REPRINTED FROM:

LIFE SCIENCES AND SPACE RESEARCH II

A SESSION
OF THE FOURTH INTERNATIONAL SPACE SCIENCE SYMPOSIUM
WARSAW, JUNE 3–12, 1963

COSPAR
(COMMITTEE ON SPACE RESEARCH)

EDITORS

M. FLORKIN A. DOLLFUS

MULTIVATOR – A BIOCHEMICAL LABORATORY FOR MARTIAN EXPERIMENTS

E. LEVINTHAL, L. HUNDLEY AND J. LEDERBERG
Instrumentation Research Laboratory, Genetics Department, Stanford University

1964
NORTH-HOLLAND PUBLISHING COMPANY — AMSTERDAM

MULTIVATOR – A BIOCHEMICAL LABORATORY FOR MARTIAN EXPERIMENTS*

E. LEVINTHAL, L. HUNDLEY and J. LEDERBERG

Instrumentation Research Laboratory, Genetics Department, Stanford University

Abstract: The Multivator represents a particular choice of a detection system to serve in the quest for signs of life on Mars. We will discuss the establishment of a critique for making such a selection, the present restrictive limitations, and other choices that might be made when less restrictive requirements are imposed.

The details of Multivator as a device will be discussed. The design, or minor modifications of the design, permit many different kinds of biochemical experiments to be carried out, particularly those which can be arranged to use a photomultiplier as an output transducer. Such experiments include fluorometry, nephelometry and scintillometry. Our efforts have largely centered on photometric assays of fluorescence, particularly those to determine a widely distributed enzyme, phosphatase.

Of this abstract no Russian translation has been received.

1. Introduction

We wish to discuss the philosophical concepts underlying the activities of the exobiology group of the Instrumentation Research Laboratory and their embodiment in the Multivator as an instrument. A modification of the instrument has also been designed by Professor John E. Arnold† of the Department of Mechanical Design at Stanford University. While we clarify those types of assays which the Multivator can carry out and give a little

* This research was supported by the National Aeronautics and Space Administration under grant NsG 81–60.

† Since Professor Arnold's death in September, 1963, Professor Peter Bulkeley has assumed the responsibility for the continued development of Multivator mechanical design.

detail on some that have been investigated in our laboratory, a complete presentation of these experiments is left to future publication by Dr. Elie Shneour and others in our laboratory carrying out this research.

2. Signs of life

The field of exobiology is growing both in the scope of its problem and in the number of scientists devoting attention to them. It therefore becomes a matter of importance to attempt to state what these concerns are in an organized way. This should serve to indicate where new experimental and theoretical effort is needed and to provide a framework for the evaluation of specific experimental possibilities as they arise.

There are an increasing number of reasons for accepting the possibility that life in the form of reasonably complex replicating molecules is prevalent throughout the universe. This same kind of reasoning leads one to further suppose that intelligent forms of life are perhaps an order of magnitude less prevalent. The quest for signs of life is the overriding immediate concern of exobiology and leads to different experimental criteria than the concern which follows; namely, understanding the nature of the life where signs have been discovered. Obviously the successes and even the failures of the search give some insights pertinent to the later analytical concern, but the instrumentation for search can be different than for analysis.

The difference between criteria for search and analysis is illustrated particularly clearly in connection with the question of intelligent life in other solar systems. At first one has to explore the complete domain of space and possible communication links, asking only for evidence of non-random phenomena unexplainable by *known* physical laws. A positive response to such a query is not at all convincing as far as the question of the existence of intelligent life elsewhere and says nothing of its nature. It, however, rationalizes an enormous narrowing of the region of search and thereby allows a corresponding increase in the analytical possibilities.

Besides the distinction between search and analysis there is another meaningful classification. Efforts to find simple forms of life by direct contact will, for at least the next decade, be limited to our solar system, where there is no evidence for the existence of intelligent forms of life* on any planet other than the earth. Thus, for the present, the search outside

* There may be other manifestations of well developed intelligence on earth than human intelligence (i.e. Cetacea). If this is so, attempts to communicate with these species permit the development of analytical techniques independently of search techniques, and require no development of technology and manipulative skills on the part of the species with whom we are attempting to communicate.

the solar system is necessarily limited to intelligent forms of life capable of communication, and that within the solar system for simple forms.

The dichotomy between search and analysis enters into the search for signs of simple forms of life within our planetary system. This is sometimes obscured by the desire to avoid a two-step process and combine both functions in one experiment. This demands an experiment that is at the same time completely general and yet sufficiently rich in analytical details to give an unambiguously interpretable answer. This combined goal is sought not only because the prize of definitive proof of the existence of life elsewhere is so great, but also because of the problems associated with contamination. This latter concern puts a high premium on the first landed capsule giving an unequivocal answer. It also supports the conclusion that the first capsule should not be landed until fly-by search missions have served to narrow either or both the geometrical volume and the volume of a matrix of possible chemical and biological properties of living systems that must be analyzed.

It is such a matrix that needs to be established in order to have a basis for choosing experiments for either search or analytical missions.

A possible format for such a matrix is shown in fig. 1. The concept of this matrix will be clarified by the following comments.

(a) Here we list the elements common to terrestrial living systems.

(b) In this column the typical chemical structure and function remain the same, but there are substitutions within a class. For example, arsenic rather than phosphorus is used for formation of energy storage bonds; or selenium or sulphur is used to play the role of oxygen; alpha aminobutyric acid might be an amino acid.

(c) Under remote analogues we simply specify inorganic, organic and macro-molecules that play functional roles similar to those found in the terrestrial system but do not ask that they have the same structure, for example, polyesters might be the basis of enzymatically active polymers in place of proteins (polypeptides).

(d) Included here is DNA (as well as protein, polysaccharides, etc.) but in the corresponding box under remote analogues we look for a more general description of information-storing and -replicating molecules.

(e) None of these molecules affords a sufficient criteria for the presence of life, but the accumulation of some of these molecules as components of organisms in high local concentrations is a necessary condition.

(f) An important observation can be made concerning a property of organic compounds in carbon-based living systems that might be extended to all living systems. If, in a living system, there are organic compounds of even moderate complexity, and the production of these compounds involved

← First priority scope of Mars investigation → ← High temperature systems →
← Interstellar space →
← Jupiter →

	The earth system	(b) Close analogues with class generalization	(c) Remote analogues	Non-aqueous	Non-carbon Non-aqueous	(g) Non-liquid
Atoms	C, N, O, H, P, S, Na, K... (a)				Si, N, B, P...	
Molecules in environment in organisms	H_2O, CO_2, O_2, CH_4, NH_3 (e)					
Organic molecules	amino acids nucleotides (f) metabolites					
Macromolecules	DNA, RNA proteins (d) polysaccharides					
Cells organisms societies	Intelligent communication					

Fig. 1.

a template which preserves their steric form, it must follow that organisms exhibit concentrations of compounds containing asymmetric carbon atoms and net optical activity of particular molecular species.

(g) Biochemical criteria for non-carbon, non-aqueous or certainly non-solvated systems of life require a philosophical generalization of biological theory not attempted at this point. The temperature of the habitat is perhaps the single, most important parameter that defines the range of research possibilities of biochemical constructions. Most of our thinking is of course conditioned by the biospheric range of 250–400 °K, defined primarily by the properties of water. For our first considerations we have decided to concentrate our efforts on this familar territory before planning distracting expeditions into even less accessible regions outside this range.

Having constructed such a matrix, the next task is to give probability values to its dimensions and adjust the scales so that equal areas are equally probable. A rational experiment or series of experiments minimizes the

effort required to explore this surface. Our experiments are based on the expectation that the maximum probability in our dimension lies in the region of close analogues. While we recognize the existence of a region of high temperature systems we have presently assigned it a negligible probability for Martian investigations.

For this procedure to be useful it is neither necessary nor presently possible for it to be carried out with rigor: the uncertainties of the solutions will, however, become more deductively ascertainable functions of the imprecisions of the stated premises.

The usefulness of a matrix of the chemical and biological properties of living system versus premises of different order of generalities would be enhanced if there also existed an exhaustive categorization of methods of experimental observation. Then one could be sure that one had arrived at a logical choice of experimental objective and at the same time the optimum technique. It is not possible at present to make such an exhaustive categorization. To be able to do so would imply that there existed a complete theory for chemical behavior. At present there is hardly a complete description of the behavior of large molecules, let alone a theory. It still may be worthwhile to attempt such an enumeration even though it be limited to a specific region of possible experiments. For example, a listing of all the possible ways of detecting hydrogen and its isotopes would demonstrate the enormous range of techniques the exobiologists should keep in mind in arriving at his research decisions. It is obvious that this is a matter of continuing concern. The means of satisfying the parameters such as incident flux and detectivity associated with any technique are constantly changing so that one must continually reexamine the experimental choices made. This also makes it a matter of importance to have a framework into which one can put new data pertinent to a particular experimental approach. That is to say that an exhaustive organization of experimental methods coupled with a system of scientific information storage and retrieval would be a powerful tool in making experimental decisions and might even bring to light completely new methods.

3. Multivator

Multivator represents an attempt to generalize the instrumental requirements of exobiology experiments. We are designing an instrument to meet the constraints of Mariner-type missions and which will be able to carry out a wide range of biochemical experiments. A simple set of performance characteristics of this instrument becomes the interface the biologists and

MULTIVATOR PARAMETERS

Reaction chamber	
Volume	≈ 0.1 ml
Number	15; 12 receive a soil sample of ≈ 5 mg; 3 are blanks
Mode of operation	Reagents stored dry in vessel; soil insufflated; solvent injected (water), incubated; measurements made. Note that some reagents may be stored as time-release capsules.
Temperature	≈ 30 °C (uncertain over what period of Martian diurnal cycle this would be maintained).
Measurements	
Types	Fluorometry, colorimetry, nephelometry. Wavelength range available 300–600 mμ. Lamp power may be assumed to be about 10 microwatts/mμ. Detectivity is about 1 part in 10^6 of input light for fluorometry or nepholometry and 1 part in 100 for colorimetry. Different input and output filters may be chosen for each reaction chamber.
Frequency	One reading on all chambers every 15 min.
Data form	Each reading quantitized into binary code of 6 bits (64 levels). May be linear or log.

Fig. 2.

biochemists in our laboratory have to cope with, not the total complexities of space missions. We hope, in time, to broaden the range of experiments that Multivators can carry out and to design other instruments with similar breadth of purpose.

3.1. Present multivator parameters

The interface characteristics of the current design are shown in fig. 2. The following possible changes would require only minor modifications: (a) non-aqueous solvents; (b) different solvents in each chamber; (c) measurement of conductivity, pH, and of radioactive reaction products; and (d) increase reading frequency to every five minutes and number of quantitization levels to seven bits (128 levels).

It should be remembered that the system will be in dormant storage for the six-month interval of space flight, and that all components of the experiment must withstand this rest period. Temperature will be maintained above freezing.

According to present sterilization criteria, the component of an experiment must be amenable to heating at 135 °C for 24 hours. We have not yet met this requirement for the assays we are investigating.

3.2. MARK I MULTIVATOR DESIGN

The design and fabrication of a device of the Multivator concept has been undertaken. Limitation on size, weight and power consumption as well as operation independent of orientation have led to the configuration of the present model, designated Mark I.

This particular design is based on the use of substrates tagged with a fluorogenic indicator for the detection of enzymes characteristically produced by soil microbes. The design is in accord with the parameters indicated in the proceeding section. It lends itself to the use of radioactive tagging techniques with only minor modification of the existing instrument.

Mark I is designed to analyse a dust sample, collected from the planet surface and delivered to the reaction chambers as an aerosol. A number of sample collection methods are being investigated including vacuum cleaning and electrostatic pick up.

The photographs depict the bench model of the device. The complete Multivator capsule is about 25 cm long, 7 cm in diameter and weighs 380 g.

Fig. 3 shows the device as it is removed from the capsule. From the upper left the components are: base plate, solvent chamber piston spring, solvent chamber, lamp connector ring, photomultiplier tube shield and photomultiplier.

In fig. 4 the aerosol inlet tube is visible running through the center of the solvent liquid chamber piston spring. The reaction chamber exhaust ports can be seen cut into the lower end of the solvent chamber. The ports in the photomultiplier housing are for the fluorescent exitation lamps, one for each reaction chamber. These lamps will be turned on sequently to obtain a reading from each reaction chamber.

Fig. 5 affords a view of the solvent chamber piston and the reaction chamber cover. The guide rod for the aerosol inlet tube shut-off valve may be seen protruding from the center of the reaction chamber cover. This valve is spring loaded, fuse-wire actuated and closes after filling is complete.

Fig. 6 shows the individual reaction chambers, the aerosol distribution channels, the solvent loading ports in the channels, the end of the aerosol loading tube and the air exit ports in the bottom of the reaction chamber. This configuration with 15 reaction chambers is designed for three or four experiments, each with several controls. The reliability and interpretibility of any assay is obviously dependent on adequate control. As an example of one of the controls, those chambers which do not have inlet channels to

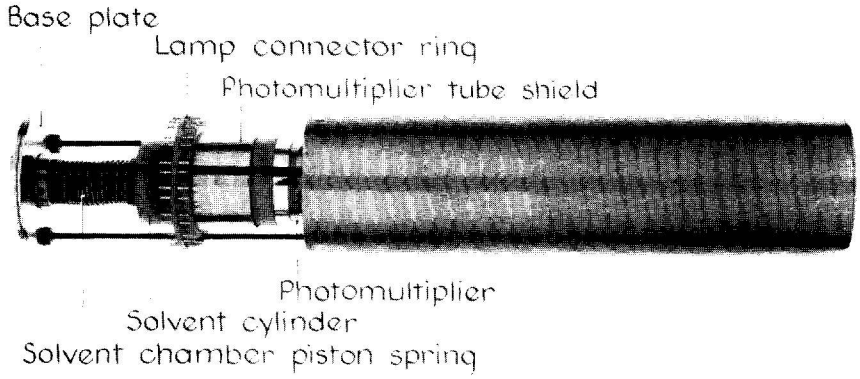


Fig. 3.

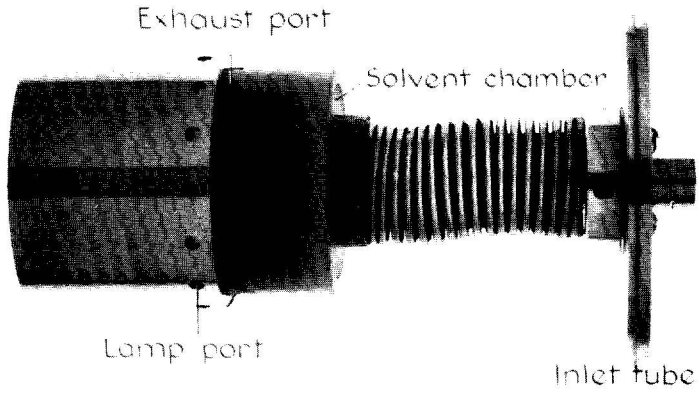


Fig. 4.

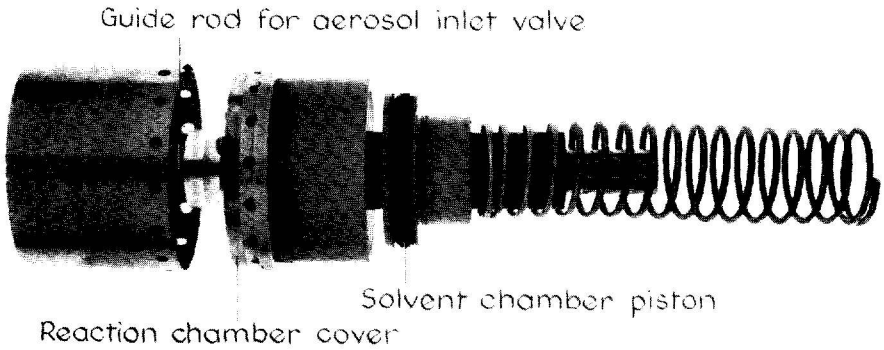


Fig. 5.

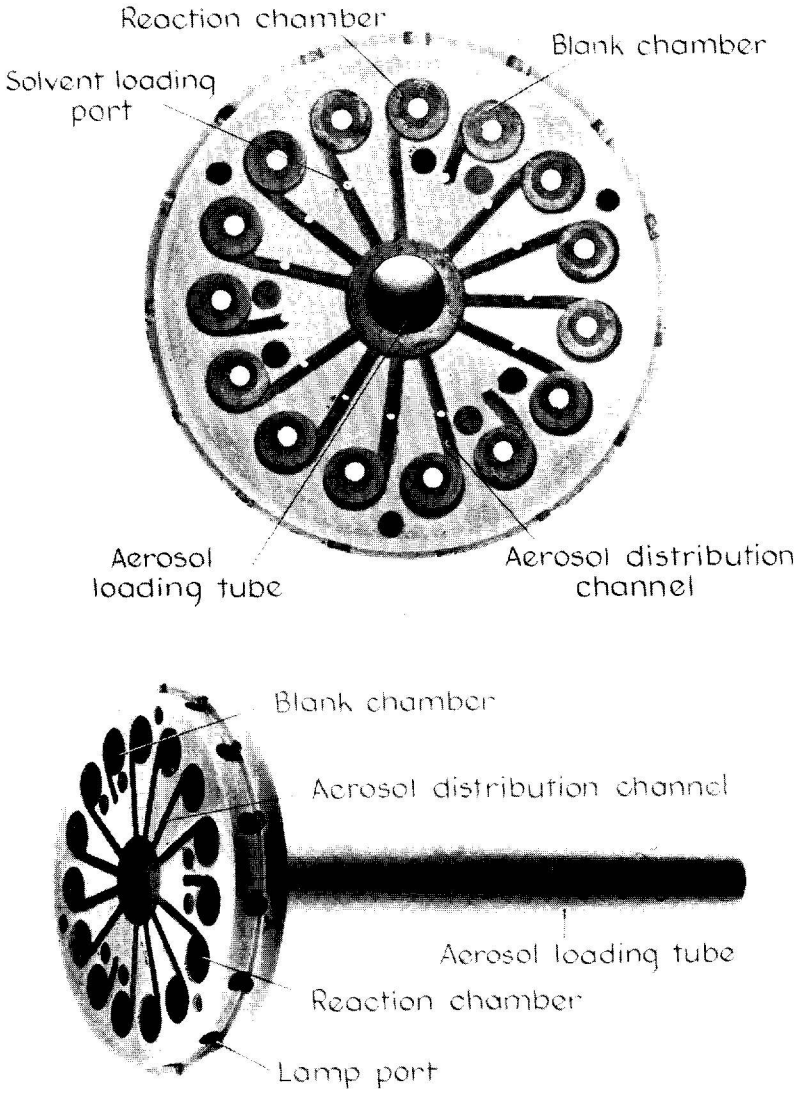

Reaction chamber
Blank chamber
Solvent loading port
Aerosol loading tube
Aerosol distribution channel
Blank chamber
Aerosol distribution channel
Aerosol loading tube
Reaction chamber
Lamp port

Fig. 6.

the aerosol inlet tube are designed to test the stability of the fluorescent substrate after the long space voyage.

The operation is as follows. Dust carrying the microbial sample is filtered to ensure that there are no oversize particles and is then blown through the inlet tube and into the reaction chambers. The dust is collected by a sticky coating on the chamber walls, and the dust-free air exhausts out the port in the rear of the chamber. This method of collection has been experimentally shown to collect about 96% of the dust in the aerosol. The aerosol inlet valve then closes, sealing off the individual reaction chamber. A small rotation of the solvent chamber closes off the air exhaust ports and aligns solvent filling ports in the solvent chamber with those in the inlet channel. The solvent chamber piston then injects the solvent into the reaction chambers. The substrates which have been stored dry in the chambers are dissolved and the reaction begins. After a preset reaction time, the exitation lamps are then turned on sequentially, and the light level in each chamber is detected by the photomultiplier tube. This information is then reduced to digital form and transmitted to earth.

3.3. Mark II Multivator

Further refinements of the mechanical design are being carried out by a separate program under the direction of Professor J. Arnold in the Mechanical Design Department. We are working closely with Professor Arnold's group in this endeavor.

For the sake of completeness we will briefly review the present state of design of the Mark II Multivator and point out some respects in which it differs from Mark I.

The Mark II Multivator is mechanically different but is electrically and optically the same. Seals are replaced by bellows, and explosive charges are used to supply the energy for mechanical operations. The overall size, weight and shape remain about the same. The light source system, photomultiplier detector and assay techniques remain essentially the same. Fig. 7 gives an overall diagramatic view. The Multivator cylinder is ejected from the capsule and erects itself by means of the adjustable legs. The collection system is being designed as a separate element and is not shown. Dust from this system is blown into the test chambers. The chambers and the aerosol flow path is shown in fig. 8. The firing of a squib charge seals the air passage as shown in the upper part of fig. 9. A second squib is fired resulting in a piercing of membrane containing the solvent in its chambers and the release of solvent to the assay chambers. Bottoming of the solvent chamber piston seals the fluid injection ports. This is illustrated in the lower part of fig. 9.

The reactions and measurements then proceed as in the previous model. The Multivator is attached to the capsule which contains the telemetry equipment.

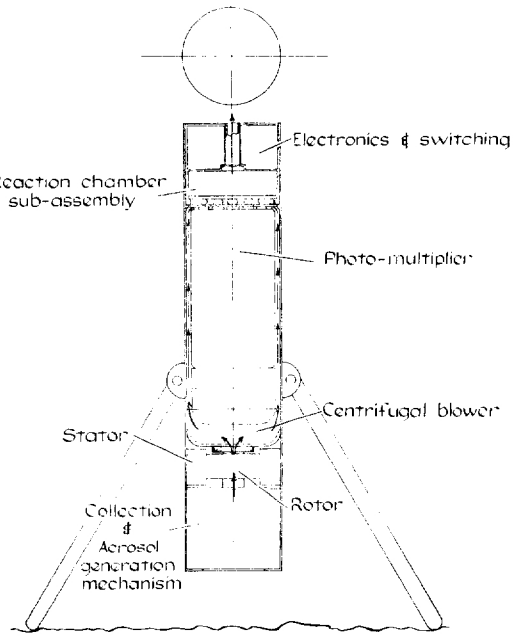


Fig. 7.

3.4. Mark III Multivator

We have set as a goal the ability to measure abundances as low as 100 to 1000 bacteria in samples of 1 to 10 mg of Martian soil. We have not yet reached this goals with the present instrument. The limitations do not seem at present to be the sensitivity of the fluorescent assay itself but rather the chemical noise introduced by the rate of hydrolysis of the substrate. We need to find methods of improving this stability or of finding substrates with similar fluorescent cross sections and improved stability. We are now working on a Multivator operating in a manner which gets around these difficulties, as well as on improved substrates. To the extent that the chemical noise is distributed throughout the volume of the sample being observed, while the fluorescent signal of biological origin is not, we can improve the sensitivity in proportion to the reduction of the observed volume. We are thus taking advantage of the fact that the great majority of soil bacteria grow in colonies which are rather firmly bound to the soil particles, giving

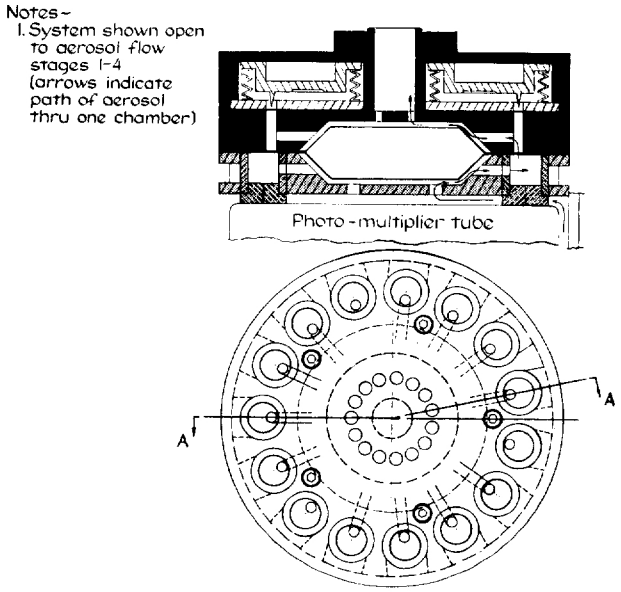
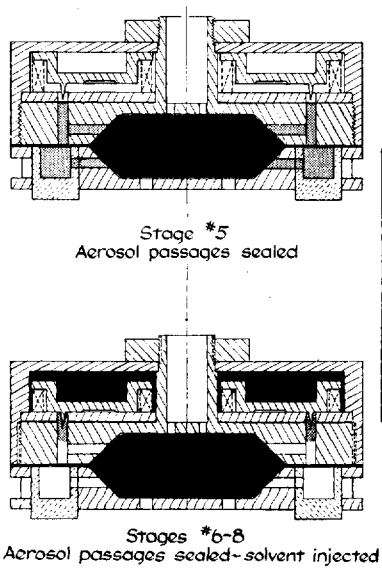


Fig. 8.



Sequence of events

Stage	Description
1	Eject from capsule
2	Erect on legs
3	Start collection pump
4	Pump air & dust thru chambers
5	Fire valve squib & seal air passages
6	Fire solvent & fill chambers
7	Incubate
8	Turn on lights sequentially & note results

Fig. 9.

much higher local metabolic activity in the vicinity of a particular soil particle than the average activity of a complete sample.

With the foregoing thought in mind, the following system is being considered. The soil particles are to be more or less evenly distributed over the surface of a substance, such as a gel, which contains the fluorescent substrate. If the diffusion rate of the product is low compared to the rate of enzymatic activity, there will then be found concentrations of fluorescent product in the vicinity of bacterial colonies. This local concentration may then be detected above the background by a suitable scanning technique. If these local concentrations occupy a volume equal to or less than the resolution of the system, the improvement of signal over background is in the ratio of total enzymatically active points to total points scanned. It should be possible to make this improvement 10^3 or better. As any device which is to be sent on a mission must of necessity be power-limited, the best possible instrumental signal-to-noise ratio for fixed power input must be determined. It can be shown that for a given light source flux, the signal-to-noise ratio becomes dependent only on the absolute noise of the detector, regardless of any other considerations. This immediately indicates that the flying spot scanning system with the low-noise photomultiplier detector is an appropriate choice. A laboratory flying spot scanning system has been built to test the proposed techniques. Preliminary experiments with the scanner have been encouraging. With this laboratory instrument, further studies of this technique will be pursued. If they are successful, design parameters of a Mark III Multivator will be determined and a model constructed. In addition we will consider what other new dimensions this adds to the experimental possibilities of Multivator.

4. Multivator assays

The assays we are investigating fall into two general categories. The first detect the presence of hydrolytic enzymes by fluorometry, testing for the catalysis of $RX + H_2O \rightarrow RH + XOH$. The bases of the test is the release of XOH which differs from XR in being highly colored, or, better, fluorescent. For example, R = phosphate; X = alpha naphthol to constitute a fluorometric assay of phosphatase. The second uses the techniques of membrane separation to measure the production of molecules with membrane transmission properties differing widely from those of the substrate.

The specific permeability of membranes could thus furnish a simple approach to biochemical analysis especially suited to the constraints of exobiological experiments. At present we have been principally concerned

with the separation of gaseous or monomeric reaction products released from larger molecules under the action of specific enzymes or of whole organisms. The identification of the end-product molecules could be dependent on the use of radioactive isotopes, for example, the detection of metabolic CO_2 from C^{14} labelled nutrient substrates. Fluorometry, however, could still be the detection mechanism if the polymer substrate were labelled with a fluorescent molecule. Our biochemical studies have been directed mainly towards evaluation of the phosphatase assay and achieving an understanding of membrane permeability which would permit exploitation of membrane operation techniques. Surprisingly little previous work has been done on the permeance of membranes on a wide enough dynamic range to be pertinent to the present applications.

Phosphatase activity has been given particular attention for the following reasons:

(a) It is widespread among terrestrial organisms.

(b) It catalyses a wide range of reactions with moderate specificity.

(c) It is involved with the unique role of phosphorus in metabolism and energy transfer, which may very well be a universal characteristic of carbon-based aqueous living systems.

(d) It is capable of being detected with relatively high sensitivity.

Because its design philosophy is not tied to a particular assay, Multivator opens the possibility that scientists in laboratories other than our own could design experiments using Multivator as an instrument, and take advantage of the simplification of the interface problem. This is important because there can never be a simple, unique, definitive detection experiment that can cover all possible manifestations of life. It becomes more important after the first sign of life has been found and a large community of scientists wish to examine a wide range of characteristics of this life. Our laboratory looks forwards to these co-operative possibilities and intends to explore them.

COSPAR BOOKS
(Committee on Space Research)

LIFE SCIENCES AND SPACE RESEARCH
VOLUME I
A Session of the Third International Space Science Symposium
Washington, D.C., 1962
Editors: R. B. LIVINGSTON, Washington
A. A. IMSHENETSKY, Moscow
G. A. DERBYSHIRE, Washington
6½ × 9½″ xvi + 184 pages 14 plates

SPACE RESEARCH I
Proceedings of the First International Space Science Symposium
Nice, 1960
Editor: H. KALLMANN-BIJL, *University of California*
6½ × 9½″ xvi + 1195 pages 62 plates

SPACE RESEARCH II
Proceedings of the Second International Space Science Symposium
Florence, 1961
Editors: H. C. VAN DE HULST, *University of Leyden*
C. DE JAGER, *University of Utrecht*
A. F. MOORE, *The Royal Society, London*
6½ × 9½″ xvi + 1242 pages 94 plates

SPACE RESEARCH III
Proceedings of the Third International Space Science Symposium
Washington, D.C., 1962
Editor: W. PRIESTER, *University of Bonn*
6½ × 9½″ xvi + 1276 pages 72 plates

SPACE RESEARCH IV
Proceedings of the Fourth International Space Science Symposium
Warsaw, 1963
Editor: P. MULLER, Observatoire de Meudon
6½ × 9½″ xvi + 998 pages 36 plates

THE USE OF ARTIFICIAL SATELLITES FOR GEODESY
Proceedings of the International Symposium on the Use of Artificial Satellites for Geodesy
Washington, D.C., 1962
Editor: G. VEIS, *Smithsonian Institution, Astrophysical Observatory, Cambridge, Mass.*
6½ × 9½″ xii + 424 pages 30 plates

NORTH-HOLLAND PUBLISHING COMPANY
AMSTERDAM P.O. BOX 103 THE NETHERLANDS

Sole distributors for U.S.A.: Interscience Publishers, a division of John Wiley & Sons, Inc. — New York

Printed in the Netherlands