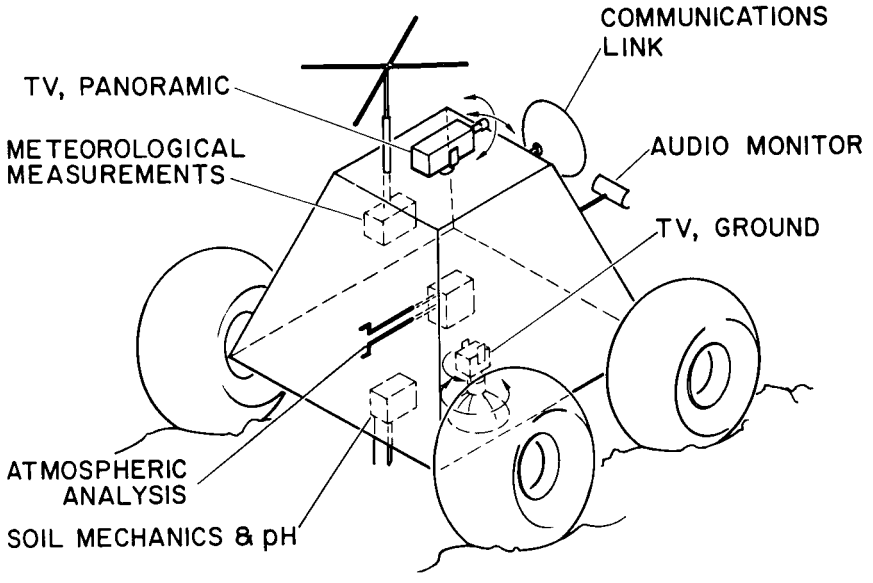


Figure 1. - Preliminary observations.

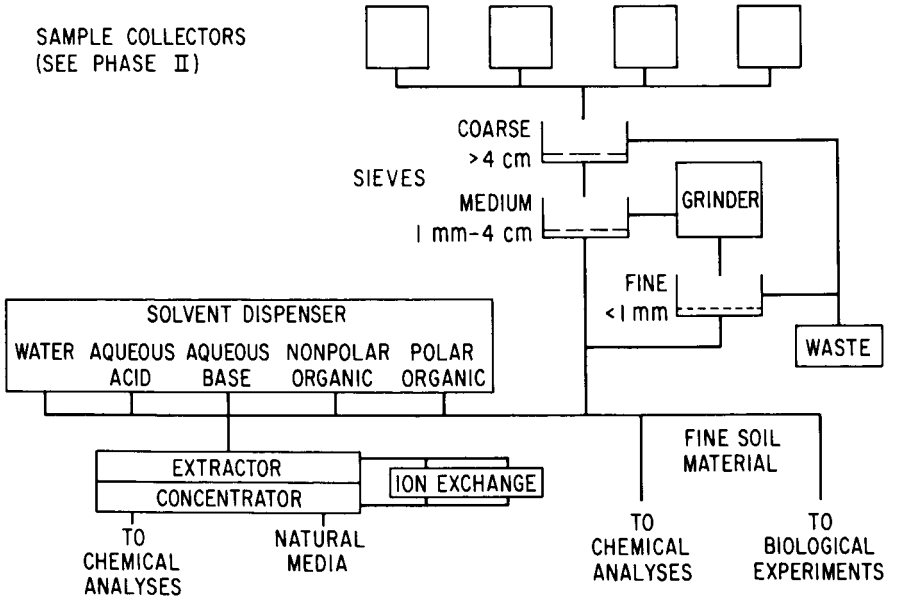


Figure 2. - Sample preparation.

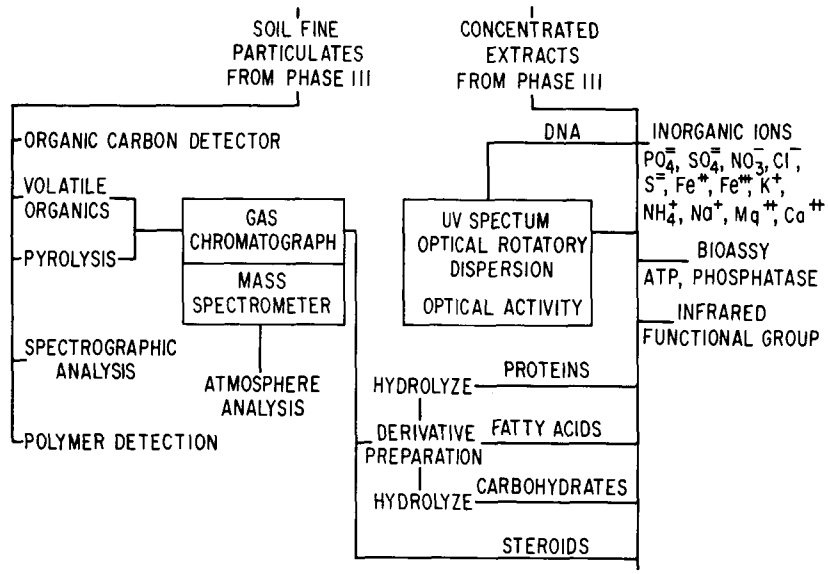


Figure 3. - Chemical analysis.

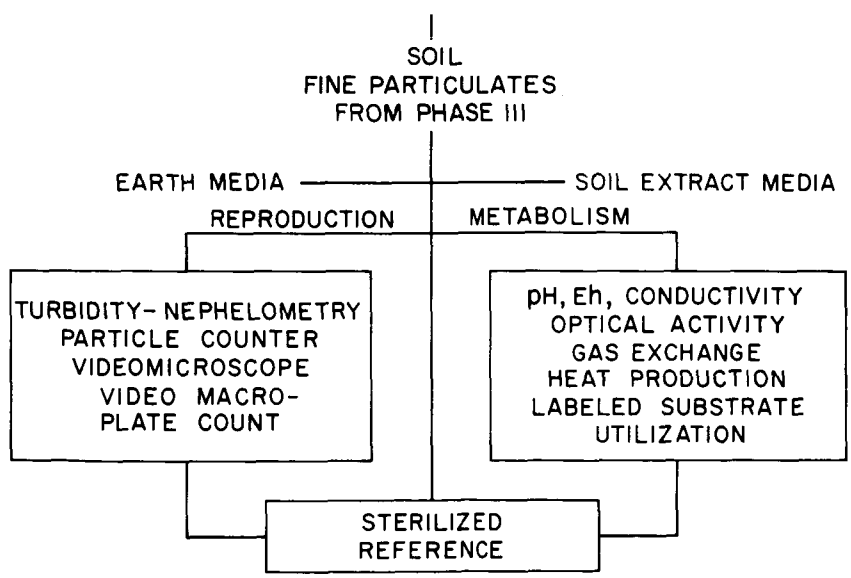


Figure 4. - Biological measurements.

"We believe that the scientific desirability of sterility control is absolute; but the degree of sterilzation required must be based on our judgements of the risks acceptable so planetary exploration will not be impossibly difficult. The probability that a single viable organism is aboard any space vehicle intended for planetary impact can then be computed as the solution of a waiting time problem in probability theory. Adopting values for the acceptable risk during approximately a decade of planetary exploration by landing vehicles, and for the biological and spacecraft reliability parameters involved - values which we consider conservative - we conclude that:

1. the probability that a single viable organism be aboard any vehicle intended for planetary landing must be less than 1×10^{-4}, and that
2. the probability of accidental planetary impact by an unsterilized fly-by or orbiter must be less than 3×10^{-5} during the interval terminating at the end of the initial period of planetary exploration by landing vehicles (approximately one decade).

"We appreciate the considerable technical difficulties involved in realizing these probabilities in practice, but we consider that they are attainable by known means. The probabilities also apply to contamination by spacecraft propulsion and attitude-control systems. The probability of contamination by accidental impact of fly-bys and orbiters can be minimized by:

1. initial trajectory control,
2. initial spacecraft sterilization, or by
3. inclusion of programmed or commanded terminal precautionary systems for assuming nonintercept trajectories or for initiating destruction sterilization.

The probabilities given above are obviously subject to future revision as our knowledge of planetary environments, microbial ecology, and spacecraft design improves.

"We feel that while our recommendations apply immediately to fly-by, orbiter, and lander missions planned for Mars, the same recommendations should apply to any planet, which, on the basis of current information, cannot firmly be excluded as a possible abode of extraterrestrial life."

The constraints imposed by such rigorous standards on the development of spacecraft and payloads for extraterrestrial probes are obvious. The difficulties in sterilization of large boosters and complex instrumented capsules are enormous, and some feel that the task is nearly impossible if we are to explore other planets in our lifetime. Some attempt to reassess the contamination problem seems appropriate. Two kinds of data available are suitable for inclusion in such an evaluation.

There are really two aspects to the possibility of contamination which can be assessed independently. First, there is the question of the likelihood of landing viable organisms on the planet under such conditions that they will be able to proliferate, and thus pose a biological threat to any indigenous population before the native organisms can be studied. The second problem is that of carrying microorganisms which may not necessarily pose a threat to the indigenous population, but which are subject to detection by instruments carefully designed for the detection of life. In the first case, to be a menace the organisms must be able to grow under the conditions prevailing on the planet in question. In the second case, the organisms must only survive the landing.

Clearly, if organisms will survive on Mars, all precautions must be taken to see to it that none are available to contaminate the experiments. This means that the experiments and their immediate surrounding must be rigorously sterilized.

Horowitz (personal communication) has analyzed the problem in the following way:

"The chance that a spacecraft will contaminate Mars with terrestrial microorganisms is a function, not only of the number of bacteria carried to the planet, but also of their location in the spacecraft. Bacteria on exposed surfaces will obviously have a better chance of infecting the planet than those which are sequestered within electronic or other components. Fortunately, exposed bacteria are also accessible to bactericidal agents such as ethylene oxide and can easily be destroyed. Sequestered cells, however, can be killed only by heat or other drastic treatments, the use of which would seriously jeopardize the mission.

"The bacterial load. Data on the extent of internal contamination of electronic components have been published by Phillips and Hoffman. These authors determined the number of items with internal contamination among 150 assorted electronic components - transistors, capacitors, resistors, etc. The surfaces were first sterilized with ethylene oxide, and each component was then pulverized aseptically and used to inoculate a flask of broth. After incubation, the flasks were scored for growth or no growth. In this way, it was determined that 21 or 14 percent of the items were contaminated. The remainder carried no detectable microorganisms. If the plausible, but unproven, assumption is made that bacteria are distributed in a Poisson distribution in electronic components, it becomes possible to estimate the average number of bacteria per component. This number is 0.15, an intuitively obvious result. If it is assumed that the average weight of the components was 3 grams, then there is one bacterium per 20 grams of electronic apparatus, or 1000 bacteria per 20 kilos.

"Release of sequestered bacteria. Bacteria carried to Mars inside electronic components do not constitute a contamination hazard as long as the components remain intact. If the spacecraft is broken up as in a crash landing, then there is a certain probability of release (P). An estimate of P as a function of the extent of fragmentation has been made, based on the following model: Bacteria are assumed to be spherical cells of diameter 1μ. Spacecraft components are assumed to be fragmented into spherical particles of radius r. A bacterial cell is considered to be released if it comes to lie within 5μ of the surface of any fragment; thus, for example, a bacterium contained anywhere in a 10μ diameter particle is considered released.

"The probability that a bacterium will lie within 5μ of the surface of a spherical particle is equal to the volume of the spherical shell of thickness 5μ, divided by the volume of the particle:

$$P = \frac{4/3\pi r^3 - 4/3\pi(r-5)^3}{4/3\pi r^3}$$

where r is the radius of the particle. Some representative values of P are shown in the following table:

r	P
5μ	1.000
10μ	0.875
50μ	0.37
100μ	0.14
150μ	0.1
200μ	0.07
500μ	0.03
1000μ	0.015
1500μ	0.011

It can be seen that fragmentation to millimeter size liberates only a few per cent of the sequestered bacteria; fragmentation to the micron range is necessary to free more than 50%.

"Conclusions and recommendations. The limited data at hand suggest that electronic components are very clean from a bacteriological viewpoint. Furthermore, the organisms which are the most dangerous from a cosmoecological point-of-view are those which, being located on exposed surfaces, are most easily killed by gaseous sterilants such as ethylene oxide. Organisms that are located within components can be released only by considerable fragmentation of the components. These considerations suggest the possibility that reasonable sterility levels might be attained without terminal heat-sterilization of spacecraft. In order to make a judgment of this possibility, further information is urgently needed."

In addition to the above considerations, several laboratories have shown that some bacteria are able to survive simulated Martian conditions, but it is not easy to demonstrate bacterial growth under these conditions. However, only slight modifications of the "Martian environment" are required for growth of certain organisms.

The sterilization problem requires much more research and technological development before any changes in current procedures can be recommended.

SUMMARY

The exploration of Mars by automated devices during the next decade for evidence of life can provide man with the basis to ascertain the universality of laws pertaining to biology, and a wealth of information pertaining to planetary evolution in general. The opportunities for exploration occur at limited intervals and with such constraints as to require early and careful planning. The expense and complexity of these missions necessitate optimum design, both in engineering and science. Since contamination of Mars by unsterilized spacecraft will occur eventually, Martian biology must be studied at the earliest opportunity which will permit successful exploration. Success is likely only with an extensive effort by large payload systems. The delay of several years for the large boosters is considered to be preferable to the risk of contamination and inconclusive results from small early missions.

The selection of life detection instrumentation on the basis of the most significant attributes of life must consider those experiments which are the most indicative of

life, for example, reproduction and metabolism versus those which are the most likely to give informative results, for example, chemical experiments. A confirmation of individual experiments and correlation between the results of different types of experiments will be necessary for meaningful interpretation. The sampling system must be both complex and versatile in order or obtain sufficient quantities of material under a variety of conditions. The system must have at least limited mobility for sampling away from the retrorocket burn area. It is desirable to sample from both the Martian dark and light areas, as well as throughout the seasons and during the wave of darkening. Adequate power, telemetry rates, spacecraft lifetime, and reliability rate are requirements as essential as the biological experiments themselves.

Since the primary purpose of the mission is biological, extraneous experiments must not be included in any instance where the life detection capability is jeopardized. It is important to realize, however, that the more sophisticated biological experiments demand a thorough familiarity with the Martian environment, its chemistry, meteorology, geography, etc., not only for data interpretation, but in many instances to properly prepare samples, design and program experiments, and prepare growth media.

In the interval remaining before the availability of adequate boosters, astronomical observations, fly-bys, orbiters, and sterilized crash-probes are needed to define more clearly the crucial Martian planetary environment, such as atmospheric density, temperature, gas composition, and abundance of water. The spacecraft design should be flexible to allow for these redefinitions, not only for the atmosphere entry problem but also for revisions in the biological instrumentation requirements.

It should be added in conclusion that if life is not found on Mars, a determination of why this is the case will also add much to our understanding of the nature of life, and the role of planetary evolution in its genesis.

APPENDIX

ENVIRONMENTAL PARAMETERS OF MARS

Atmosphere

Only two molecules have been identified in the atmosphere of Mars. One is carbon dioxide, in amounts estimated at from about 3 to 30 percent. The other molecule is water, in amounts only about 1/1000 of that in the Earth's atmosphere. It is assumed that nitrogen and argon are present. Molecular oxygen has not been detected and if it exists at all, it must be in very low concentrations. Oxides of nitrogen have been reported, but in general, this observation is not accepted and has not been confirmed.

Radiation

The radiation flux on Mars at the surface is unknown; however, it is presumed that the ultraviolet flux which reaches the surface is rather high, since there appear to be no ultraviolet absorbing materials in the atmosphere. The estimates of particulate radiation at the surface are about 6×10^{-4} rads per hour if the atmosphere has a total pressure of approximately 40 mb, but at around 10 mb, this figure increases by a factor of 2 or 3.

Surface Composition

The surface of Mars is considered to be limonite from polarization data obtained by Dollfus. This is a hydrated iron oxide and is appealing from a biological point of view because of its water binding capabilities.

Pole Caps

The polar caps of Mars are thought to be ice. They are seen to wax and wane with the seasons and are probably a frost layer a few centimeters thick. In the summer season the pole cap recedes at a rate of about 35 km per day. As one pole cap is receding, the pole cap of the opposite pole is apparently being formed under a cloud, implying a pole-to-pole transport of water vapor. A dark band, which seems to be from 100 to 200 km wide, has been observed to follow the receding pole cap. This is not generally thought to be a wetting phenomenon since the total amount of water in the pole cap of Mars must be very small, and the conditions of temperature and pressure in these regions seem to preclude the possibility of liquid water. More measurements are needed to clarify this point.

Dark and Light Regions

The surface of Mars is divided into dark regions and light areas. Color changes have been observed in the dark areas, but these color changes are probably illusory. There is a wave of darkening that proceeds at a rate of 35 km per day from pole to equator in the dark areas in the spring. It has been suggested that the wave of darkening is correlated with the movement of water vapor through the atmosphere from one pole through the equator toward the opposite pole, but the evidence for this is poor. It has also been suggested that the wave of darkening is correlated with a living process. However, it seems quite unlikely that the wave of darkening could be directly correlated with a living process, because of the low temperature which appears to prevail at its leading edge. If it could be shown that the temperatures in these regions are considerably warmer than indicated, this picture could change appreciably.

Clouds

Three different types of clouds have been observed in the atmosphere of Mars. There are white clouds which are generally considered to be composed of ice crystals, and yellow clouds which are considered to be dust. It has been shown that dust storms are possible in spite of the thinness of the Martian atmosphere. In addition, there is a blue haze which appears in the atmosphere of Mars, which is a transient and little understood phenomenon.

Temperature

The average temperature of Mars is considerably lower than the average temperature of the Earth; however, the temperature extremes on Mars are not incompatible with life. The highest observed daytime temperature near the equator is about 30^{o} C while the nighttime temperatures apparently go well below freezing (about -70^{o} C) so that even during the summer when life on Mars may be attempting to proliferate the diurnal temperature cycling is very severe from a biological point of view. It also seems likely that the time during a Martian summer day when the temperature may be significantly above zero is very brief, possibly only about 5 or 6 hours. Farther from the equator, of course, this time is even shorter. These temperature estimates, however, are average figures and do not preclude the possibility of microenvironments on Mars in which the temperatures may be considerably less harsh.

Pressure

Estimates of the total atmospheric pressure at the surface of Mars vary from around 100 mb to as low as 10 mb. The most recent determination shows the surface pressure to be about 25±15 mb. Pressure, per se, is not a particularly important factor for microorganisms, except in that it affects the availability of water. For example, at 11 mb, water is in its liquid phase only from 0^{o} to 8^{o} C, while at 25 mb, it is only liquid from 0^{o} to 20^{o} C, and so forth.

Water

Water has recently been identified in the Martian atmosphere at about 14±7 microns of precipitable water. This is approximately 1/1000 of the amount of water that one would find in the Earth's atmosphere, and this is an extremely important factor from a biological point of view. Indeed, it seems that there must be more water than this, at least periodically, for biological activity to occur. Again, microenvironments, in which above average accumulations of water exist, must be sought.

Meteoritic Influx

The meteoritic influx on the Earth is approximately 7×10^4 tons per year. The meteoritic influx on Mars must be considerably larger (10^6 to 10^7 tons per year), because the orbit of Mars is closer to the asteroid belt, and since the atmosphere of Mars is considerably rarer than that of the Earth, the survival rate of meteorites entering the atmosphere will be greater. It is possible that the contribution of organic material and water to the surface of Mars by meteoritic influx may be appreciable.

Sinton Bands

Sinton has observed peaks in the infrared spectrum of Mars at 3.45, 3.58, and 3.69 microns, which have been interpreted as evidence of carbon-hydrogen bonding and, thus, organic compounds on the surface. Sinton found these peaks to be restricted to the dark areas. Colthup interpreted Sinton's peaks as being indicative of an aldehyde, and specifically acetaldehyde. Recent observations indicate that two of the three Sinton bands are probably due to DHO in the Earth's atmosphere, and not Martian features at all. The question of organic materials on Mars remains open.

Microenvironments

Since the planet Earth is really a composite of a vast number of microenvironments, from one extreme of dryness and temperature to the other, there is no reason to think that a similar geographical situation should not exist on Mars. For example, it is conceivable that considerable amounts of water could be tied up below the surface of the planet in the form of an ice layer which, through localized geothermal activity, could give rise to an oasis or warm spring at the surface.

MARINER IV OBSERVATIONS

The recent (July 1965) Mariner spacecraft, which was able to return photographs of the surface of Mars from as close as 12,000 km, contributes little to the question of life on Mars. It should be remembered, however, that this vehicle was not intended to contribute directly to that question. Although at the time of this writing the analysis of the Mariner data are not complete, preliminary interpretation indicates no severe deviations from the environmental parameters believed to exist earlier.

The Mariner probe did indicate the lack of a magnetic field around Mars which indicates the probability of a high radiation flux at the surface.

The surface pressure indicated by the Mariner probe is at the lower end (10-11 mb) of the scale previously estimated. Large numbers of impact craters are observed in the Mariner IV photos whose size range up to 120 km in diameter. Because of the unweathered appearance of these craters, it is concluded that the visible surface of Mars is very old (as much as $2\text{-}5\times10^{9}$ years); and for that time has had neither a dense atmosphere nor large bodies of liquid water. This does not preclude the existence of water and a denser atmosphere earlier in the history of the planet, and in fact, as suggested by the Mariner IV experimenters, the surface of Mars may be the only place in this solar system which still holds clues to primitive organic chemistry, all traces of which are long gone from the earth because of biological activity. This concept, of course, strengthens our case for placing chemical studies first in priority for Martian experiments.

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