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FINAL REPORT

STUDY OF THE AUTOMATED BIOLOGICAL LABORATORY PROJECT DEFINITION

VOLUME VI OF VI
TECHNICAL APPENDICES

PART ONE OF TWO
APPENDICES 1 THROUGH 6

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FOREWORD

This volume contains the results of certain detailed analyses of particular relevance to discussions in Volumes II and III of this report. Also included is certain specific reference material that was particularly relevant to some of the analyses and developments in this study.

SUMMARY TABLE OF CONTENTS

CONTENTS

VOLUME VI - TECHNICAL APPENDICES
PART ONE OF TWO
APPENDICES 1 THROUGH 6

CONTENTS (Continued)

CONTENTS (Continued)

ILLUSTRATIONS

VOLUME VI - TECHNICAL APPENDICES
PART ONE OF TWO
APPENDICES 1 THROUGH 6

ILLUSTRATIONS (Continued)

TABLES

VOLUME VI - TECHNICAL APPENDICES
PART ONE OF TWO
APPENDICES 1 THROUGH 6

TABLES (Continued)

APPENDIX 1

ANALYSIS OF INTERVIEWS

1.1 INTRODUCTION

The first step in the preliminary design study of the Automated Biological Laboratory (ABL) was the definition of the scientific objectives of ABL. As part of the defining of scientific objectives, a survey of the scientific community was carried out to obtain opinions and suggestions relative to the desirability and objectives of the ABL mission. In this section the goals and techniques of the interviews are described; and the results are tabulated, discussed, and analyzed to show how the scientific objectives selected for ABL seem to be a valid reflection of the views of the scientific community.

1.2 SOURCES OF DATA

While the actual direct considered responses of scientists to interview questions (either oral or written) constitute the most tangible part of the effort to define the scientific objectives of ABL, they by no means represent the total raw material from which the final recommendations were synthesized. Attendance by Philco scientists at meetings (e.g., NAS Exobiology Summer Meeting at Stanford), participation in and observation of informal conversations with scientists, articles and letters published in the current literature and frequent discussions among the Philco scientists engaged in ABL project all contributed indirectly to the definition of the scientific objectives. In this section, however, the material analyzed was taken from the direct contributions by the scientific community. Interviews were conducted by senior members of Aeronutronic's Bioscience staff and included persons whose professional cognizance includes zoology, botany, biochemistry, organic chemistry, physical chemistry, and physiology.

1.3 THE SAMPLE

The initial effort to contact the scientific community consisted of the preparation of a letter which asked for an oral interview at the scientist's convenience and explained briefly the goals of the interview. The letter said, in part:

> "In order to accomplish this objective (definition of biological experiments and associated support equipment that could comprise an integrated experimental package known as the Automated Biological Laboratory (ABL) we invite your opinions concerning definitive experiments or general properties, including environmental parameters, which you consider to be useful or essential for describing life. Of particular interest would be experiments which you feel could be performed on Martian life in order to obtain information useful in current scientific inquiry and as an extension of your own research interests. However, you will make a substantial contribution to this program by simply discussing with us the feasibility of the ABL concept and its objectives in regard to your own scientific specialty."

A return postcard was included on which the scientist could indicate his willingness to contribute by granting an interview at his convenience or by making a written contribution. In addition, he could indicate that he was not interested in participating.

This letter was sent to the approximately 350 prominent scientists in various disciplines throughout the country. (The distribution list for these letters is included at the end of this Appendix.) When favorable replies were received, the respondents were contacted and interviews arranged. A number of scientists contacted in this way arranged for informal group discussions with other interested scientists in their institution or area. A follow-on letter explaining the project in more detail was sent to a number of scientists who wished additional information prior to the interview.

In addition to contacts with the scientific community through the formal letters, interviews were also arranged in other ways. In many cases, scientists interviewed were able to suggest others who were actively working in some areas related to exobiology or particularly interested in or qualified to give opinions on some aspect of ABL. Interviews were subsequently scheduled in a less formal manner.

In all, 144 scientists contributed to the survey, 3 by correspondence only, and 141 through oral interviews. Four of the latter also expressed some of their views by letter either before or after the oral interview. In almost all cases, the scientists were interviewed at their home institutions. The list of scientists interviewed is contained in Paragraph 1.7 of this Appendix. In Table I the responses to the original letter requesting interviews are analyzed.

TABLE I

RESPONSES TO ORIGINAL REQUEST FOR CONTRIBUTIONS

Letters Sent	325[1]		
Responses to Letters	125 (38.5%)		
Declined to Participate	80[2]		
Subsequently Interviewed		3	
Subsequently Wrote		3	
Agreed to Interview	42[3]		
Subsequently Interviewed		33	
Interviewed and Wrote	4		
Agreed to Write	3		
Subsequently Contributed		0	
Did not Respond to Letter but were Subsequently Interviewed		18	
TOTAL CONTRIBUTIONS IN RESPONSE TO LETTERS		57[4]	
Percent of Total Contacted who Contributed			17.5%
OTHER CONTRIBUTIONS (Not Contacted by Letter)		87	
TOTAL CONTRIBUTIONS		144	

(1) At least 2 deaths in this group.

(2) Some reasons for declining were given: "Too busy" or "am not able" (10); "Not in my field" or "nothing to contribute" (13)" declined for policy reasons (2); declined (not qualified) but suggested other contacts (5); "Not at present" (4); "Later if I have any ideas" (1).

(3) Two required consultation fees.

(4) Some of these individuals arranged group meetings or other interviews.

The interviews with individuals or groups were handled as free informal discussions, not as answers to specific questions on a checklist. However, within the context of the free discussion, interviewers attempted to guide the interview as much as possible along the following lines:

(1) Explanation of what Aeronutronic is trying to do.

(2) Outline of current NASA life detection techniques - statement that this is a fundamental biology program.

(3) Outline of what is known about Mars from terrestrial observations.

(4) Questions and statements designed to stimulate thinking and to obtain information.

(a) What are the aims of the biological exploration of Mars?

(1) To detect life.

(2) To determine if such life has a common origin with life on Earth.

(3) To establish the evolutionary pathway of Martian life.

(4) If life is not found, to discover the factors which prevented development.

(5) If only fossil forms of life are uncovered, to determine the factors associated with the extinction of life.

(b) Should a life-detection technique be based on an analysis for specific substances such as DNA, chlorophyll, phosphatases, steroids, or metabolic pathways (which in essence detect specific enzyme systems) or upon the detection of less specific factors such as energy conversion, the presence of molecular aggregates and growth?

(c) Would the occurrence of a right-handed screw sense in Martian life indicate a separate origin? If not, what factors would?

(d) The occurrence of lipoprotein membranes in living systems appears to be a universal requirement. How can such systems be detected in Martian life and characterized to an extent which will allow us to say they are similar to or different from those present on Earth?

To a large extent, the subject matter discussed was centered around the particular interests of the scientist interviewed; and so the material covered does not necessarily correlate from interview to interview.

A major difficulty in this type of interview program was guiding the interviews in such a manner that the broad view of the problem (the real purpose of the interview), namely, what should the specific aims of the biological exploration of Mars be and how should these aims be implemented, was kept in mind. It is obvious that a number of scientists interviewed tended to think of the problem of exobiology in terms of their own specialized areas of research. In some cases it proved virtually impossible to prevent the interview from becoming an expostulation on the part of the scientist being interviewed of the merits, demerits, etc., of a system or piece of apparatus in which he was vitally interested. In retrospect, it seems clear that the selection of the type of scientist to be interviewed contributed to this difficulty. Naturally, a large percent of the scientists who came to our attention and were contacted were eminent in one field of science related to exobiology. The eminence most frequently derived, as would be expected in the present state of development of the sciences, from specialization in one fairly narrow field of endeavor, or from the development of a highly useful technique.

In view of the seemingly vociferous expression of concern on the part of some of the scientific community about the proposed extra-terrestrial life program of NASA (letters in Science, etc.) and critical commentaries on proposed life-detection systems, it is of interest to note the percentage of scientists contacted for interviews who refused to grant them (Table I).

Table II shows the breakdown of the interviews by the size of the group interviewed. Some characteristics of the sample of scientists interviewed are given in Table III, including the scientific disciplines represented by the scientists interviewed, their affiliation (academic institutions, industry, or government), and whether they were actively involved in work directly related to exobiology. Most of the scientists (110) were biologists; those giving their field as biochemistry were the largest single group (40). In addition, there were 17 chemists, 8 physicists, 9 engineers, and 1 geographer. The latter was an expert on interpretation of aerial photographs.

Three-quarters of the scientists interviewed had academic connections. The same proportion had no direct connection with work in exobiology, while most of those interviewed who were professionally concerned with exobiology were in industry. However, the coincidence of a quarter of the total sample so concerned with the quarter of the sample employed in industry is not complete.

TABLE II

TYPES OF INTERVIEWS CONDUCTED

Number of Scientists Interviewed Individually	54	
Number of Scientists Interviewed in Small Groups (2-3)	15	
Number of Small Group Interviews		6
Number of Scientists Interviewed in Large Groups (6-22)	72	
Number of Large Group Meetings		6*
Scientists Interviewed	141	
Scientists Sending Written Contributions	3	
TOTAL CONTRIBUTIONS	144	

*These were as follows: University of Oklahoma-Civil Aeromedical Research Institute (22); Rice University (20); Jet Propulsion Laboratory, Cal Tech (10); Marine Biological Laboratory, Woods Hole, Massachusetts (8); Abbott Laboratories (6); Research Institute for Advanced Studies, Martin-Marietta Corp., Baltimore (6).

TABLE III

COMPOSITION OF SAMPLE OF SCIENTISTS INTERVIEWED

I. Discipline

Biochemistry	40
Biophysics	4
Biochemistry-Biophysics	2
Physical Biochemistry	1
Microbiology (unspecified)	10
Bacteriology	6
Mycology	3
Protozoology	1
Virology	1
Zoology-Medical (unspecified)	5
Physiology	8
Embryology	5
Parasitology	4
Genetics	3
Ecology	3
Cytology	2
Entomology	1
Oncology	1
Botany (unspecified)	3
Plant Physiology	6
Agronomy	1
Chemistry (unspecified)	9
Physical Chemistry	2
Geochemistry	2
Organic Chemistry	1
Physical Organic	1
Polymer Science	1
Nuclear Chemistry	1
Physics (unspecified)	4
Oceanography	1
Metallurgy	1
Radiation Physics	1
Engineering	9
Geography	1
TOTAL	144

II. Institutional Affiliation

Academic	107
Industrial	34
Government	3
TOTAL	144

III. Professional Activities Directly Related to Exobiology

No	108
Yes, Research and/or Development	31
Yes, Writing or Official	5
TOTAL	144

1.4 SUMMARY OF FINDINGS

Because of the informal and minimally directed nature of the interviews, which were designed in this way specifically to increase their creative value, the responses are difficult to summarize and tabulate in a satisfactory manner. As discussed in the previous section, the responses listed in the tables were not, as a rule, prompted by questions but represent voluntary contributions on the part of the scientist being interviewed. A more complete sampling of the scientific community on these specific points could obviously have been obtained had the interviews been conducted on a set question-answer basis. We feel the informal, relatively unprompted interview gave a truer reflection of the real feeling of the scientific community and was more productive of creative suggestions and ideas. In the final analysis, some scientists interviewed had contributed relatively little, others a great deal. Figures in the tables, although frequently representing only a small percentage of the total sample, probably represent in most cases a fair representation of the view of the scientific community. Certainly, of the scientists contacted, those who had any definite original ideas or opinions or comments on published ideas of others in the area of exobiology were given ample opportunity to express them.

It was considered desirable to list responses elicited from scientists interviewed individually separately from those interviewed in a group. In the tables an entire group is included in a figure if one individual in the group expressed the opinion in question. The rationale for this is, again, that possible dissenters within a group were given ample opportunity to express their dissent to a statement made by any member of the group. Lack of such expression was assumed to connote at least a reasonable degree of accord with the statement. Actually, on no occasion during group discussions did an important disagreement arise. However, since it is evident that various restraints and inhibitions operating in group discussions are absent or attenuated in individual interviews, it is not felt that the figures for group responses should be weighed as heavily as those obtained in individual interviews.

1.4.1 GENERAL CONSIDERATIONS ON THE BIOLOGICAL EXPLORATION OF MARS

a. The Expressed Desirability of the Biological Exploration of Space. The majority of scientists interviewed expressed enthusiasm for a program directed toward the search for extraterrestrial life. There were a few dissenters who were completely opposed to the program and a larger number who, although favorable to the idea of extraterrestrial life search, had certain reservations or felt that the political advantages of the scientific and technological fallout would outweigh any intrinsic scientific merits of the program. The opinions of the scientists interviewed are summarized in Table IV.

TABLE IV

SUMMARY OF OPINIONS OF SCIENTISTS ON THE BIOLOGICAL EXPLORATION OF MARS

	Should Extraterrestrial Life be Sought?									Type of System Preferred		
		Yes			Neutral	No						
		For Intrinsic Reasons	For Political Reasons	For Fallout			Except For Political Reasons	Fallout Nil	Little Chance of Success	ABL	Multiple Probes	Single Experiment
Individual	21	2	4	2	2	2	1	2	1	19	3[1]	4[2]
Group	47										9	
TOTAL	68				2	2				19	11	4

(1) One of these prefers ABL if it has roving capability; another favors either ABL or multiple probes with a slight bias toward the latter.

(2) One favors a single experiment first, ABL later.

b. Type of Life-Detection System. The scientists interviewed were in favor of one or another of the following systems for the biological exploration of Mars: (a) a single, integrated automated biological laboratory (ABL); (b) a series of multiple, sequentially integrated probes; (c) a single-package, small payload relying on a single life-detection technique or instrument (Table IV).

Most respondents were in favor of either the ABL concept or multiple probes; and there was little enthusiasm for a single-package life-detection experiment or for sole reliance on methods depending on the culture of Martian organisms on artificial media. Many who favored multiple probes over ABL implicitly or explicitly based their decision on three arguments: (a) some preliminary exploratory experiments will have to be performed and the data analyzed before more definitive experiments can be designed; (b) considering the difficulty and expense involved in an ABL, the chances of selecting the wrong landing site for the probe (i.e., an abiotic region); or (c) failure of the system to operate properly mitigates against the ABL concept. Some who favor the ABL concept suggest overcoming the first objection by selecting the experiments and designing the system so that with command control from Earth, exploratory experiments can be performed, the data analyzed, and appropriate additional experiments conducted. The second objection, failure to select the proper landing site, could be met by designing the ABL with orbiting capability prior to landing. With surveillance facilities and command control, the probability of selecting a profitable landing site will be greatly increased. A number of those interviewed also stressed the point that ABL should have a roving capability. In reference to the third objection, several scientists emphasized the necessity of selecting equipment and experiments in such a way that complexity is minimized and instrument reliability maximized. According to several respondents, the latter should be stressed even at the expense of sacrificing a good deal of sensitivity and selectivity in the instruments chosen.

It was apparent that many of the respondents favoring the idea of multiple probes had not taken cognizance of one of the main scientific objections to this approach. Namely, the possibility of contamination of the planet with terrestrial life forms with the first probe perhaps making forever unanswerable the question of whether life existed on Mars prior to the first landing. Many who favored ABL based their decision on this argument. (See also Paragraph 1.4.4.)

c. Assumptions Concerning Martian Biology. Most of the scientists interviewed made certain operating assumptions or guesses, which seemed reasonable to them, concerning the general properties to be expected of Martian organisms, if life exists on Mars. These assumptions covered such things as the density and species-abundance of Martian organisms, the degree of similarity to terrestrial organisms, and the general types of

organisms which might be expected. Most of the assumptions were implicit and impossible to isolate from the context of the interview. However, a few explicit statements which were made are summarized in Table V. Obviously, from the general discussions, many more scientists than shown in the table assumed that Martian life would be carbon-based; and many believed it reasonable or necessary to search for specific compounds (DNA, chlorophyll, adenosine triphosphate, etc.) associated with life on Earth (see Paragraph 1.4.5 and Table IX). Very few scientists interviewed (half a dozen or so) cautioned specifically against reaching foregone conclusions about what to expect of Martian life on the basis of what we know of terrestrial life, or suggested that Martian life might be exotically different from life on Earth. Many apparently felt that Martian organisms would be quite similar to terrestrial organisms, whether or not life on the two planets had a common origin.

TABLE V

SPECULATIONS ABOUT MARTIAN LIFE

	Probably	Possibly	Possibly Not	No
Similar to Life on Earth	2			
Carbon-based	4		1(1)	
Phosphorus-based(2)	1			
Water-based			2	
Protein-based	1			
Enzymes Similar to Those of Terrestrial Organisms	1			
Growth Factors Same as Those of Terrestrial Organisms	1			
Evolutionary State Primitive	1			
Life "Marginal"	1		1	
Intelligent Life		1		
Type of Organism Likely:				
Bacteria	1			
Viruses	1			
Lichens	1			1
Multicellular "Vegetation"(3)	2 2			

(1) Unless common origin.

(2) i.e., phosphorus transformations are important in energy storage and utilization.

(3) i.e., photosynthesizing organisms, probably containing chlorophyll.

1.4.2 GENERAL SCIENTIFIC OBJECTIVES FOR THE BIOLOGICAL EXPLORATION OF MARS

The scientific objectives for the biological exploration of Mars which emerged from the interviews can be grouped under the following headings:

(1) Detect life.

(2) Chemically and physiologically characterize the life forms detected.

(3) Determine if such life has a common origin with life on Earth. (This includes both the inter-transfer of life between Earth and Mars and a common chemical evolution.)

(4) Establish the evolutionary pathway of Martian life.

(5) Determine the interaction of life forms with the environment.

(6) Look for fossil life and if only fossils are found, determine the factors associated with the extinction of life.

(7) If life is not found, discover the factors which prevented its development.

These represent overall goals of the program; and it is not contemplated, of course, that they can all be achieved with one, or probably even many landings. The responses are summarized in Table VI. As with some of the other data reported, the tabulated values are not entirely objective. This is due, as discussed previously, to the nature of the interviews. Thus, obviously, the responses tabulated in Table VI were not always explicitly stated in the form given. Many statements given in the interviews were so general that they might be interpreted to indicate a desire to explore any facet of biology which could be listed. However, an attempt has been made to interpret these statements in terms of the goals which seem to be implied. These seven categories seem to cover essentially all the goals brought up in the course of the interviews. The aim, in short, has been to give an analysis which appears to represent the thinking of the scientists interviewed. It is evident that items (1) and (2) (detection and characterization of life) are, in a real sense, inseparable considerations from an operational point of view; and they have not been separated in Table VI. Not all of those interviewed are listed as favoring the detection and characterization of life, even though they may have expressed agreement to the idea of an extra-terrestrial life search. In these cases, when more specific points about the probe were discussed, it was evident that the respondent was more

TABLE VI

SUMMARY OF OPINIONS ON THE SCIENTIFIC OBJECTIVES FOR THE BIOLOGICAL EXPLORATION OF MARS

		Detect and Characterize Life[1]	Investigate Common Origin	Investigate Evolutionary Pathway[4]	Investigate Ecology	Search for Fossil Life	If Life Absent, Investigate Reason
Yes	Individual	56	9	9	4	2	2
	Group	64		23	20		
TOTAL FAVORABLE		120	9	31	24	2	2
No	Individual	1[2]	2[3]				

[1] Those opposed to the idea of searching for extraterrestrial life (Table IV) are not represented here. A few of those interviewed who never specifically mentioned life detection or seemed to ignore the biological purpose of the proposed ABL are not included in the total.

[2] This individual thought life detection was too limited a goal and that it should be incidental to a chemical and physical investigation of Mars.

[3] These scientists felt that the question would probably be impossible to answer and, while it might be investigated at a later time, it should not be considered for the first ABL.

[4] Investigation of genetics, study of information transfer, etc., are included in this category.

interested in geochemical or meteorological than of biological exploration of the planet. Investigation of the evolutionary pathways of Martian life has been considered to include suggestions to investigate genetics, information transfer, etc., as well as statements (always vague) about "investigation of evolution," etc. The suggestions to investigate the ecological relationships of Martian organisms included consideration of both the effect of physical and biological environment on organisms and (in two interviews) the exploitation of possible effects of organisms on the environment as an indicator of life.

1.4.3 THE PRIME OBJECTIVE OF THE FIRST PROBE

In stating their views on the proposed ABL and its relation to the probable total extraterrestrial-life exploration effort, various scientists stressed different goals for the initial Mars probe (Table VII). These can be categorized fairly comprehensively as follows: (1) adequate background data on the environmental parameters which might be expected to determine the existence and type of life on the planet is not available; therefore, the primary (or sole) objective of the first probe should be to gather such data (e.g., accurate temperature ranges and diurnal and seasonal fluctuations, atmospheric composition, atmospheric and surface radiation level data, water availability, soil chemistry, etc.) so that more reliable and successful life-detection experiments can be planned for later probes; (2) the obligatory goal of the first probe must be to detect life on Mars or demonstrate its absence conclusively; and, if life exists, to characterize it as far as possible; (3) the first probe should stress, or be limited to, visual examination of the planet (both long-range and close-range and/or microscopic). These goals represent to some extent arbitrary categories in a spectrum of responses. Several scientists mentioned two or even all three of the goals listed as obligatory. Some scientists specifically recommended not attempting to detect life (except incidentally) with the first probe, but concentrating all efforts on goal (1). Others specifically suggested not making any physical or chemical measurements which were not essential to the objective of getting an immediate answer to the question of whether or not life exists on Mars. Goal (2) was urged by many scientists on the grounds that the certainty of avoiding contamination of Mars with terrestrial organisms on the first probe could not be assured with present techniques or techniques likely to be developed in the near future. A few scientists felt, largely on the basis of the known environmental differences between Earth and Mars, that the chances of significant replication of foreign life forms on Mars is very slight. The proponents of goal (1) generally argued the impossibility of devising crucial life-detection experiments without background information on the physical and chemical environment of Mars. Those favoring goal (3) felt that visual evidence (either seeing living organisms or large-scale visual evidence of life) is prerequisite to planning meaningful experiments and selecting a favorable landing site, or at least essential corroborative evidence in conjunction with physical and chemical experiments.

TABLE VII

PRIMARY OBJECTIVE OF FIRST PROBE

	Favors Emphasizing			Exclusively		Scientists Favoring
	Individual	Group	TOTAL	Individual	Type of Study	Individual
Emphasis on Study of Martian Environment and Physical and Chemical Characterization of Planet	14	37	51	3	Atmospheric Radiation Levels	1
					Surface Radiation	2
					Seismographic	3
					Geomagnetic	2
					Petrographic	1
					Electric and Dielectric Properties of Surface	2
					Complete Elemental Analysis of Soil and Rocks	10
					Organic Chemistry	3
					"Soil" or "Surface Chemistry"	3
Emphasis on Detection of Life	24	41	65	4	(See Table IX)	
Emphasis on Visual Exploration	15	9	26		(See Table IX)	

Division of opinion into these categories was not sharp. Several scientists mentioned two or even all three of these goals. The majority of opinion was clearly that the first probe should attempt to establish the presence or absence of life on Mars.

1.4.4 ABL GENERAL CONSIDERATIONS

a. General Features Which Should be Included in ABL. Specific mention was made of some general features, facilities, and capabilities which should be incorporated into ABL. These are listed, with the number of scientists referring to each item, in Table VIII.

TABLE VIII

GENERAL FEATURES WHICH SHOULD BE INCLUDED IN THE ABL

	Yes			No
	Individual	Group	Total	Individual
Orbiting Capability:				
Prelanding	2		2	1
Separate Orbiter	1		1	
Extensive Visual Scanning and Transmitting Facilities	3		3	
Roving Capability	4(1)	20	24	
Command Control	2	22	24	
Long Life (2 years)	2		2	
Means of Collecting and Preserving Sterile Soil Samples	3		3	
Light Power Supply; Low Power Requirements	1		1	
Complete Quantitative Chemical Data on Vehicle	1		1	

(1) One scientist specified a several-mile roving capability; another specified both horizontal and vertical roving capability.

b. Sterilization of ABL; the Problem of Contamination of Mars. Relatively few (9) scientists interviewed explicitly considered the question of sterilization of the vehicle and the possibility of contaminating Mars with terrestrial organisms. Several felt that the problem would not be serious, implying that terrestrial organisms should not survive and grow under the rigorous Martian environmental conditions.

Dr. D. G. Rea (Space Sciences Department, University of California, Berkeley) expressed a general criticism of the multiple probe concept as opposed to the ABL concept implicit in responses from many others: "An enduring contamination by one of a series of probes would greatly reduce the value of following probes." Mr. M. G. Koesterer (Wilmot Castle Research Laboratory) feels that the probability of contamination is a direct function of the mass of terrestrial material placed on Mars and that, comparing one large ABL and several smaller vehicles whose combined mass was equal to that of the ABL, the chances of contamination would probably be the same. He thinks that sterilization procedures for present space probes are not adequate for the Martian lander and estimates the probability of contamination with present techniques is 1/100 and that these could be improved to reduce the risk to less than 1/10,000.

1.4.5 SPECIFIC TECHNIQUES AND EQUIPMENT WHICH SHOULD BE INCLUDED IN ABL

a. Life Detection and Characterization Techniques. The opinions of the scientists interviewed varied as to the characteristics of life upon which life-detection should be based. Some suggested methods for examination of these characteristics are given in Table IX.

b. Techniques for Pursuing Other Scientific Objectives in the Biological Exploration of Mars. Experiments designed to investigate a common origin of Martian and terrestrial life (except for the study of optical rotation of molecules on Mars) or to study evolutionary pathways, ecology, or the fossil history of the planet were not specified in detail by any of the scientists interviewed. Reference to these objectives was general, which probably indicates a feeling that life detection and chemical characterization should be the prime objective of the first Martian probe with the other objectives being incidental or more suitable for later investigation.

c. Specific Instrumentation Which Should be Included in ABL. In connection with life detection and characterization, as well as for physical and chemical measurements of the Martian environment, many suggestions were made for specific instruments or systems which should be included in the ABL. These instruments are listed with the number of scientists mentioning them in Table X.

TABLE IX

CHARACTERISTICS OF LIFE WHICH SHOULD BE SOUGHT WITH THE ABL

Type of Evidence	Compound, Class Activity, etc.	Yes			No	Methods; Remarks
		Individual	Group	Total	Individual	
Chemical[1]	Characteristic compounds (unspecified)	1	22	23		
	Proteins	6		6		UV absorption; ninhydrin reaction
	Enzymes (unspecified)	4	8	12	1*	*Low reliability
	Alkaline phosphatase	1		1		
	Urease	1		1		Cited as virtually ubiquitous in humus-like soil; stable
	Nucleic acids	7[2]		7		UV absorption; specific dyes

(1) Two respondents were opposed to emphasizing chemical detection methods as an indication of life, one feeling that even molecules as complex as proteins and nucleic acids would not be conclusive evidence of life. Another was opposed to reliance on detection of any compounds except enzymes.

(2) A molecule with the double helical structure (like DNA) was cited by several as ideally suited to serve an information storage and transfer function since the structure allows the optimum degree of reactivity and plasticity together with a high degree of stability.

TABLE IX (Continued)

Type of Evidence	Compound, Class Activity, etc.	Yes			No	Methods; Remarks
		Individual	Group	Total	Individual	
Chemical (Continued)	Nucleotides (e.g., ATP)	1		1		On the assumption that "phosphorus transformations" are necessary
	ATP (cited as essential to life)	2		2		Luciferase Assay
	Lipids	4		4		
	Steroids	1		1		
	Complex phosphatides, sterols, terpenoids	1		1		
	Polysaccharides	3		3		
	Amino acids	3		3		
	Monosaccharides	1		1		
	Hemes		3	3		
	Chlorophyll	1	3	4		
	Pristane[3]	1		1		Gas chromatography
	Macromolecules	4		4	1	Dye assays (e.g., J-band)
	Molecular aggregates	2		2		Viscosity measurements

[3]Pristane is a degradation product of phytol. It is easily detected and identified; and it is suggested that its origin, other than from living organisms, would be difficult or impossible to explain.

TABLE IX (Continued)

Type of Evidence	Compound, Class Activity, etc.	Yes			No	Methods; Remarks
		Individual	Group	Total	Individual	
Chemical (Continued)	Informational macromolecules	9(4)	2	11		
	"Life molecules"(5)	1		1		Fluorescence spectrum should be characteristic
	Electron-transport molecules	2		2		
	"Energy sources"	1		1		
	"Humic acids"(6)	1		1		
	Optical activity	10		10		
	Fluorescence	2		2	1*	*Lacks specificity; high background
Visual	Long range	2		2		High resolution facsimile
	Close range	2		2		High resolution facsimile
	Microscopic	1	9	10	2	

(4) One respondent suggested preliminary experimental work to determine what kinds of molecules, other than DNA, might be suited to information storage and transfer functions; several stressed the importance of the double helical structure (see also Footnote 1).

(5) This respondent suggested that characteristic conjugated, heat-labile molecules specialized electronically to perform life functions (like ATP, flavines, etc.) are essential to life.

(6) Compounds characteristically found in humus.

TABLE IX (Continued)

Type of Evidence	Function	Yes			No		
		Individual	Group	Total	Individual	Group	Total
Functional	METABOLISM (measured by:)	20		20			
	Photosynthesis	2		2			
	Heat production	1		1	1		
	Synthesis from labeled substrates	4	4	8	2		2
	"Energy conversion and transfer"	1		1			
	Gas exchange	5	24	29			
	Isotope enrichment	1		1			
	Entropy decrease	1		1			
	REPRODUCTION AND GROWTH (culture on:)	17	14	31			
	Synthetic medium	5	5	10	3	22	25
	Soil (Martian)	2	22	24			
	Bacteria (for phage)	1		1			
	(measure as:)						
	Increase in mass	1	25	26			

TABLE IX (Continued)

Type of Evidence	Function	Yes			No		
		Individual	Group	Total	Individual	Group	Total
Functional (Continued)	REPRODUCTION AND GROWTH (Cont.)						
	Increase in cells (turbidimetry)	2		2			
	Increase in DNA	2		2			
	Increase in protein	1		1			
	Increase in some material	3		3			
	IRRITABILITY	1	9	10			
	TYPE OF RESPIRATION	1		1			

TABLE X

MAJOR PIECES OF EQUIPMENT OR SYSTEMS WHICH SHOULD BE INCLUDED IN THE ABL

	Yes			No	Comments
	Individual	Group	Total	Individual	
Visible-range Spectrophotometer	1*				*Favored by implication by a majority
Gas Chromatograph	12*	2	14		*Two specify combination with mass spectrograph
Mass Spectrograph	10	2	12	1*	*For organic compounds identification is very difficult
Ultraviolet Spectrophotometer	6		6		
Infrared Spectrophotometer	5		5	1*	*Almost useless except for purified material
Microcalorimeter	3	2	5	1	
High Resolution Facsimile Equipment (macro and micro)	2	24	26		
Polarimeter or Optical Rotary Dispersion	3		3		
Fluorescence Spectrophotometer	2		2	1*	*Lacks specificity; high background reading
Thin Layer Chromatography	2		2		
Seismograph	2		2		
α-scatter Spectrograph	2		2		

TABLE X (Continued)

	Yes			No	Comment
	Individual	Group	Total	Individual	
Radio-carbon Dating Equipment	1	6	7		
Microscope-Spectrophotometer	1		1		
Microwave Rotational Spectroscope	1		1		
Paper Chromatography Equipment	1		1		
Electron Probe	1		1		
NIL-Baroza Carbon Skeleton Determinator	1		1		
pH Meter	1		1		
Acoustical Device	1		1		
Solid Scintillation Counter	1		1		
Light-scattering Instrumentation	1		1		
Multivator	1		1	1	
Gulliver		2	2	2*	*One objected to the requirement for specialized substrates
Wolf Trap	1		1		
Microscope, Optical	1		1		
Inelastic Neutron Scatter Instrumentation	1		1		

TABLE X (Continued)

	Yes			No	
	Individual	Group	Total	Individual	Comments
Raman-Spectroscope				1*	*Lacks specificity; high background
Electron Microscope				3*	*All cited the weight and complexity as objections; one cited the difficulty of knowing what you are looking at even under optimum conditions

1.4.6 **PREPARATORY EXPERIMENTS ON EARTH**

Several scientists interviewed were of the opinion that preliminary experiments on Earth using a simulated Martian environment might be of value in planning experiments for inclusion in the ABL. Three suggested studying the growth of terrestrial microorganisms of various kinds under conditions of extreme temperature fluctuation, low moisture and other conditions which are likely to exist on Mars. The object of these studies would be twofold: (1) to determine if survival and growth of unadapted terrestrial organisms can take place under these conditions; (2) to find if adaptation of terrestrial organisms to an environment of this type is possible. Such studies might be valuable both from the standpoint of designing fruitful experiments for the ABL and for assessing the probability of reproduction and growth of terrestrial microorganisms inadvertently introduced into the Martian environment with the probe.

One scientist felt that a broad program of study of desert ecology, desert soil composition, etc., would be rewarding. Another respondent suggested that the study of terrestrial enzyme systems under simulated Martian conditions would aid in designing more efficient biochemical experiments for inclusion in the ABL.

1.5 OTHER COMMENTS BY SCIENTISTS

A number of observations were made concerning extraterrestrial life search which do not lend themselves well to summarization and tabulation or which constitute amplification of some of the material summarized in the last section.

1.5.1 BIOLOGICAL EXPLORATION OF MARS: GENERAL CONSIDERATIONS

Several scientists felt that the probability of life on Mars was low enough to render the value of a biological exploration of the planet questionable. Dr. Barry Commoner (Washington University, St. Louis) was representative of the extreme view. He thought that from what we already know of Mars the conditions are such that the probability of life is slight and does not justify a probe designed specifically to detect life. He pointed out that life was so intimately dependent on the hydrogen bonding influence of water that in an environment with as little water as Mars, life could not exist. He considered that NASA was remiss in its duty to the public in not sponsoring a full public discussion on the problems of seeking life on Mars prior to carrying out engineering studies.

Some of the scientists interviewed pointed out the desirability of including in the ABL some experiments which will virtually guarantee some positive and meaningful results (e.g., concerning the geology or the meteorology of Mars) simply to ensure continued public support for the space program in case the biological portion of the probe is not successful.

A number of scientists conveyed, through various comments, the general impression that they felt the entire area of exobiology was being controlled too extensively along disciplinary lines. Much of the general feeling seems to be summarized in the atatement of Dr. T. D. Brock, Indiana University, that molecular biologists are dominating the planning of the exobiology program and that ecologists should have a stronger hand in the planning of ABL. Others thought that the concepts and techniques of classical bacteriology were being relied on to the exclusion of those of other potentially profitable areas.

Dr. K. Jarmalow and his group (Research Institute for Advanced Studies, Martin-Marietta Corp.) expressed a somewhat unusual philosophy for the biological exploration of Mars. Their idea was that the main purpose of the Martian life-detection mission should be to collect and preserve soil samples under sterile conditions for later removal and study on Earth. The remainder of the effort should be devoted to establishing Martian environmental conditions and an attempt to obtain a crude (i.e., approximate) answer as to whether any form of life exists on the planet.

1.5.2 ABL: GENERAL CONSIDERATIONS

William Wilhite (Jet Propulsion Laboratory, California Institute of Technology) suggested that ABL should include experiments which can be completed and communicated during descent so that even if the ABL is destroyed on impact some information can be gained. Dr. W. O. Fenn (University of Rochester Medical School) was concerned with the possibility of false positive information and suggested that this might be controlled in part by testing the systems first on the moon, which is presumably abiotic.

Dr. Fenn also urged, as did others, that the more generalized or nonspecific tests should be accented in planning the ABL. Dr. S. Scher (Department of Space Sciences, University of California, Berkeley) thought a complete quantitative inventory should be kept on the chemical composition of any lander so that, in case of disintegration, allowances could be made in subsequent analyses for its contribution in trace elements and compounds. Dr. D. G. Rea (Space Sciences Department, University of California, Berkeley) suggested that an orbiter (1969) might provide valuable information about the Martian surface for aid in designing ABL experiments. He suggested that impacting an object from the orbiter on the surface could provide useful information, since the impact could be monitored from the orbiter and this would give some data on the nature of the surface.

1.5.3 SPECIFIC TECHNIQUES

a. Life Detection and Characterization Techniques. Dr. Myles Maxfield (Hancock Foundation, University of Southern California) suggested the possibility of including a germ-free animal as a culture medium for infectious agents which might occur on Mars. Dr. W. W. Umbreit (Rutgers University) suggested culturing organisms from Martian soil on a complex medium based perhaps on ground algae or algal extracts supplemented with extracts of various animal and plant tissues and fortified with a number of substrates. Attempts would then be made to detect metabolism by measuring gas uptake or production, formation of products from labeled substrates, etc. Since soil may be physically necessary, the culture medium should be injected into Martian soil *in situ* beneath a hood-like device which would contain the measuring instruments. He specifically cited microcalorimetry to be an unfeasible tool since heat production is very difficult to detect and measure even under optimum conditions in the laboratory.

Dr. J. Oro (University of Houston) cautioned against measurement of contaminants introduced as part of the probe. He has studied organic compounds reported to be present in meteorites and shown that these were probably contaminants introduced onto the surface of the meteorites by handling prior to analysis. Fingerprints contain certain amino acids and other compounds in quantities sufficient for detection by gas chromatography. This finding emphasizes the necessity of ensuring not only that the Martian lander is sterile in the bacteriological sense, but also free of organic contaminants which might be detected by the ABL and interpreted as evidence of Martian life.

b. Common Origin of Life on Earth and Mars. As indicated in the previous section, at least two scientists interviewed felt that the question of a common origin of life on different planets would probably never be answered unequivocally. Dr. W. W. Umbreit suggested that even if virtually identical organisms were found on Earth and Mars, this would not necessarily be an indication of common origin but might be explained as the result of a common evolutionary pattern acting on organisms which originated separately.

c. Specific Instrumentation. Dr. Myles Maxfield (Hancock Foundation, University of Southern California) discussed the possible use of Technicon automatic analysis equipment in the ABL. He has used this equipment extensively and has encountered some difficulties. He doesn't feel that these are insurmountable. The major problems concern the tubing which readily becomes dirty. This leads to uneven bubble formation and clogging. The tubing is also attacked by many reagents and incubation times, using present equipment, are limited.

Dr. W. W. Umbreit (Rutgers University) discussed a system currently under development in his laboratory for performing colorimetric analyses on filter paper tape. It can be very simply and reliably automated and has been found to be more sensitive than test tube procedures for a number of standard colorimetric analyses.

1.6 SCIENTISTS CONTACTED BY LETTER

Name	Institution	Field
Abelson, Philip H.	Geophysical Laboratory Carnegie Institute of Washington	Geology
Adey, W. Ross	Brain Research Institute UCLA	
Allen, R.	Princeton University	Chemistry
Alverez, L. W.	UC - Berkeley	Physics
Anderegg, Doyle	Oklahoma University	Microbiology
Anders, Edward	University of Chicago	Chemistry
Anderson, C. D.	U.S. Geological Survey	Physics
Anderson, Chas.	Oklahoma University	Botany
Arnold, W. A.	Oak Ridge National Laboratories	Botany
Arnon, D. I.	UC - Berkeley	Botany
Aronoff, Samuel	Iowa State University	Biochemistry
Augenfeld, John	Oklahoma University	Zoology
Ball, Eric G.	Harvard Medical School	Biochemistry
Balls, Arnold K.	Purdue University	Biochemistry
Bard, Philip	Johns Hopkins Medical School	Physiology
Barker, Horace A.	UC - Berkeley	Biochemistry
Bartlett, Paul D.	Harvard University	Chemistry

Name	Institution	Field
Beadle, G. W.	University of Chicago	Botany
Beinert, H.	Institute for Enzyme Research University of Wisconsin	Biochemistry
Bellamy, Albert W.	Department of Medicine UCLA	Biochemistry
Beloff, Ruth	Yale University	Dev. Biology
Benson, A. A.	Department of Marine Biology UC - San Diego	Chemistry
Benzer, Seymor	Purdue University	Botany
Berger, R.	Lockheed Aircraft	Physics
Bethe, Hans A.	Cornell University	Physics
Biemann, K.	Massachusetts Institute of Technology	Biochemistry
Blei, I.	Melpar, Inc.	Biophysics
Blinks, L. R.	Hopkins Marine Station	Botany, Biochemistry
Bloch, Konrad E.	Harvard University	Biochemistry
Blois, M.	Stanford University	Chemistry
Blomquist, Alfred T.	Cornell University	Chemistry
Bock, R. M.	University of Wisconsin	Biochemistry
Bogorad, Lawrence	University of Chicago	Botany, Biochemistry
Bonde, Eric	Colorado University	Botany
Bongers, L. A.	Martin-Marietta Corp.	Biochemistry
Bonner, James F.	Cal Tech Department of Biology	Biochemistry, Botany
Borris, Robt. H.	University of Wisconsin	Botany, Biochemistry
Bostrom, K.	UC - San Diego	Chemistry
Boyer, Paul D.	Department of Chemistry UCLA	Biochemistry
Bradbury, N. E.	Los Alamos Scientific Laboratory	Physics
Bramlette, M. N.	UC - San Diego	Geology
Briggs, Robert W.	Indiana University	Zoology

Name	Institution	Field
Brink, Frank	Rockefeller Institute	Physiology, Biochemistry
Brock, T. D.	Indiana University	Microbiology
Bronk, Detley W.	Rockefeller Institute	Physiology, Biochemistry
Brown, H. S.	Cal Tech	Geochemistry
Buchanan, J. M.	Massachusetts Institute of Technology	Biochemistry
Bullock, Theodore H.	Department of Zoology - UCLA	Zoology
Calvin, Melvin	UC - Berkeley	Chemistry, Biochemistry
Cameron, Roy E.	Jet Propulsion Laboratory	Chemistry
Carpenter, C. C.	Oklahoma University	Zoology
Chance, Britton	University of Pennsylvania	Biochemistry
Chandrasekhar, S.	Yerkes Observatory University of Chicago	Astronomy
Charzaff, Erwin	Columbia University	Biochemistry
Ciereszko, Leon	Oklahoma University	Chemistry
Clark, Bennett	Oklahoma University	Bacteriology
Clarke, Hans T.	Yale University School of Medicine	Biochemistry
Cleland, Ralph E.	Indiana University	Botany, Biochemistry
Cleverdon, Robert	University of Connecticut	Bacteriology
Cohn, Waldo	Oak Ridge National Laboratories	Biochemistry
Colowick, Sidney P.	Vanderbilt University	Microbiology, Biochemistry
Commoner, Barry	Henry Shaw School of Botany Washington University	Biochemistry
Cope, Arthur C.	Massachusetts Institute of Technology	Chemistry
Cori, Carl F.	Washington University School of Medicine	Biochemistry
Cozad, George	University of Oklahoma	Microbiology

Name	Institution	Field
Craig, Lyman C.	Rockefeller Institute	Biochemistry
Crawford, Bryce L.	University of Minnesota	Chemistry
Crane, Charles	Civil Aeromedical Research Institute	Biochemistry
Creseitelli, F.	UCLA	Zoology
Crow, James F.	University of Wisconsin	Zoology, Genetics
Davidson, Norman	Cal Tech	Chemistry
Davies, Richard W.	Jet Propulsion Laboratory	Engineering
Davis, G.	Union Carbide Research Institute	Chemistry
Debye, Peter	Cornell University	Physics
Delbruck, Max	Cal Tech	Botany
Demerec, Milislav	Brookhaven National Laboratories	Zoology
Dingle, John H.	Western Reserve University School of Medicine	Pathology, Microbiology
Dische, Z.	Columbia University	Chemistry, Biochemistry
Djerassi, Carl	Stanford University	Chemistry
Doering, William	Yale University	Chemistry
Doisy, E. A., Sr.	St. Louis University School of Medicine	Biochemistry
Doty, Paul	Harvard University	Chemistry
Doudoroff, Michael	UC - Berkeley	Microbiology, Biochemistry
Dragstedt, Lester R.	University of Florida	Pathology, Microbiology
Dubos, Rene J.	Rockefeller Institute	Pathology, Microbiology
DuBridge, L. A.	Cal Tech	Physics
DuVigneaud, Vincent	Cornell University Medical College	Biochemistry
Eckart, Carl	UC - San Diego	Geophysics
Edsall, John T.	Harvard University Biology Laboratories	Biochemistry

Name	Institution	Field
Ehrlich, R.	Armour Research Foundation	Bacteriology
Elderfield, Robert C.	University of Michigan	Chemistry
Eisenstadt, Jerome	Yale University	Bacteriology
Elsasser, W. M.	UC - San Diego	Physics
Emerson, Alfred E.	University of Chicago	Zoology
Emmett, Paul H.	Johns Hopkins University	Chemistry
Evans, H. M.	UC - Berkeley	Physiology
Eyring, Henry	University of Utah Chemistry Department	Chemistry, Biochemistry
Fenn, Wallace O.	University of Rochester	Physiology
Ferry, John D.	University of Wisconsin	Chemistry
French, C. Stacey	Carnegie Institute of Washington	Biochemistry
Friedburg, W.	Civil Aeromedical Research Institute	Biochemistry
Friser, Louis F.	Harvard University	Chemistry
Folkers, Karl A.	Merck, Sharp & Dohme Research Laboratories	Chemistry
Fox, Sidney	Florida State University	Biochemistry
Fredriksson, K.	UC - San Diego	Geochemistry
French, Dexter	Iowa State University	Chemistry, Biochemistry
French, John D.	Director, Brain Research Inst. UCLA	Physiology
Frieden, Earl	Florida State University	Chemistry
Fruton, Joseph F.	Yale University	Biochemistry
Gaffron, Hans	Florida State University	Biochemistry
Gamow, George	University of Colorado	Physics
Gates, Marshall D.	University of Rochester	Chemistry
Geiger, P. J.	Jet Propulsion Laboratory	Biology
Giaugue, Wm. F.	UC - Berkeley	Physics
Gibbs, Martin	Brandeis University	Biochemistry
Gileman, Henry	Iowa State University	Chemistry
Glaser, D. A.	UC - Berkeley	Physics

Name	Institution	Field
Glass, Bentley	Johns Hopkins University	Biochemistry
Goddard, David R.	University of Pennsylvania	Botany, Biochemistry
Goebel, Walter F.	Rockefeller Institute	Biochemistry
Goff, Richard A.	Oklahoma University	Zoology
Goles, Gordon	UC - San Diego	Geochemistry
Gowdy, B.	Armour Research Foundation	
Gray, Frank B.	Jet Propulsion Laboratory	Physics
Green, V. M.	General Mills	Microbiology
Green, David E.	University of Wisconsin	Biochemistry
Griffin, Donald R.	Harvard University	Zoology
Haines, Howard	Oklahoma University	Zoology
Halpern, L. A.	Union Carbide Research Institute	Biology
Haley, Floyd C.	Jet Propulsion Laboratory	
Hamburger, Viktor	Washington University	Zoology
Hammett, Louis P.	Columbia University	Chemistry
Handler, Philip	Duke University	Biochemistry
Harshbarger, J. C.	UC - Irvine	Pathology
Haskins, Carlyl P.	Carnegie Institute of Washington	Zoology
Hassid, Wm. Z	UC - Berkeley	Biochemistry
Hastings, A. Baird	Scripps Clinic and Research Foundation	Biochemistry
Hauser, Charles R.	Duke University Chemistry Department	Chemistry
Hawrylewicz, E. J.	Armour Research Foundation	Microbiology
Heidelberger, Michael	Institute of Microbiology Rutgers State University	Microbiology
Hermann, H.	University of Connecticut	Biochemistry
Herriott, Roger M.	Johns Hopkins University	Biochemistry
Hershey, Alfred D.	Carnegie Institute of Washington	Genetics, Botany
Hess, S. L.	Florida State University	Biochemistry

Name	Institution	Field
Hildebrand, Joel H.	UC - Berkeley	Chemistry
Hirschfelder, Jos. O.	University of Wisconsin	Chemistry
Hitchcock, Diane	Hamilton Standard	Statistics
Hobbey, G. L	Jet Propulsion Laboratory	Sterilization
Hoftreter, Johannes	University of Rochester	Zoology
Hollaender, Alexander	Oak Ridge National Laboratories	Biochemistry
Hopla, C. E.	Oklahoma University	Zoology
Horecker, B. L.	Einstein College of Medicine	Biochemistry
Horowitz, N. H.	Cal Tech	Biology
Hoshizake, Takashi	Space Biology Laboratory UCLA	Biology
Hotchkiss, Rollin D.	Rockefeller Institute	Biochemistry, Physiology
Huang, S.	Princeton University	Biochemistry
Hubbs, Carl L.	UC - San Diego	Biology
Hundley, L.	Stanford University	
Hutchinson, George E.	Yale University	Zoology
Irwin, Malcolm R.	University of Wisconsin	Zoology, Genetics
Jagendorf, A. T.	Johns Hopkins University	Biochemistry
Jaffe, L. D.	Jet Propulsion Laboratory	Sterilization
Jahn, Theodore L.	UCLA	Zoology, Physiology
Johnson, John R.	Cornell University	Chemistry
Jukes, T.	UC - Berkeley	Biochemistry
Kalckar, Herman M.	Harvard University	Biochemistry
Kamen, Martin D.	UC - San Diego	Biochemistry
Kaplan, Joseph	UCLA	Geophysics
Kaplan, Nathan O.	Brandeis University	Biochemistry
Kasha, Michael	Florida State University	Biochemistry, Chemistry
Kaufman, B. P.	Carnegie Institute of Washington	Botany

Name	Institution	Field
Kendall, Edward C.		Biochemistry
Kimball, George E.	Arthur D. Little, Inc.	Chemistry
Kistiakowsky, G. B.	Harvard University	Chemistry
Kleinpell, Robt. M.	UC - Berkeley	Paleontology
Klotz, Irving M.	Northwestern University	Biochemistry
Koesterer, Martin G.	Wilmot Castle Research Laboratory	Microbiology
Kornberg, Arthur	Stanford Medical School	Biochemistry
Koshland, D. E.	Brookhaven National Laboratories	Biochemistry
Kramer, Paul J.	Duke University	Botany
LaMer, Victor K.	Columbia University	Chemistry
Lancaster, John	Oklahoma University	Microbiology
Land, Edwin H.	Polaroid Corporation	Chemistry, Physics
Landis, Eugene M.	Harvard Medical School	Physiology
Lardy, Henry S.	Institute for Enzyme Research University of Wisconsin	Chemistry
Larsh, Howard	Oklahoma University	Microbiology
Lederberg, Joshua	Stanford University	Botany, Biochemistry
Lehninger, S. L.	Johns Hopkins University	Biochemistry
Lerner, I. M.	UC - Berkeley	Genetics
Levin, Gilbert	Hazelton Laboratories	Engineering
Levinthal, E.	Stanford University	Biophysics
Lewis, Warren H.	Winstar Institute of Anatomy and Biology	Zoology
Libby, Willard F.	UCLA	Chemistry
Link, Karl P.	University of Wisconsin	Biochemistry
Lippmann, Fritz S.	Rockefeller Institute	Biochemistry
Lipscomb, William N.	Harvard University	Chemistry
Lloyd, David P. C.	Rockefeller Institute	Physiology
Long, Cyril N. H.	School of Medicine Yale University	Physiology

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Long, Franklin A.	Cornell University	Chemistry
Longsworth, Lewis G.	Rockefeller Institute	Chemistry
Lorento de No, Rafael	Rockefeller Institute	Physiology
Lowe, C.	New York State University	Biochemistry
Lowenstam, Heinz A.,	Cal Tech	Geology, Paleontology
Lowry, Oliver H.	Washington University School of Medicine	Biochemistry
MacLeod, Colin M.	New York University School of Medicine	Pathology, Microbiology
Mangelsdorf, Paul C.	Harvard University	Botany
Maxfield, Myles	USC	Biophysics
Mayer, Maria G.	La Jolla, California	Physics
Maynard, Leonard A.	Cornell University	Biochemistry
Mayr, Ernst	Harvard University, Museum of Comparative Zoology	Zoology
Mazia, Daniel	UC - Berkeley	Zoology, Biochemistry
Mason, Brian	American Museum of Natural History	Zoology
McClintock, Barbara	Carnegie Institute of Washington	Botany
McCollum, E. V.	Johns Hopkins University	Biochemistry
McDonald, Gordon J.	UCLA	Geophysics
McElroy, W. D.	McCollum Pratt Institute Johns Hopkins University	Biochemistry
McKenzie, Jess	Civil Aeromedical Research Institute	Biochemistry
McMillan, Wm. G., Jr.	Department of Chemistry UCLA	Chemistry
McLaren, Arthur D.	UC - Berkeley	Biochemistry
Mead, James F.,	UCLA	Biochemistry
Meister, Alton	Tufts University School of Medicine	Biochemistry
Metz, Charles W.	Woods Hole	Zoology
Metzler, David E.	Iowa State University	Biochemistry

Name	Institution	Field
Miller, S. L.	UC - San Diego	Chemistry
Mills, Kenneth	Oklahoma University	Zoology
Mirsky, Ezra S.	Rockefeller Institute	Physiology
Moore, Stanford	Rockefeller Institute	Biochemistry
Morris, John W.	Oklahoma University	Geography (Aerial Photo)
Morrison, Martin	City of Hope Duarte, California	Biochemistry
Muller, H. J.	Indiana University	Zoology
Mullin, D. D.	General Mills	
Murray, Bruce C.,	Cal Tech	Astronomy
Nachmanson, David	Columbia University	Biochemistry
Nagy, B.	UC - San Diego	Geochemistry
Nason, Alvin	Johns Hopkins University	Biochemistry
Nelson, S. S.	Melpar, Inc.	Biochemistry
Nicholas, John S.	Yale University	Zoology
Northrup, J. H.	UC - Berkeley	Biochemistry
Noyes, W. Albert	University of Rochester	Chemistry
Ochoa, Severo	New York University School of Medicine	Biochemistry
Oncley, J. L.	University of Michigan	Biochemistry
Oro, J.	University of Houston	Biochemistry
Painter, Theophilus S.	University of Texas	Zoology
Palade, George E.	Rockefeller Institute	Biochemistry, Cytology
Pardee, Arthur B.	Princeton University	Biochemistry
Pattee, H.	Stanford University	Biophysics
Pease, Daniel C.,	UCLA	Anatomy
Peterson, R. E.	General Mills	
Petrunkevitch, Alex.	Osborn Zoological Laboratory	Zoology
Phinney, B. O., Jr.	UCLA	Botany
Pinchot, G. B.	Johns Hopkins University	Biochemistry
Pitts, Robert F.	Cornell University Medical College	Physiology

Name	Institution	Field
Pitger, Kenneth	Rice University	Chemistry
Pruitt, W. O.	Oklahoma University	Zoology
Prusoff, William	Yale University	Biochemistry
Puck, Theodore T.	University of Colorado Medical Center	Biochemistry
Raper, Kenneth B.	University of Wisconsin	Bacteriology
Rea, D. G.	UC - Berkeley	Engineering
Redfield, Alfred C.		Physiology
Revelle, R. R.	Scripps Institute of Oceanography UC - San Diego	Geophysics
Rho, Joon	Jet Propulsion Laboratory	Chemistry
Rhoades, M. M.	Indiana University	Botany
Rice, Elroy	Oklahoma University	Botany, Physiology, Ecology
Rich, Alexander	Massachusetts Institute of Technology	Biochemistry
Rich, Arnold R.	Johns Hopkins Hospital	Microbiology
Riker, A. J.	University of Wisconsin	Pathology
Rittenberg, David	Columbia University College of Physicians and Surgeons	Biochemistry
Roberts, Eugene	City of Hope Medical Center	Biochemistry
Roberts, Richard B.	Carnegie Institute of Washington	Botany
Roberts, Walden K.	UC - Berkeley	Biochemistry
Rohrbaugh, L. H.	Oklahoma University	Physiology
Roth, Jay S.	University of Connecticut	Biochemistry
Rouser, D. G.	City of Hope Medical Center	Biochemistry
Sabin, Albert B.	Children's Hospital Research Foundation	Microbiology, Pathology
Sagan, C.	Harvard University	Astronomy
Salisbury, F. B.	Colorado State University	Botany
Saltman, Paul D.,	USC	Biochemistry

Name	Institution	Field
Sartorelli, Alan	Yale University	Oncology
Scher, S.	Space Sciences Laboratory UC - Berkeley	Microbiology
Scheraza, H. S.	Cornell University	Biochemistry
Schmitt, Francis O.	Massachusetts Institute of Technology	Physiology
Scholander, P. F.	UC - San Diego	Physiology
Seto, Frank	Oklahoma University	Physiology
Shedlovsky, Theodore	Rockefeller Institute	Chemistry
Sheehan, John C.	Massachusetts Institute of Technology	Chemistry
Shemin, David	Columbia University College of Physicians and Surgeons	Biochemistry
Shoemaker, E. M.	U.S. Geological Survey	Geology
Shope, Richard E.	Rockefeller Institute	Pathology
Siegel, S. M.	Union Carbide Research Institute	Physiology
Simha, Robert	USC	Chemistry
Sjostrand, Fritiof,	UCLA	Zoology
Skoog, Folke K.	University of Wisconsin	Botany
Slater, J. V.	Space Sciences Laboratory UC- Berkeley	Biophysics
Smith, Emil L.,	UCLA	Biochemistry
Smith, Lee Irvin	University of Minnesota	Chemistry
Smyth, Charles P.	Princeton University	Chemistry
Snell, E. E.	UC - Berkeley	Biochemistry
Soffen, G. A.	Jet Propulsion Laboratory	Biochemistry
Sonneborn, Tracy M.	Indiana University	Zoology
Spedding, Frank H.	Institute for Atomic Research Iowa State University	Chemistry
Spikes, John D.	University of Utah	Biochemistry
Sprinson, D. B.	Columbia University	Biochemistry

Name	Institution	Field
Stanley, W. M.	UC - Berkeley	Biochemistry, Virology
Stein, William H.	Rockefeller Institute	Biochemistry
Steinhaus, E. A.	UC - Irvine	Entomology
Stern, Curt	UC - Berkeley	Zoology
Stockmayer, W. H.	Dartmouth College	Biochemistry
Stone, Wilson S.	Genetics Foundation University of Texas	Zoology
Stork, Gilbert J.	Columbia University	Chemistry
Straus, William L.	Johns Hopkins University	Anatomy
Stuart, Jerry L.	Jet Propulsion Laboratory	Engineering
Szent-Gyorgyi, Albert	Institute for Muscle Research	Biochemistry
Tanford, C.	Duke University	Biochemistry
Tarbell, Dean S.	University of Rochester	Chemistry
Tatum, Edward L.	Rockefeller Institute	Biochemistry, Genetics
Terry, Richard	Oklahoma University	Bionics
Tishler, Max	Merck and Company	Chemistry
Touster, Oscar	Vanderbilt University	Biochemistry
Tyson, J. W.	George Washington University	Biochemistry
Umbreit, W. W.	Rutgers State University	Biochemistry
Urey, Harold C.	UC - San Diego	Chemistry
Utter, Merton F.	Western Reserve University	Biochemistry
Vallee, Bert L.	Harvard Medical School	Biochemistry
van Niel, C. B.	Hopkins Marine Station	Biochemistry
Vennesland, Birgit	University of Chicago	Biochemistry
Visscher, Maurice B.	University of Minnesota	Physiology
Wakil, S. J.	Duke University	Biochemistry
Waksman, Selman A.	Rutgers State University	Microbiology
Wald, George	Harvard University	Physiology
Walker, J. C.	University of Wisconsin	Botany
Warren, Stafford L.,	Department of Medicine UCLA	Biochemistry

Name	Institution	Field
Watson, James D.	Harvard University	Biochemistry
Webb, John L.	USC, School of Medicine	Biochemistry
Weiss, Paul A.	Rockefeller Institute	Physiology, Biochemistry
Westheimer, Frank H.	Harvard University	Chemistry
Wetmore, Ralph H.	Harvard University	Botany
Whipple, Fred L.	Astrophysical Observatory Smithsonian Institute	Astronomy
Willhite, William F.	Jet Propulsion Laboratory	Chemistry
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Williams, John W.	University of Wisconsin	Chemistry
Williams, Robley C.	UC - Berkeley	Biophysics
Williams, Roger J.	Chemistry Department University of Texas	Chemistry
Wilson, D. Wright	University of Pennsylvania School of Medicine	Biochemistry
Wilson, E. Bright, Jr.	Harvard University	Chemistry
Winstein, Saul	Department of Chemistry UCLA	Chemistry
Wolfrom, Melville L.	Ohio State University	Biochemistry
Wood, Harland G.	Western Reserve University	Biochemistry
Wood, Wm. Barry	Johns Hopkins University	Microbiology
Woodward, R. B.	Harvard University	Chemistry
Woollet, Dilworth	Rockefeller Institute	Biochemistry
Woolsey, Clinton N.	University of Wiconsin	Physiology
Young, Andrew T.	Harvard College Observatory	Astronomy
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ZoBell, Claude E.	UC - San Diego	Microbiology

1.7 SCIENTISTS INTERVIEWED

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Bremann, K.
Brock, T. D.
Burns, D.

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Chesters, G.
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Commoner, B.
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Doty, P.

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Eisenstadt, J.

Fenn, W. O.
French, D.
Freidburg, W.

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Graves, D. J.
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Prusoff, W.

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Rice, E.
Rittenberg, D.
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Rouser, G.

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Senft, A. W.
Seto, F.
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Soffen, G.
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Wilson, E. B.
Woodward, V. N.

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APPENDIX 2

COMPUTATION OF SENSITIVITIES

2.1 INTRODUCTION

The sensitivities of the various techniques examined in this analysis have been expressed in terms of the minimum number of cells required to give a positive result. Since it is difficult to determine all the variables in a method, the calculations have been based upon experimental results published in the literature rather than theoretically derived values. For convenience, the round numbers 1μ 1 cell volume, 1 mg wet weight, 1/4 mg dry weight, and 10^9 cells have been used to represent the content of 1 ml cell suspension of optical density 0.300 (650 mμ 1 cm path length).

2.2 ANALYSIS

2.2.1 DETECTION OF ENERGY CONVERSION AND TRANSFER

a. Evolution of Heat

The most sensitive microcalorimeter available appears to be the "heatburst microcalorimeter" of Kitzinger and Benzinger[1]. An appropriately

modified instrument of this type could conceivably be constructed which will detect 4 μcal/sec.

The heat output of a typical bacterium (E. coli) during division is 1.4×10^{-7} μcal/sec[2] and by analogy with the hear output from a resting wheat grain the output for a resting bacterium should be about 10^3 times less than that for a dividing cell or 1.4×10^{-10} μcal/sec.

Therefore, the number of dividing cells detectable (that is, the number of organisms which divide during the observation period) is:

$$\frac{4\mu \text{ cal/sec}}{1.4 \times 10^{-7} \mu\text{cal/sec/cell}} = 2.9 \times 10^7 \text{ cells}$$

and the number of nondividing cells detectable is:

$$\frac{4 \ \mu\text{cal/sec}}{1.4 \times 10^{-10} \ \text{cal/sec/cell}} = 2.9 \times 10^{10} \text{ cells}$$

b. Net Change in Heat of Combustion

The material is synthesized is E. coli with a composition of 78% protein, 13% nucleic acids, 5% lipid and 4% carbohydrate and each E. coli cell weighs 0.25×10^{-12}g.

During the observation the population will double.

The sensitivity with which heat of combustion can be measured is 10^{-3}cal[2]. The heat of combustion per cell will therefore be:

$$(0.25 \times 10^{-12}) \ (0.78 \times 4 + 0.13 \times 3.8 + 0.05 \times 9 + 0.04 \times 4) \ (10^3)$$

$$= 0.78 \times 10^{-9} + 0.095 \times 10^{-9} + 0.112 \times 10^{-9} + 0.04 \times 10^{-9}$$

$$= 1.0 \times 10^{-9} \text{ cal/cell.}$$

Thus, the number of original cells required is:

$$\frac{10^{-3}}{1.0 \times 10^{-9}} = 1 \times 10^{6} \text{ cells}$$

c. Light Emission by Organisms

Organism: Photobacterium phosphoreum

3.35×10^{5} molecules O_2 respired/quanta of light emitted (personal communication C. B. Van Niel).

Respiration rate of typical bacteria is $50\mu\ell\, O_2/4 \times 10^{9}$ cells/hr[3].

Therefore, $\frac{50 \times 10^{-3}}{4 \times 10^{9} \text{ cells/hr}} \times \frac{1}{22.4 \times 10^{3}} \times 6.023 \times 10^{25}$ molecules

$= 3.35 \times 10^{8}$ molecules O_2/cell/hr

Thus, $3.35 \times 10^{8} \frac{\text{molecules } O_2}{\text{cell/hr}} \cdot \frac{1}{3.35 \times 10^{5} \frac{\text{molecules } O_2}{\text{quanta}}} = 10^{3}$ quanta/cell/hr are emitted

Photosensitive detector requires 15-50 quanta in the visible region of the spectrum per photoelectron generated[4].

100 photoelectrons/sec are required for measurable steady current.

Therefore: 1500 quanta/sec = 5.4×10^{6} quanta/hr required for measurable steady current[4].

Therefore:

$$5.4 \times 10^{6} \times \frac{1}{10^{3}/\text{cell/hr}} = 5.4 \times 10^{3} \text{ cells} = \text{minimum detectable}$$

d. Unpaired Electrons

The sensitivity of ESR measurements is 10^{-12} molar[5].

10^{-21}M of free radicals are formed in each dying S. lactis organism[6].

Therefore:

$$\frac{10^{-12}}{10^{-21}} = 10^{9} \text{ dying cells must be available}$$

e. **Bioelectric Potential or pH Change**

Assume:

Organism is E. coli

Minimum detectable EMF change = 0.1 mv = 10^{-4} volts

Two electrons are transferred in the oxidation-reduction reaction

Substrate concentration = 1 m mol.

Bioelectric potential $E-E_o = 2.303 \frac{RT}{nF} \log \frac{[Ox]}{[Red]}$ (7)

$$\log \frac{[Ox]}{[Red]} = \frac{1}{2.303} \times \frac{2 \times 96500}{8.314 \times 300} \times 10^{-4} \text{volt} = 0.00338$$

$$\frac{[Ox]}{[Red]} = 1.0078$$

for substrate conc. of 1 m molar

$$\frac{1 + X}{1 - X} = 1.0078$$

$$2.0078X = 0.0078$$

Therefore, detectable concentration change $= \frac{0.0078}{2.0078} = 0.0039$ m mol.

for change in EMF of 10^{-4} volts $= 3.9$ μ mol.

Respiration rate (at 32°C) of E. coli = 272 $\mu\ell$ / 4×10^9 cells/hr.(3)

$$\frac{272\ \mu\ell}{22.4\ \mu\ell/\mu \text{ mole}} / 4 \times 10^9 \text{ cells/hr} = 3 \times 10^{-9}\ \mu \text{ mol/ cell/hr}$$

Therefore, no. of organisms required =

$$3.9\ \mu \text{ mole} / 3 \times 10^{-9} \frac{\mu \text{ mole}}{\text{cell}} = 1.3 \times 10^9 \text{ cells}$$

acting for 1 hour

This calculation also establishes the relative sensitivity of measuring changes in pH.

f. **Metabolism of Substrate**

Use Glucose-E-C^{14} and detect $C^{14}O_2$

Assume:

Specific activity of glucose is 1.0 mc/mM

Counting efficiency is 50%

95% confidence limit is satisfactory

Counting time is 1 hour

3×10^{-12} mM/cell-hr of CO_2 is obtained from the oxidation of glucose by *E. coli* (3).

Therefore:

$$3 \times 10^{-12} \times 222 \times 10^{7} \times 0.5 = 3.33 \times 10^{-3} \text{ cpm/ cell /hr}$$

To fulfill counting requirements indicated, the total cts. needed in 1 hour are 1500 and the cpm are 25.

Therefore:

$$\frac{25}{3.33 \times 10^{-3}} = 7500 \text{ cells}$$ required to liberate a detectable amount of $C^{14}O_2$ in 1 hour

g. Dark Fixation of $C^{14}O_2$

Assume:

Specific activity of $C^{14}O_2$ is 30 mc/mM

Counting efficiency 50%

95% confidence limit required and counting time is 1 hour

1 mM of $C^{14}O_2$ supplied

$$\frac{30 \times 222 \times 10^{7} \times 0.5 \times 0.2}{8 \times 10^{8} \times 80} = \text{cpm } C^{14}O_2 \text{ fixed/cell}$$

$$= 0.1 \text{ cpm/cell for } \textit{E. coli}\ (9).$$

To fulfill counting requirements indicated the total cts. needed in 1 hour are 1500 and the cpm are 25

Therefore:

$$\frac{25}{0.1} = 250$$ dividing cells fixing $C^{14}O_2$ over a 1 hour period are required to fix a detectable quantity of $C^{14}O_2$.

h. Light Fixation of $C^{14}O_2$

Organism: Chlorella saccharophila

Photosynthesis rate = 0.452 ml $CO_2/10^8$ cells/hr

= 0.02 m mole $CO_2/10^8$ cells/hr[3]

Assume:

Specific activity of $C^{14}O_2$ = 30 mc/m mole

= 30 x 22 x 10^7 dpm/ m mole

= 6.6 x 10^{10} dpm/ m mole

Counting efficience of 50%

Specific activity of $C^{14}O_2$ = 3.3 x 10^{10} cpm/m mole

Sensitivity per organism = $0.02 \times \frac{1}{10^8 \text{ cells/hr}} \times 3.3 \times 10^{10}$ cpm =

6.6 cpm/cell/hr of photosynthesis

Total counts needed for 95% confidence with 0.05 probability of error greater than 5% is 1500 counts[8].

Therefore, sensitivity of method = $\frac{1500 \text{ counts}}{60 \text{ min}}$ = 25 cpm assuming 1 hr counting time and no. of cells required

= $\frac{25}{6.6}$ = 3.8 cells fixing $C^{14}O_2$ for a period of 1 hour

i. Detection of ATP

There is 8.3 x 10^{-10} µg of ATP per cell (yeast)[10].

The sensitivities of various methods for detecting ATP according to Strehler et al.[11] are:

(a) Chemical - 10 µg

(b) Ion exchange - 5 µg

(c) Enzymic (firefly) - 10^{-4} µg (quantum counter)

Therefore, the number of cells required is:

(a) chemical $\frac{10}{8.3 \times 10^{-10}}$ = 1.2 x 10^{10} cells

(b) Ion exchange $\frac{5}{8.3 \times 10^{-10}}$ = 6 x 10^9 cells

(c) Enzymic (firefly) $\frac{10^{-4}}{8.3 \times 10^{-10}}$ = 1.2 x 10^5 cells

j. Detection of Porphyrins

(1) Photosynthetic organism

On an average, photosynthetic micro and macro organisms contain 0.2% porphyrins or about 2×10^{-9} µg/organism[12].

Less than 10^{-4} µg of porphyrins can be detected fluorometrically[13] and 2.9×10^{-2} µg/ml by absorption spectrophotometry (chlorophyll)[14].

Therefore,

$$\frac{10^{-4}}{2 \times 10^{-9}} = 5 \times 10^{4} \text{ cells are required for fluorometric method.}$$

and $\frac{2.9 \times 10^{-2}}{2 \times 10^{-9}} = 1.45 \times 10^{7}$ photosynthetic cells are required for absorption method

(2) Non-photosynthetic organism

On the average, non-photosynthetic micro and macro-organisms contain 0.00025% porphyrin (haematin) on a wet weight basis[10].

Sensitivity of porphyrin by fluorometry is less than 10^{-4} µg[13] and by absorbance spectrophotometry it is 7.7×10^{-3} µg/ml[14].

The content of porphyrin per cell is:

2.5×10^{-4} mg/100 mg $\times 10^{9}$ $= 2.5 \times 10^{-15}$ mg/cell

$= 2.5 \times 10^{-12}$ µg/cell

Number of cells required for detection by fluorometry is,

$= 10^{-4}$ µg/2.5×10^{-12} µg/cell

$= 4 \times 10^{7}$ cells

and by absorption spectrometry

$$\frac{7.7 \times 10^{-3}}{2.5 \times 10^{-12}} = 3.1 \times 10^{9} \text{ cells}$$

k. Light stimulated evolution of oxygen

(1) Tracer Method (O^{18})

Sensitivity for detection of O^{18} by mass spectrometer in presence of water background is 10^{-6} parts (personal communication, L. Theard)

Assume reaction can be carried out in 99% H_2O^{18}, at 40 mb pressure (Mars atmospheric pressure) and a gas volume of 5 ml (9µ moles)

$20\,\mu$ moles O_2 are produced photosynthetically in one hour by 10^8 cells[3].

Therefore, $\frac{20}{108}$ = $2 \times 10^{-7}\,\mu$ moles O_2 is produced per cell /hr. The O^{18} concentration in the gas phase produced by 1 cell is $\frac{2 \times 10^{-7}}{9} = 0.22 \times 10^{-7}$ parts

Thus, $\frac{10^{-6}}{0.22 \times 10^{-7}}$ = 45 cells photosynthesizing for 1 hour are required.

(2) Cartesian Diver Method

Sensitivity of method is $10^{-4}\,\mu$ l/hr[15].

4.5×10^{-6} µl oxygen/hr/cell is liberated photosynthetically (chlorella saccharophila)[3].

Therefore,

$\frac{10^{-4}}{4.5 \times 10^{-6}}$ = 22 cells photosynthesizing for 1 hour can be detected.

(3) Microrespirometry

Sensitivity of method is $10^{-2}\,\mu$ l/hr[15].

$4.5 \times 10^{-6}\,\mu$ l oxygen/hr/cell is liberated photosynthetically[3].

Therefore,

$\frac{10^{-2}}{4.5 \times 10^{-6}} = 2.2 \times 10^3$ cells can be detected.

(4) Magnetic Susceptibility

The sensitivity of this method is $4 \times 10^{-3}\%$ O_2[16].

Assume measurement can be made on 1 ml sample.

Therefore, minimum conc. change of O_2 by this method in 1 ml sample =

$4 \times 10^{-3} \times .01$ ml/ml sample = 4×10^{-5} ml O_2/ml sample

4.5×10^{-9} ml oxygen/cell/hr is liberated photosynthetically[3].

Therefore:

$\frac{4 \times 10^{-5}}{4.5 \times 10^{-9}} = 9 \times 10^3$ cells can be detected in 1 hour.

(5) Oxygen electrode method

The sensitivity of the method is 1 μl[16].

4.5×10^{-6} μ l oxygen/hr/cell is liberated photosynthetically[3].

Therefore,

$$\frac{1}{4.5 \times 10^{-6}} = 2.2 \times 10^{5} \text{ cells can be detected}$$

1. Uptake of Oxygen

(1) Cartesian Diver Method

Sensitivity is 10^{-4} μ l/hr[15].

6.8×10^{-8} μ l oxygen/hr/cell is taken up (E. coli)[3].

Therefore;

$$\frac{10^{-4}}{6.8 \times 10^{-8}} = 1.47 \times 10^{3} \text{ cells can be detected in 1 hour.}$$

(2) Microrespirometry

Sensitivity is 10^{-8} μ l/hr[15].

Therefore,

$$\frac{10^{-3}}{6.8 \times 10^{-8}} = 1.47 \times 10^{4} \text{ cells can be detected in 1 hour.}$$

(3) Oxygen Electrode

Sensitivity is 1 μ l[16].

Therefore, $\frac{1}{6.8 \times 10^{-8}} = 1.47 \times 10^{7}$ cells can be detected in 1 hour

(4) Magnetic Susceptibility

The sensitivity of this method is $4 \times 10^{-3}\%$ O_2[16].

Assume measurement can be made in 1 ml sample.

Therefore, the minimum concentration change of O_2 by this method is 4×10^{-5} ml O_2/ml sample.

6.8×10^{-11} ml O_2/cell/hr are taken up by E. coli[3].

Therefore;

$$\frac{4 \times 10^{-5}}{6.8 \times 10^{-11}} = 6 \times 10^{5} \text{ cells can be detected in 1 hour}$$

m. Evolution of CO_2

(1) Cartesian Diver Method

1. Sensitivity is 10^{-3} μl/hr[15].
2. 6.8×10^{-8} μl CO_2/hr/cell is evolved (E. coli)[3].

Therefore,

$$\frac{10^{-3}}{6.8 \times 10^{-8}} = 1.47 \times 10^4 \text{ cells can be detected in 1 hour.}$$

(2) Microrespirometry

Sensitivity is 10^{-2} μ 1/hr[15].

Therefore,

$$\frac{10^{-2}}{6.8 \times 10^{-8}} = 1.47 \times 10^5 \text{ cells can be detected in 1 hour.}$$

(3) CO_2 Electrode

Assume: (a) total volume of 1.0 ml, (b) temperature of 25°C, (c) standard pCO_2 and (d) no substances in the medium which react with CO_2.

The amount of dissolved CO_2 is therefore 0.759 ml[17].

A change in pCO_2 of 1% can be detected[16].

Therefore, a change of

$0.01 \times 0.759 = 7.6$ μ 1 CO_2 can be detected.

6.8×10^{-8} μl CO_2/hr/cell is evolved (E. Coli)[3]

Thus,

$$\frac{7.6}{6.8 \times 10^{-8}} = 1.1 \times 10^8 \text{ cells can be detected in 1 hour.}$$

(4) Change in solution conductivity

1. 6.8×10^{-8} μ 1 CO_2/cell/hr evolved by E. coli[3].
2. 0.002 mg CO_2 can be detected by method[18].

Therefore:

2 μg CO_2 = $\frac{2}{44}\mu$ mole CO_2 = 1 μ1 CO_2 can be detected

and Thus:

$$\frac{1\ \mu\ 1}{6.8 \times 10^{-8}\ \mu\ 1/\text{cell}} = 1.5 \times 10^7 \text{ cells evolving } CO_2 \text{ for } \underline{1 \text{ hour}} \text{ can be detected.}$$

2.2.2 DETECTION OF MACROMOLECULES

a. **By Absorption Spectrophotometry**

(1) **Assume macromolecule is DNA.**

An increase in absorbance of 0.005 is detectable

The amount of DNA per cell (E. coli) is 10^{-8} μ g[3].

The amount detectable is:

a. **J-band dye - 0.10μ g/ml[19]**

b. **UV absorption - 0.20 μg/ml[20]**

Therefore:

J-band dye $\frac{0.10}{10^{-8}} = x\ 10^7$ **cells are required**

UV absorbance $\frac{0.20}{10^{-8}} = 2 \times 10^7$ **cells are required.**

(2) **Assume macromolecule is a protein**

An increase in absorbance of 0.005 is detectable

The amount of protein per organism (E. coli) is 2×10^{-7} μg[3].

The amount detectable is:

a. **J-band dye -0.01 μg/ml[19].**

b. **UV absorption[21].**

E = 76,000 280 mμ - 2.5 μg/ml

E = 900,000 190 mμ - 0.2 μg/ml

Therefore:

J-band dye $\frac{0.01}{2 \times 10^{-7}} = 5 \times 10^4$ **cells are required**

UV absorbance 280 mμ $= \frac{2.5}{2 \times 10^{-7}} = 1.25 \times 10^7$ **cells**

$190\ m\mu = \frac{0.2}{2 \times 10^{-7}} = 1 \times 10^6$ cells are required.

b. **By Optical Rotation**

(1) **Assume macromolecule is DNA**

The specific rotation of a solution is given[22] by

$$[\alpha]_\lambda = \frac{a}{\ell} \cdot \frac{1}{c}$$

a = angle of rotation (degrees)
ℓ = path length (decimeters)
c = conc (g/ml)

The specific rotation of DNA at 257 mμ is -2000[23].

A change in angle of rotation of 0.001 deg. can be detected.

Assume a measuring cell of 10 ml with a path length of 1 dm.

Therefore, minimum concentration detectable

$$c = \frac{a}{\ell} \cdot \frac{1}{[\alpha]_\lambda} = \frac{0.001}{1} \cdot \frac{1}{2000} = 5 \times 10^{-7} \text{ g/ml}$$

$$= 5 \times 10^{-6} \text{g/10 ml cell} = 5 \times 10^{-3} \text{ mg in 10 ml cell}$$

DNA per organism (E. coli) = 10^{-11} mg/cell[3].

Therefore $\frac{5 \times 10^{-3} \text{ mg}}{10^{-11} \text{ mg/cell}} = 5 \times 10^8$ cells required for detection

(2) **Assume macromolecule is Protein**

The specific rotations averaged for a series of proteins were found(20) to be about -7000 deg. at 233 mμ.

A change in angle of rotation of 0.001 deg. can be detected.

Assume 1 ml measuring cell with a path length of 0.1 decimeter.

Therefore, the minimum concentration detectable

$$C = \frac{a}{\ell} \frac{1}{[\alpha]} = \frac{.001}{0.1} \times \frac{1}{7000} \text{ g/ml} \cong 1.4 \times 10^{-6} \text{ g/ml} = 1.4 \text{ μg/ml}$$

The amount of protein per cell (E. coli) 2×10^{-7} μg/cell[3].

Therefore $\frac{1.4 \text{ μg}}{2 \times 10^{-7} \text{ μg/cell}} = 7 \times 10^6$ cells are required for detection

(3) Assume that a planar dye can be attached to the macromolecule. In this case, the cotton effect will be shifted into the visible with apparently very little change in sensitivity as regards the detection of the macromolecule by OR[24a].

c. By Change in Refractive Index

A. Assume macromolecule is protein

Refractive index increment = 0.00185 change in refractive index per 1% change in conc[15].

Index of refraction can be measured to ± 0.00002. Hence changes in index of refraction of .00008 should be readily detectable.

Therefore,

$$\frac{0.00008}{0.00185} \times 0.01 = 4.3 \times 10^{-4} \text{ g/100 ml}$$

$$= 4.3 \times 10^{-6} \text{ g/ml change in conc. should be detectable.}$$

The amount of protein per cell (E. coli) = 2×10^{-7} µg[3].

Therefore,

$$\frac{4.3 \text{ µg}}{2 \times 10^{-7} \text{ µg/organism}} = 2.2 \times 10^{7} \text{ cells are required for detection.}$$

d. Viscosity:

There is a striking difference in the viscosity effects produced by globular (i.e. albumin) and linear (i.e. DNA) macromolecules. Globular macromolecules have an insignificant effect on viscosity of aqueous solutions, whereas linear macromolecules increase the viscosity greatly. The effectiveness of this method will therefore depend on the structural configuration of the macromolecule[15a].

4 µg of DNA can be detected in a 1 ml sample by measuring change in viscosity[15b].

The DNA content of cells is 10^{-8} µg/cell[3].

Therefore: $\frac{4 \text{ m.l.m}}{10^{-8} \text{ µg/cell}} = 4 \times 10^{8}$ cells are detectable by measuring the change in viscosity of a solution due to DNA.

e. Light Scattering

Relatively low concentrations of some macromolecules in solution have been shown to produce measurable light scattering, for example, using light scattering dissymmetry techniques, 20 μg/ml of tobacco mosaic virus can be detected [15c]. However, the use of light scattering as a positive detection tool leaves much to be desired. Scattering has been shown to be greatly dependent on molecular shape, nature of solvent, pH and wavelength of incident light[15a]. Furthermore, the presence of dust, air bubbles and similar coarse impurities greatly distort the results.

2.2.3 REPRODUCTION, REPLICATION AND GROWTH

a. **Turbidity Measurement as a Function of Time**

According to Vishniac (24b) the minimum reliable detection level for turbidity measurement is around 10^5 bacteria/ml.

b. **Increase in Mass as a Function of Time**

(1) Change in weight

Microbalances are available which will detect changes in weight (mass) of 1.0 μg[25].

On Mars, this corresponds to about 3.3 μg (assuming 0.3 g for Mars) of mass.

Therefore, $3.3 \times 10^{-3} \times 10^9 = 3.3 \times 10^6$ cells are required.

(2) Change in crystal frequency

Mass deposited on a piezoelectric quartz crystal will cause a change in frequency. Thus, it is conceivable that they can be used to detect a change in mass. In fact, the frequency change is directly related to the mass deposited on the crystal and theoretically the detection limit is 10^{-6} μg[26].

Special sorption detectors based on this principle have been constructed which will detect a mass increase of 10^{-1} μg.

Therefore, $10^{-4} \times 10^9 = 10^5$ cells could be detected.

c. **Increase in Macromolecules as a Function of Time**

(1) Assume macromolecule has anionic sites

A change in absorbance of 0.005 is required

For the J-band method a change of 0.005 in absorbance is equal to a change in anionic macromolecule concentration of about 0.01 μg/ml[19].

The macromolecule content of a micro-organism is about 20 percent of the moist weight[10].

Therefore,

$\frac{0.01}{0.2}$ = an increase of 0.05 μg of organisms can be detected

this equals 0.05×10^{-3} mg $\times 10^9$/mg = 5×10^4 cells

(2) Assume the macromolecule exhibits optical activity.

Assume the macromolecule has a specific rotation which approximates that of DNA.

Then the sensitivity is the same as for the detection of DNA by OR. That is, 5×10^8 microorganisms/ml.

d. Increase in Nucleic Acids as a Function of Time

(1) By spectrophotometric absorbance or Optical Rotation-UV , dye, and dye + OR.

The same sensitivity applies, within reasonable limits, as for the detection of DNA, but the amount of nucleic acid must be increased to take into consideration the RNA content of the cell. The RNA content of bacterial cells is about 3 times the DNA content[3]. Therefore, the amount of nucleic acid per organism is about 4×10^{-8} µg.

Thus to detect an increase in nucleic acids requires the production of the following number of cells:

UV absorption - $\frac{.2}{4 \times 10^{-8}} = 5 \times 10^6$ cells

Dye - $\frac{.1}{4 \times 10^{-8}} = 2.5 \times 10^6$ cells

OR + Dye - $\frac{5}{4 \times 10^{-8}} = 1.25 \times 10^8$ cells

(2) By Colorimetry

(a) DNA

2 µg of DNA can be determined colorimetrically[27].

The DNA content of cells is 10^{-8} µg/cell[3].

Therefore:

$\frac{2\ \mu g}{10^{-8}\ \mu g/cell} = 2 \times 10^8$ cells are detectable by determining DNA colorimetrically.

(b) RNA

5 μg of RNA can be determined colorimeterically[27].

The RNA content of cells is 3×10^{8} μg/cell[3].

Therefore,

$\frac{5\ \mu g}{3 \times 10^{8}\ \mu g/cell} = 1.7 \times 10^{8}$ cells are detectable by determining RNA colorimeterically

e. Increase in Proteins with Time

(1) Modified Lowry Method

Sensitivity of method is 0.2 μg of protein[28].

Protein per organism (E. coli) = 2×10^{-7} μg.

Therefore,

$\frac{0.2}{2 \times 10^{-7}} = 10^{6}$ cells detectable by determining protein by modified Lowry method

(2) By UV or J-Band Absorption Spectrophotometry - See page 10.

f. Increase in Lipids as a Function of Time

(1) Steroids

The sterol content of organisms is about 0.3 percent of the dry weight and the dry weight is about 25 percent of the fresh weight[3].

The sterol content of a cell is therefore about:

$0.003 \times 0.25 \times 10^{-6}\ \mu g = 7.5 \times 10^{-10}\ \mu g/cell$

a. In chloroform solution, 25 μg of steroid can be detected by infrared methods (29).

b. By gas chromatography about 10^{-2} μg of steroid can be detected[30].

c. About 1.0 μg of steroid can be detected colorimetrically by its reaction with $FeCl_3$.

d. By the use of ORD about 100 μg of steroid can be detected[31].

Thus, if it is assumed that the most abundant sterol species comprises 25% of the total, the following number of cells is required for each method.

Infrared - $\frac{25}{7.5 \times 10^{-10}} = 3.3 \times 10^{10}$ cells

Gas Chromatography - $\frac{10^{-2}}{7.5 \times 10^{-10} \times .25} = 5.2 \times 10^{7}$ cells

Colorimetric - $\frac{1.0}{7.5 \times 10^{-10}} = 1.3 \times 10^{9}$ cells

ORD - $\frac{100}{7.5 \times 10^{-10} \times .25} = 5.2 \times 10^{11}$ cells

Mass Spectrometer - $\frac{10^{-2}}{7.5 \times 10^{-10} \times .25} = 5.2 \times 10^{7}$ cells

(2) Fatty acids

The fatty acid content (both esterified and unesterified) of an organism is about 8 percent of the dry weight[33]. The dry weight is 25 percent of the wet weight.

The most abundant single fatty acid species comprises about 25 percent of the total fatty acid content.

Therefore, there is $0.08 \times 0.25 \times 10^{-6} = 2 \times 10^{-8}$ µg fatty acid/cell and 0.5×10^{-8} µg of a single fatty acid specie.

The sensitivites of the following methods are:

a. Gas chromatograph - 5×10^{-3} µg[34].

b. Infrared - 20 µg[35].

c. Colorimetric - 50 µg by ferric chloride color reaction with methylated fatty acid.

d. Mass spectrometer - 10^{-2} µg[32].

Therefore, the following numbers of cells are required:

Gas chromatograph - $\frac{5 \times 10^{-3}}{0.5 \times 10^{-8}} = 10^6$ cells

Infrared - $\frac{20}{2 \times 10^{-8}} = 10^9$ cells

Colorimetric $\frac{50}{2 \times 10^{-8}} = 2.5 \times 10^9$ cells

Mass Spectrometer - $\frac{10^{-2}}{0.5 \times 10^{-8}} = 2 \times 10^6$ cells

2.2.4 DETECTION OF SUBSTANCES ASSOCIATED WITH LIVING SYSTEMS

a. **Detection of Amino Acids**

Assume all of the protein can be hydrolyzed to amino acids.

2×10^{-7} μg of amino acid would be available per cell.

The most abundant amino acid species comprises about 15 percent of the total amino acids[36].

The minimum amount of amino acid(s) which can be detected by the following methods is:

Gas chromatography - 5×10^{-3} μg[37].

Mass spectrometer - 10^{-2} μg[32].

Colorimetric - 0.1 μg - (ninhydrin)

Thus, the following number of cells are required for each method:

Gas chromatography $\frac{5 \times 10^{-3}}{2 \times 10^{-7} \times 0.15} = 1.67 \times 10^5$ cells

Mass spectrometer $\frac{10^{-2}}{2 \times 10^{-7} \times 0.15} = 3.3 \times 10^5$ cells

Colorimetric $\frac{10^{-1}}{2 \times 10^{-7}} = 5 \times 10^5$ cells

b. Detection of Nucleotides (or Purines & Pyrimidines)

Assume all of the nucleic acids are hydrolyzed to monomeric bases. The amount of nucleotide available per cell is then

$$(\%\ \text{N.A./D.Wt})\ \frac{12.84}{100} \times \text{D.Wt/cell}\ \frac{10^{-6}\ \mu g}{4} = 3.21 \times 10^{-8}\ \mu g/\text{cell}^{(3)}.$$

The minimum amount of nucleotide which can be detected by UV absorbance -

$$0.2 \times 0.67 = 0.13\ \mu g/ml^{(20)}.$$

Therefore, the following number of cells can be detected by UV absorbance

$$\frac{1.3 \times 10^{-1}}{3.21 \times 10^{-8}} = 4 \times 10^{6}\ \text{cells}$$

c. Detection of Monosaccharides

Whereas bacteria and animal cells contain perhaps 4 percent carbohydrate on a dry weight basis, plant material is very rich in carbohydrate. Therefore, as a compromise estimate of the carbohydrate composition of cells a value of 25 percent of the dry weight is assumed.

Thus a cell contains,

$$\frac{10^{-6}\ \mu g}{4} \times 0.25 = 6.25 \times 10^{-8}\ \mu g\ \text{carbohydrate/cell}$$

The minimum amount of carbohydrate which can be detected by the following methods is:

Gas chromatography (methyl esters) - 5×10^{-3} μg[34].
Mass spectrometer 10^{-2} μg (32)
Colorimetric 5 μg (38).

Assuming that 50 percent of the carbohydrate will be composed of a single species the following number of cells will be required:

Gas chromatography - $\frac{5 \times 10^{-3}}{0.5 \times 6.25 \times 10^{-8}} = 1.6 \times 10^{5}$ cells

Mass spectrometer $\frac{10^{-2}}{0.5 \times 6.25 \times 10^{-8}} = 3.2 \times 10^{5}$ cells

Colorimetric $\frac{5}{6.25 \times 10^{-8}} = 8 \times 10^{7}$ cells

d. Detection of Porphyrins

See page 2-6

e. Detection of Fatty Acids

See page 2-16

f. Detection of Steroids

See page 2-15

g. Detection of Phospholipids

About 4 percent of the dry weight of a cell is phospholipid[28].

Therefore a cell contains:

$0.04 \times 0.25 \times 10^{-6} = 10^{-8}$ μg phospholipid/cell

Colorimetric analysis for chloroform soluble phosphorus will detect 2×10^{-3} μg of P or about $\frac{560}{31} \times 2 \times 10^{-3}$ μg phospholipid[39].

Therefore, $\frac{3.6 \times 10^{-2}}{10^{-8}} = 3.6 \times 10^{6}$ cells are required.

h. Detection of Total Flavins

As a general average, there appears to be about 40 μg of flavin per g moist weight in bacteria[10].

Therefore, there is $\frac{40}{10^{12}} = 4 \times 10^{-11}$ μg flavin/cell

As little as 1×10^{-4} μg of flavin can be detected by the lumiflavin method[40].

Thus $\frac{1 \times 10^{-4}}{4 \times 10^{-11}} = 2.5 \times 10^{6}$ cells are required

2.2.5 CATALYTIC ACTIVITY

a. Dehydrogenase, dye reduction

Assume: (a) a change in color of 1 percent is detectable.

(b) The oxidation rate of succinate is representative of dehydrogenase activity and equal to 10 μl O_2/mg dry wt./hr or 0.893 μ moles/ 4×10^{9} cells/hr[3,10].

If the concentration of the dye is 10^{-4} M or 100 μ molar and one hydrogen is required to reduce the dye the number of cells required is:

$$\frac{0.01 \times 100}{0.893} \times 4 \times 10^{9} = 4.5 \times 10^{9} \text{ cells}$$

b. Phosphatase - Fluorometric

The method depends upon the detection of fluorescein liberated from phosphorylated fluorescein. Therefore the sensitivity is about 10^{-3} μg/ml or 3×10^{-6} μ moles/ml[41].

A representative concentration of non-specific alkaline phosphatases in cells appears to be 0.06 percent of the protein and the turn over number is about 100,000 moles/min/100,000 g protein[42].

The protein content of bacterial cells is about 75 percent of the dry weight[3].

Therefore, $0.0005 \times 0.75 \times 0.25 = 9.37 \times 10^{-11}$ μ g of non-specific alkaline phosphatase is present in each cell.

Thus in 1 hour one cell liberates.

$$60 \times \frac{9.37 \times 10^{-11} \times 100{,}000}{100{,}000} = 0.56 \times 10^{-9} \text{ μmoles of fluorescein}$$

Therefore, $\frac{3 \times 10^{-6}}{0.56 \times 10^{-9}} = 5.3 \times 10^{3}$ cells are required.

c. Catalase - Evolution of Oxygen from H_2O_2

On an average, there is about 5×10^{-5} μ moles of catalase per mg dry weight of cells[10].

The turn over for catalase is 10^7 molecules of H_2O_2 per molecule of catalase per minute[3].

For each mole of H_2O_2 utilized 0.5 moles of O_2 is produced.

The evolution of 10^{-2} μ 1 per hour of O_2 can be detected manometrically[15].

The liberation of 10^{-2} μ 1 $O_2 = \frac{2 \times 10^{-2}}{22.4} = 9 \times 10^{-4}$ μ moles H_2O_2 utilized

To utilize 9×10^{-4} μ moles of H_2O_2

$$\frac{9 \times 10^{-4}}{60 \times 10^{7}} = 1.5 \times 10^{-12} \text{ μ moles of catalase is required.}$$

Thus the number of cells required = $\frac{1.5 \times 10^{-12}}{5 \times 10^{-5}} \times 4 \times 10^{9} = 1.2 \times 10^{2}$ cells.

d. Oxygen Exchange Between Water and Phosphate

The rate of oxygen exchange catalyzed by inorganic pyrophosphatase is:

44 μ moles oxygen/μg enzyme/hr[43].

About 10^{-10} μg of inorganic pyrophosphatase per cell appears to be a generous estimate (see paragraph 2.2.5b).

4.4×10^{-6} moles of O^{18} exchange/hr can be detected with a mass spectrometer[43].

Therefore, $\frac{4.4 \times 10^{-6} \times 10^{6}}{44 \times 10^{-10}} = 10^{9}$ cells are required

2.2.6 DETECTION OF ORGANIZATION

a. Optical Purity

Assume optically active molecule is a protein

The specific rotations averaged for a series of proteins were found[20] to be about -7000 deg. at 233 mμ.

A change in angle of rotation of 0.001 deg. can be detected.

Assume 1 ml measuring cell with a path length of 0.1 decimeter.

Therefore, the minimum concentration detectable

$$C = \frac{a}{\ell}\,\frac{1}{[\alpha]} = \frac{0.001}{0.1} \times \frac{1}{7000}\ \text{g/ml} = 1.4 \times 10^{-6}\ \text{g/ml or } 1.4\ \mu\text{g/ml}$$

The amount of protein per cell (E. coli) 2×10^{-7} μg/cell[3].

Therefore $\frac{1.4\ \mu g}{2 \times 10^{-7}\ \mu g/\text{cell}} = 7 \times 10^{6}$ cells are required for detection

b. Detection of Macroscopic Organisms by Visual Scan

Assume:

(1) That Martian macroforms are composed of cellular units having a diameter of about 10 μ.

(2) The Martian macroform can be composed of a single sheath of cells.

(3) The resolution of the viewing system is 0.5 mm.

A spot 0.5 mm in diameter occupies an area of:

$$0.7854 \times 0.5^2 \times 10^6 = .2 \times 10^6$$

$$= 2 \times 10^5 \mu^2$$

One cell occupies an area of about $0.7854 \times 10^2 = 78\ \mu^2$

Therefore:

$\frac{2 \times 10^5}{78} = 2.5 \times 10^3$ cells are required to form a 0.5 mm macroscopic organism.

c. Detection of Microorganisms by Light Microscopy

Assume:

(1) The observations are made during a single scan of a movable tape under a 95 X objective which gives a field of view of about 20,000 μ^2 (diameter 159 μ).

(2) The density of particles on the tape is such that they occupy 10% of the available area, the average particle has a diameter of 10 μ and the density of the non-biological material is 2.2 gm/cm^3.

(3) 10^3 views can be obtained

$$\frac{2 \times 10^4}{0.785 \times 10^2} = 2.6 \times 10^2 \text{ particles can be accommodated in one field.}$$

Thus $0.1 \times 2.6 \times 10^2 = 26$ particles will be present in one field and $10^3 \times 26 = 2.6 \times 10^4$ total particles are viewed

$$\text{Total vol. of particles} = \frac{2.6 \times 10^4 \times 4.189 \times 1.25 \times 10^2 \ \mu^3}{10^{12} \mu^3/cm^3} = 1.36 \times 10^{-5} cm^3$$

Total weight of particles

$$= 1.36 \times 10^{-5} \times 2.2 = 3 \times 10^{-5} \text{ g}$$

Thus,

$$\frac{1}{3 \times 10^{-5}} = 3.3 \times 10^4 \text{ cells/g must be present if one organism is to be found in } 10^3 \text{ views}$$

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APPENDIX 3

RATING OF BIOLOGICAL EXPERIMENTS

I. DETECTION OF ENERGY TRANSFER AND CONVERSION

The detection of energy transfer and conversion can be accomplished by direct measurements of energy changes or by indirect procedures which measure changes due to energy transfer and conversion or detect specific substances necessary for energy transfer and conversion in terrestrial systems. The direct procedures do not depend upon a knowledge of the mechanism by which the energy transfer or conversion is carried out. On the other hand, to employ the indirect methods it is necessary to assume that the same processes or substances utilized by terrestrial life are essential for energy conversion and transfer in Martian life.

ANALYSIS OF RATINGS FOR LIFE DETECTION COMPLEMENT

I. DETECTION OF ENERGY TRANSFER AND CONVERSION

TECHNIQUE *

1. Evolution of Heat

Parameter	Pertinent Information or Factors Considered **	
A 0.7	a. The function occurs in all microorganisms the distribution of which are probably adequate.	2
	b. The function is ubiquitous in living forms.	2
	c. Only endogenous substrates are required.	2
	d. It does not appear possible that the atomic vibrations necessary for heat evolution will be absent in Martian life.	2
	e. Many non-biological events liberate heat.	0
Weighted Score 5.6	Score	8
B 0.7	a. The test simply records the evolution of heat and is therefore not related to any one specific reactive group.	2.5
	b. The controlled production of heat is considered to be a useful biological property.	2.5
	c. The technique tests the overall process of heat liberation and not heat due to a single reaction.	2.5
	d. It does not appear to be possible that the atomic vibrations necessary for heat evolution will be absent in Martian life.	2.5
Weighted Score 7.0	Score	10.0
C 1.0	a. The technique relies on a voltage developed by a series of thermopiles. It is conceivable, but not likely, that other processes could generate a voltage (chemical cell).	3.0
	b. The production of heat by living organisms depends upon metabolic and reproductive processes. Both of these processes are easily affected by changes in the environment. In addition, abiogenic processes can be initiated by small changes in the environment. For example, a small increase in temperature may supply enough energy to surmount an activation energy barrier for an exothermic chemical reaction.	0
Weighted Score 3.0	Score	3.0

* Numbers refer to weighting factor

** Numbers refer to score awarded

Parameter	Pertinent Information or Factors Considered **	
1. Evolution of Heat, cont'd		
D	a. It is conceivable that the procedure can be applied directly to a crude soil sample.	4
0.7	b. The sensitivity of the method indicates that 3×10^7 dividing cells are required.	1.0
	c. The test does not depend upon the organism undergoing a physiological change, but the heat output is 1000 times greater for dividing organisms.	1.5
Weighted Score 4.55	Score	6.5
E	a. Heat should be evolved continuously, but much larger amounts are liberated during cell division. Therefore, the optimum circumstance may have a long time constant.	1.0
0.7	b. 3×10^7 dividing cells are required.	1.18
	c. The apparatus is sensitive to rate of heat liberation rather than total heat evolved.	0
	d. The function is not observed in a static system.	0
Weighted Score 1.54	Score	2.2
F	a. Heat evolution does not necessarily depend upon atmospheric composition.	5
0.4	b. The Martian temperatures are variable enough to make the selection of an appropriate incubation temperature difficult.	2
Weighted Score 2.8	Score	7

TOTAL WEIGHTED SCORE - 24.5

Parameter	Pertinent Information or Factors Considered **	
2. Change in Heat Content		
A		
0.7	a. The function occurs in all microorganisms, the distribution of which is probably adequate.	2.0
	b. The function is ubiquitous in living forms.	2.0
	c. In order for an adequate change to take place the function requires the utilization of relatively large amounts of substrates which would probably have to be supplied.	0
	d. Life can not exist without thermodynamic changes.	2.0
	e. A loss in heat content can be easily explained by abiogenic processes, but an increase in heat content is less likely to be abiogenic in nature.	1.0
Weighted Score 4.9	Score	7.0
B		
0.7	a. The test is not related to the occurrence of any one particular reactive substance.	2.5
	b. Thermodynamic changes appear to be essential to life.	2.5
	c. The technique tests and overall thermodynamic change.	2.5
	d. Synthesis or degradation of material is not likely without a net change in heat content.	2.5
Weighted Score 7.0	Score	10.0
C		
1.0	a. The technique relies on a voltage developed by a series of thermopiles. It is not likely that other processes will generate a voltage in this system.	3
	b. The quantity to be measured is net synthesis or degradation brought about by the life processes of metabolism and reproduction. These processes are easily altered by environmental changes.	0
Weighted Score 3.0	Score	3

Parameter	Pertinent Information or Factors Considered **	
2. Change in Heat Content, cont'd		
D	a. To make a meaningful observation it is likely that a change in heat content of part of a system will be necessary. For example one might wish to separate large molecules from small molecules and measure the gain or loss of heat content in each category.	0
0.7	b. The sensitivity of the method indicates that a gain or loss of 10^6 cells is required.	1.8
	c. The greatest possibility for observing a change in heat content would probably be in dividing cells, but this would not be a requirement.	1.0
Weighted Score 1.96	Score	2.8
E	a. The overall change depends upon the metabolic rate which could be very slow.	1.0
0.7	b. A gain or loss of 10^6 cell is required.	1.5
	c. The experiment can be designed to detect differences which occur over long periods of time (i.e. growth)	2.5
	d. A dynamic system is necessary.	0
Weighted Score 3.5	Score	5.0
F	a. Changes in heat content of an adapted systems do not depend upon the endogenous atmospheric composition.	5.0
0.4	b. The best results would be obtained with a growing system. The Martian temperatures are variable enough to make selection of an appropriate incubation temperature difficult.	2.0
Weighted Score 2.8		7.0

TOTAL WEIGHTED SCORE - 23.2

Parameter	Pertinent Information or Factors Considered **	
3. Light Emission		
A 0.7	a. Light emitting organisms occur in 40 different orders of the animal kingdom and in the lower plants. The distribution is however largely marine.	0
	b. The function is limited to a small number of organisms.	0
	c. Only endogenous substrates are usually necessary, but the function is often critically dependent upon oxygen, temperature and pH.	1
	d. Light emission often does not perform a function for the organism and some of the supposed functions, such as protection, can be performed better by other methods.	0
	e. Light emission can be produced by the action of ionizing radiation (e.g. from radioisotopes) on phosphors.	0
Weighted Score 0.7	Score	1
B 0.7	a. The test depends upon observing the end-product of a metabolic function rather than an intermediate in the reaction chain.	2.5
	b. The function appears to be of little biological value.	0
	c. The technique tests the overall process.	2.5
	d. The function can be replaced.	0
Weighted Score 3.5	Score	5.0
C 1.0	a. The technique utilizes a photodector which responds to visible light and it is unlikely that other factors will give rise to a response.	5
	b. The emitted light is produced enzymatically and is subject to small changes in the environment. This can produce a false negative result.	0
Weighted Score 5	Score	5
D 0.7	a. The test can be conducted on material <u>in situ</u>.	4
	b. About 5×10^3 organisms are required.	2.55
	c. Light emission does not depend on a physiological change.	3
Weighted Score 6.68	Score	9.55

Parameter	Pertinent Information or Factors Considered **	
E	a. Light emission is often a continuous process and the time constant is therefore quite short.	2.5
0.7	b. About 5×10^3 organisms are required.	2.1
	c. The process depends on the rate rather than the total emission.	0
	d. The process cannot be observed in a static system.	0
Weighted Score 3.22	Score	4.6
F	a. Light emission in terrestrial organisms is oxygen dependent. The oxygen content of the Martian atmosphere is negligible.	0
0.4	b. The Martian temperatures are variable enough to make the selection of an appropriate incubation temperature difficult (light emission in terrestrial forms is temperature dependent).	2
Weighted Score 0.8	Score	2

TOTAL WEIGHTED SCORE - 19.9

4. Unpaired Electrons

A	a. The creation of unpaired electrons by life processes occurs in microorganisms which are widely distributed.	2
0.7	b. All forms of terrestrial life produce unpaired electrons.	2
	c. Endogenous substrates are adequate.	2
	d. Many biochemical reactions appear to be mediated through free radical mechanisms, but chemical reactions can be carried out by other methods.	0
	e. Innumerous non-biological processes produce unpaired electrons.	0
Weighted Score 4.2	Score	6

Parameter	Pertinent Information or Factors Considered**	
4. Unpaired Electrons, cont'd		
B 0.7	a. The test does not depend upon any one particular reactive group.	2.5
	b. The test function is essential to biological oxidations.	2.5
	c. The test measures both overall and individual processes.	2.5
	d. Chemical reaction can be performed by other than free radical mechanisms.	0
Weighted Score 5.25	Score	7.5
C 1.0	. The technique can be made reasonably specific for the detection of unpaired electrons.	3
	. The phenomenon sought is produced by metabolic processes which are sensitive to small changes in the environment.	0
Weighted Score 3.0	Score	3
D 0.7	a. If any degree of specificity is to be achieved a separation of the biological entity is mandatory.	0
	b. The sensitivity of the system requires 10^9 cells.	0.9
	c. A physiological change is not necessary, but more unpaired electrons occur in dying preparations.	2
Weighted Score 2.0	Score	2.9
E 0.7	a. The unpaired electrons are produced by metabolic processes that are essentially continuous, and the measurement is not based on the accumulation of unpaired electrons.	2.5
	b. The sensitivity requires 10^9 organisms.	0.75
	c. Although free radicals can be trapped the possibility of increasing sensitivity by accumulation is remote.	0
	d. The phenomenon requires an active metabolic system.	0
Weighted Score - 2.28	Score	3.25

Parameter	Pertinent Information or Factors Considered **	
4. Unpaired Electrons, cont'd		
F 0.4	a. Studies indicate that free radical production is extremely low in the absence of oxygen.	0
	b. The Martian temperatures are variable enough to make it difficult to select an appropriate incubation temperature for maximum metabolism.	2
Weighted Score 0.8	Score	2
TOTAL WEIGHTED SCORE - 17.5		
5. Bioelectric Potential		
A 0.7	a. Microorganisms produce bioelectric potentials and their distribution is ubiquitous.	2.0
	b. All living systems produce bioelectric potentials.	2.0
	c. The production of a bioelectric potential which can be monitored so as to be interpretable requires the oxidation of a supplied substrate.	0
	d. Electric potentials represent either ion distributions or oxidation-reduction processes both of which appear to be irreplaceable in the mechanics of life.	2.0
	e. Many abiogenic processes produce electrical potentials.	0
Weighted Score 4.2	Score	6
B 0.7	a. The substrate provided may not be capable of being oxidized or reduced by the Martian organism.	0
	b. The maintenance of a bioelectric potential appears to be necessary for living processes.	2.5
	c. The technique tests only the oxidation-reduction potential of the substrates provided.	0
	d. Electric potentials represent either ion distributions or oxidation-reduction processes both of which appear to be irreplaceable in the mechanics of life.	2.5
Weighted Score 3.5	Score	5.0

Parameter	Pertinent Information or Factors Considered **	
5. Bioelectric Potential, cont'd		
C 1.0	a. The test can be designed so that it gives a positive result only for a redox potential.	5
	b. The test depends upon metabolic processes which are easily altered by environmental factors.	0
Weighted Score 5.0	Score	5
D 0.7	a. Many substances, when added to a medium, will cause a change in electric potential.	0
	b. The sensitivity of the method requires about 1.3×10^9 cells.	0.9
	c. The production of a redox potential does not require a physiological change.	3
Weighted Score 2.73	Score	3.9
E 0.7	a. Electrical potentials are generated by continuous metabolic processes, but the bioelectric potential observed is a consequence of the accumulation of a reduced or oxidized specie and if the metabolic rate is very low the length of time to produce the required concentration may be very long.	1.0
	b. The sensitivity of the method requires 1.3×10^7 cells.	0.75
	c. The potential developed is a result of the accumulation of specific chemical species.	2.5
	d. The function can not be observed in a static system.	0
Weighted Score 2.98	Score	4.25
F 0.4	a. The function depends upon oxidation or reduction of substrates and this is not necessarily tied to atmospheric composition (i.e., either anaerobic and aerobic metabolism will suffice).	5.0
	b. The Martian temperatures are variable enough to make it difficult to select an appropriate incubation temperature for maximum metabolism.	2.0
Weighted Score 2.8	Score	7.0

TOTAL WEIGHTED SCORE - 21.2

Parameter	Pertinent Information or Factors Considered **	
6. Metabolism of Radioactive Substrates to $C^{14}O_2$		
A 0.7	a. Microorganisms metabolize C^{14} labeled substrates to $C^{14}O_2$ and they are widely distributed.	2.0
	b. All living systems evolve $C^{14}O_2$ by metabolizing appropriate C^{14} labeled substrates.	2.0
	c. The C^{14} labeled substrate must be selected for incorporation into the incubation medium.	0
	d. The aerobic mechanism for the production of CO_2 can be obviated, but it is difficult to imagine a living system which does not have carboxyl groups and the ability to anaerobically release such groups as CO_2.	2.0
	e. The release of $C^{14}O_2$ from C^{14} labeled compounds can be catalyzed by abiogenic processes.	0
Weighted Score 4.2	Score	6
B 0.7	a. The technique detects an end-product of a biological reaction and does not depend upon characteristics which may not be biologically necessary.	2.5
	b. The evolution of CO_2 either aerobically or anaerobically is a method of carbon chain shortening which appears to be essential in order for the total metabolic cycle to function.	2.5
	c. The technique examines the liberation of $C^{14}O_2$ from only preselected substrates.	0
	d. It is possible that the function could be performed by liberation of CN or CO, but the presence of CO_2 on Mars indicates that CO_2-forming mechanisms are operative.	2.5
Weighted Score 5.25	Score	7.5
C 1.0		
	a. The technique depends upon detection of C^{14} liberated as a gas. This can be made reasonably specific for CO_2 and for the C^{14} radioisotope.	5
	b. The technique depends upon metabolic processes which are easily altered by changes in the environment.	0
Weighted Score 5	Score	5

Parameter	Pertinent Information or Factors Considered **	
D	a. $C^{14}O_2$ evolution can be monitored in relatively crude preparations.	4.0
0.7	b. The sensitivity of the method requires 7.5×10^3 cells.	2.5
	c. The rate of CO_2 evolution may be altered by physiological change, but such changes are not requisite to its evolution (in bacteria CO_2 evolution in non-dividing organisms is only a fraction of that in dividing populations).	3.0
Weighted Score 6.65	Score	9.5
E	a. The function depends upon a metabolic process and the metabolism may be quite slow.	1.0
0.7	b. The sensitivity of the method requires 7.5×10^3 cells.	2.0
	c. The technique can be based on total accumulated $C^{14}O_2$ or on rate of $C^{14}O_2$ release.	2.5
	d. The system must actively metabolize.	0
Weighted Score 3.85	Score	5.5
F	a. The lack of oxygen on Mars indicates that the aerobic system may be subservient to anaerobic metabolism and substrates labeled in positions designed to take advantage of a supposed aerobic processes may be ineffective.	3.0
0.4	b. The Martian temperatures are variable enough to make selection of a proper incubation temperature difficult.	2.0
Weighted Score 2.0	Score	5.0

TOTAL WEIGHTED SCORE - 26.9

Parameter		Pertinent Information or Factors Considered **	
7. Dark Fixation of $C^{14}O_2$			
A		a. Both micro- and macroorganisms perform the function.	2.0
0.7		b. All terrestrial organisms fix CO_2.	2.0
		c. The function does not depend upon reaction with specific substances supplied exogenously.	2.0
		d. Carbon could be fixed by Martian organisms as some compound other than CO_2 (e.g. CO, CS_2, CH_4 etc.)	0
		e. Many abiogenic processes will fix CO_2.	0
Weighted Score	4.2	Score	6.0
B		a. The test depends upon observing the end result of a function and is therefore not influenced by the details of the process.	2.5
0.7		b. The function provides a valuable mechanism for changing the carbon chain length.	2.5
		c. The technique tests the overall process of carbon chain lengthening.	2.5
		d. Carbon fixation can be accomplished by fixing compounds other than CO_2 (e.g. CO, CS_2, CH_4, etc.)	0
Weighted Score	5.25	Score	7.5
C		a. The technique monitors radioactivity above background and it can be made reasonably specific for $C^{14}O_2$.	5
1.0		b. The technique depends upon metabolic processes which are easily altered by changes in the environment.	0
Weighted Score	5.0	Score	5
D		a. If the material is combusted to CO_2 at the end of the experiment no separation is required.	4.0
0.7		b. The sensitivity requires 2.5×10^2 cells.	2.7
		c. The rate of $C^{14}O_2$ fixation may be altered by physiological change, but such changes are not requisite to the function.	3.0
Weighted Score	6.8	Score	9.7

Parameter	Pertinent Information or Factors Considered **	
7. Dark Fixation of $C^{14}O_2$, cont'd		
E 0.7	a. The function depends upon a metabolic process and the metabolism may be quite slow.	1.0
	b. The sensitivity of the method requires 2.5×10^2 cells.	2.25
	c. The technique is based on <u>total</u> fixation of $C^{14}O_2$ and is therefore time dependent.	2.5
	d. Active metabolism must occur.	0
Weighted Score 4.0	Score	5.75
F 0.4	a. The Martian atmosphere contains appreciable amounts of CO_2 and this is compatible with a CO_2 fixation function.	5.0
	b. The function depends on metabolic processes and the variation in Martian temperatures is great enough to make selection of the proper incubation temperature difficult.	2.0
Weighted Score 2.8	Score	7.0
TOTAL WEIGHTED SCORE - 23.0		
8. Light Stimulated Fixation of $C^{14}O_2$		
A 0.7	a. Photosynthetic organisms are distributed primarily in areas exposed to light for considerable periods.	0
	b. A large number of terrestrial forms do not carry out photosynthesis.	0
	c. The function does not dep end upon reaction with specific substances supplied exogenously.	2
	d. Carbon could be fixed from sources other than CO_2 (CO, CS_2, CH_4,etc.)	0
	e. Light stimulated CO_2 fixation is not readily imitated by abiogenic processes.	2
Weighted Score 2.8	Score	4
B 0.7	a. The test observes the results of an overall processes and therefore it does not depend upon the detailed steps involved.	2.5
	b. The function is essential for terrestrial life as the primary mode of converting energy and lengthening carbon chains.	2.5
	c. The technique tests the overall mechanism of carbon dioxide fixation.	2.5
	d. Carbon fixation could be mediated by fixation of carbon compounds other than CO_2 (e.g. CO, CS_2, CH_4, etc.)	0
Weighted score 5.25	Score	7.5

Parameter	Pertinent Information or Factors Considered **	
8. Light Stimulated Fixation of $C^{14}O_2$, cont'd		
C 1.0	a. The technique can be made reasonably specific for $C^{14}O_2$ liberated by combustion of the product obtained.	5.0
	b. The technique depends upon metabolic processes which are easily altered by changes in the environment.	0
Weighted Score 5.0	Score	5.0
D 0.7	a. If the material obtained in the fixation processes is combusted to CO_2 at the end of the experiment no separation is required.	4.0
	b. The sensitivity requires 4 cells.	3.0
	c. A physiological change is not a requisite.	3.0
Weighted Score 7.0	Score	10.0
E 0.7	a. The function depends upon metabolic processes which may be slow.	1.0
	b. The sensitivity requires 4 cells.	2.5
	c. The technique is based on total $C^{14}O_2$ fixation.	2.5
	d. Active metabolism must occur.	0
Weighted Score 4.2	Score	6.0
F 0.4	a. The Martian atmosphere contains an appreciable amount of CO_2 and this is compatible with light stimulated CO_2 fixation.	5.0
	b. The function depends on metabolic processes and the variation in Martian temperature is great enough to make selection of the proper incubation temperature difficult.	2.0
Weighted Score 2.8	Score	7.0
TOTAL WEIGHTED SCORE - 27.0		

Parameter	Pertinent Information or Factors Considered **	
9. Detection of ATP - Enzymic Method	(If the sterilization requirements are not modified this experiment cannot be carried out.)	
A	a. ATP is found in microorganisms and they are widely distributed.	2.0
0.7	b. ATP is found in all terrestrial life.	2.0
	c. The method requires reaction with an enzyme under specified conditions.	0
	d. ATP could be replaced by a number of substances which are able to store energy as high energy bonds and couple this energy to synthesis. For example, UTP or high energy sulfate bonds.	0
	e. ATP has been easily produced by abiogenic processes.	0
Weighted Score 2.8	Score	4.0
B	a. The high energy bond is an intergal part of the ATP and this bond is responsible for the light emission phenomenon.	2.5
0.7	b. ATP supplies the energy currency for biological synthesis and is indispensible to terrestrial life.	2.5
	c. The technique depends upon the overall function of the enzyme system.	2.5
	d. ATP could be replaced by a number of substances which are able to store energy as high energy bonds and couple this energy to synthesis. For example, UTP or high energy sulfate bonds.	0
Weighted Score 5.25	Score	7.5
C		
1.0	a. The technique is essentially specific and substances known to react, such as UTP, are also of interest.	5
	b. The technique is based on an enzymatic reaction and such reactions are sensitive to environmental changes.	0
Weighted Score 5.0	Score	5
D	a. The test can in many instances be applied to relatively crude preparations.	4.0
0.7	b. 1.2×10^5 cells are required for a positive response.	2.1
	c. A physiological change is not required.	3.0
Weighted Score 6.4		9.1

Parameter	Pertinent Information or Factors Considered **	
9. Detection of ATP - Enzymic, cont'd		
E	a. The time constant is not likely to vary with the source of the ATP.	2.5
0.7	b. 1.2×10^5 cells are required for a positive result.	1.75
	c. The technique depends upon the rate of light production not the total light produced.	0
	d. ATP can be obtained from static systems.	2.5
Weighted Score 4.72	Score	6.75
F	a. ATP can be formed anaerobically or aerobically so a lack of oxygen is not a factor.	5.0
0.4	b. The temperature requirements for the reaction are known and can be applied without regard to the Martian temperature.	5.0
Weighted Score 4.0		10.0
TOTAL WEIGHTED SCORE - 28.2		
10. Detection of ATP - Chemical (Barium separation and phosphorus analysis) - Only questions which require answers different from those in 9 are considered.		
A 0.7	c. The technique requires an analysis for organic phosphorus	0
Weighted Score 2.8	Score	4
B	a. The test analyzes for organic phosphorus and the phosphate group is an intergal part of the functional properties of ATP.	2.5
0.7	c. The technique analyzes directly for organic phosphorus and therefore it does not depend upon an indirect procedure, the details of which are a function of a Martian life form.	2.5
Weighted Score 5.25	Score	7.5
C	a. A positive result will be obtained for any organic phosphorus present in the fraction analyzed.	0
1.0	b. Small changes in the environment do not interfere with qualitative tests for phosphorus.	5.0
Weighted Score 5.0	Score	5.0

Parameter	Pertinent Information or Factors Considered **	
10. Detection of ATP - Chemical (Barium separation and phosphorus analysis) - Only questions which require answers different from those in 9 are considered. CONT'D		
D 0.7 Weighted Score 2.52	a. The method requires separation of organic phosphorus from the sample and fractionation of the organic phosphorus. b. 1.2×10^{10} cells are required for a positive response. Score	0 0.6 3.6
E 0.7 Weighted Score 3.85	b. 1.2×10^{10} cells are required for a positive response. c. The technique depends upon an analysis for total ATP, but in terrestrial organisms ATP does not accumulate to an appreciable extent. Score	0.5 0 5.5
F 0.4 Weighted Score 4.0	Score	10
TOTAL WEIGHTED SCORE - 22.4		

Parameter	Pertinent Information or Factors Considered **	
11. Detection of ATP - Ion Exchange.	Only questions which require answers different from those in 10 are considered.	
A 0.7 Weighted Score 2.8	a. The technique requires adsorption and elution from an ion exchange resin plus analysis for organic phosphorus.	0 Score 4
B 0.7 Weighted Score 5.25	c. The technique is a direct analysis for ATP and therefore it does not depend upon how the ATP is produced.	2.5 Score 7.5
C 1.0 Weighted Score 7.5	a. A positive result will be obtained for any organic phosphorus present in the fraction analyzed, but the separation process is relatively specific and therefore more reliable than the chemical procedure.	2.5 Score 7.5
D 0.7 Weighted Score 2.59	b. 6×10^9 cells are required for a positive response.	0.7 Score 3.7
E 0.7 Weighted Score 3.92	b. 6×10^9 cells are required for a positive result.	0.6 Score 5.6
F 0.4 Weighted Score 4.0		Score 10

TOTAL WEIGHTED SCORE - 26.0

Parameter		Pertinent Information or Factors Considered **	
12. Detection of Porphyrins - Photosynthetic Pigments Only			
	A	a. Photosynthetic organisms are distributed primarily in areas exposed to light for considerable periods.	0
	0.7	b. A large number of terrestrial organisms do not carry on photosynthesis.	0
		c. The technique employs a direct assay for fluorescence which does not depend upon interaction with preselected chemicals.	2
		d. Other substance can perform the functions carried out by porphyrins (i.e. electron transport, light absorption and catalysis).	0
		e. Porphyrins could be produced abiogenically, but this appears to be a more difficult task than, for example, the synthesis of ATP.	1
Weighted Score	2.1	Score	3
	B	a. The technique depends upon the fluorescence of porphyrins and the electronic properties of the porphyrins which permit fluorescence are the same ones which allow the porphyrins to perform their physiological functions.	2.5
	0.7	b. The porphyrins perform physiological functions which are essential to terrestrial life.	2.5
		c. The technique analyses directly for phorphyrins rather than an intermediate product.	2.5
		d. Other substance can perform the functions carried out by porphyrins (i.e. electron transport, light absorption and catalysis).	0
Weighted Score	5.25	Score	7.5
	C		
	1.0	a. The technique is reasonably specific if the excitation wavelength is specified and the emission region scanned.	3
		b. Small changes in the environment do not affect the qualitative results.	5
Weighted Score	8.0	Score	8
	D	a. Only a simple extraction is required.	3
		b. The sensitivity of the method requires 5×10^4 cells.	2.25
	0.7	c. A physiological change in the organism is not required.	3
Weighted Score	- 5.78	Score	8.25

Parameter	Pertinent Information or Factors Considered **	
E 0.7	a. The technique can be applied to a static system. b. The sensitivity requires 5×10^4 cells. c. Porphyrins do not accumulate with time in non-dividing cells. d. The technique analyses directly for total porphyrins and does not depend upon metabolic functions.	2.5 1.87 0
Weighted Score 4.8	Score	2.5 6.87
F 0.4	a. Porphyrins function in both anaerobic and aerobic organisms. b. The Martian temperatures are not incompatible with the stability and the accumulation of porphyrins.	5
Weighted Score 4.0	Score	5 10

TOTAL WEIGHTED SCORE - 28.9

13. Detection of Porphyrins Other than Photosynthetic Pigments. Only questions which require answers different from those in 12 are considered.

A 0.7	a. All microorganisms contain porphyrins and the distribution of microorganisms is ubiquitous. b. The prophyrins perform a number of functions at least one of which is exhibited by every organism.	2.0
Weighted Score 4.9	Score	2.0 7.0
B 0.4		
Weighted Score 5.25	Score	7.5

Parameter	Pertinent Information or Factors Considered **		
13. Detection of Porphyrins Other than Photosynthetic Pigments. Only questions which require answers different from those in 12 are considered, CONT'D			
C 1.0 Weighted Score 8.0		Score	8.0
D 0.7 Weighted Score 5.17	b. The sensitivity of the method requires 4 x 10^7 cells.		1.38
		Score	7.38
E 0.7 Weighted Score 4.3	b. The sensitivity of the method requires 4 x 10^7 cells.		1.15
		Score	6.15
F 0.4 Weighted Score 4		Score	10

TOTAL WEIGHTED SCORE - 31.6

Parameter	Pertinent Information or Factors Considered **	
14. Light Stimulated Evolution of Oxygen - Tracer (O^{18}) Method		
A	a. Many organisms do not evolve oxygen when exposed to light.	0
0.7	b. The function occurs only in photosynthetic organisms which utilize water as the reducing agent.	0
	c. The function proceeds by use of materials present in the environment and the addition of H_2O^{18}.	1.5
	d. The function can use substances other than water for reduction without the liberation of oxygen.	0
	e. Light stimulated evolution of oxygen can be accomplished abiogenically by the decomposition of peroxides or even CO_2 under appropriate conditions.	0
Weighted Score 1.05	Score	1.5
B	a. The test depends upon water acting as a reducing agent, but other substances can perform the function.	0
0.7	b. Photosynthesis provides the principle mechanism of energy conversion in terrestrial organisms.	2.5
	c. The technique tests the overall photosynthetic process when water is the reducing agent.	2.5
	d. There are other modes of photosynthetic reduction which do not liberate oxygen.	0
Weighted Score 3.5	Score	5
C		
1.0	a. The technique detects a mass of 18, substances other than O^{18} have this mass, but the number is reasonably limited.	5.0
	b. The test procedure depends upon a metabolic function which can be materially affected by small changes in the environment.	0
Weighted Score 5.0	Score	5.0
D	a. The test can be conducted with a crude preparation.	4
0.7	b. The sensitivity of the method requires 45 cells.	3
	c. A physiological change is not a prerequisite to photosynthesis.	3
Weighted Score 7	Score	10

Parameter	Pertinent Information or Factors Considered **	
E 0.7	a. Photosynthesis, in the presence of light, generally does not depend upon a function which has a long time constant. However, desiccation may result in a relatively long period for rehydration during which time photosynthesis would not be apparent. Desiccation on Mars is a highly probably situation.	1.0
	b. The sensitivity of the method requires 45 cells.	2.5
	c. The experiment can measure the total O^{18} produced in a given time period.	2.5
	d. The system must actively metabolize.	0
Weighted Score 4.2	Score	6.0
F 0.4	a. If active photosynthesis with the liberation of oxygen occurs on Mars one would expect to find a considerable amount of oxygen in the atmosphere. The oxygen concentration is at the limit of detection.	0
	b. The technique requires that the organism is actively metabolizing. The Martian temperatures are variable enough to make the selection of an appropriate incubation temperature difficult.	2
Weighted Score 0.8	Score	2

TOTAL WEIGHTED SCORE - 21.5

Parameter	Pertinent Information or Factors Considered **		
15. Light Stimulated Evolution of Oxygen - Cartesian diver method. Only question which require answers different from 14 are considered.			
A 0.7 Weighted Score 1.4	c. No exogenous materials need be added		2.0
		Score	2.0
B 0.7 Weighted Score 3.5		Score	5.0
C 1.0 Weighted Score 3.0	a. The technique detects the evolution of a gas. It is conceivable that a gas other than oxygen could be released by light stimulation.		3.0
		Score	3.0
D 0.7 Weighted Score 7.0	b. The sensitivity of the method requires 22 cells.		3.0
		Score	10
E 0.7 Weighted Score 4.2	b. The sensitivity of the method requires 22 cells.		
		Score	6.0
F 0.4 Weighted Score 0.8		Score	2.0

TOTAL WEIGHTED SCORE - 19.9

Parameter	Pertinent Information or Factors Considered **		
16. Light Stimulated Oxygen Evolution - Micro-respirometry. Only questions which require answers different from 15 are considered.			
D	b. The sensitivity of the method requires 2.2×10^3 cells.		2.55
0.7			
Weighted Score 6.65		Score	9.5
E	b. The sensitivity of the method requires 2.2×10^3 cells.		2.1
0.7			
Weighted Score 3.92		Score	5.6
TOTAL WEIGHTED SCORE - 19.4			
17. Light Stimulated Oxygen Evolution - Oxygen Electrode. Only questions which require answers different from 16 are considered.			
D	b. The sensitivity of the method requires 2.2×10^5 cells.		1.95
0.7			
Weighted Score 6.26		Score	8.95
E	b. The sensitivity of the method requires 2.2×10^5 cells.		1.6
0.7			
Weighted Score 3.57		Score	5.1
TOTAL WEIGHTED SCORE - 18.7			

Parameter	Pertinent Information or Factors Considered **	
18. Light Stimulated Oxygen Evolution - Magnetic Susceptibility. Only questions which require answers different from 15 are considered.		
C 1.0	a. The method depends upon the evolution of a gas which is magnetically susceptible. The only gas which could be confused with oxygen in this regard is nitrogen dioxide (NO)	5.0
Weighted Score 5.0	Score	5.0
D 0.7	b. The sensitivity of the method requires 9×10^3 cells.	2.4
Weighted Score 6.7	Score	9.6
E 0.7	b. The sensitivity of the method requires 9×10^3 cells.	2.0
Weighted Score 3.85	Score	5.5
TOTAL WEIGHTED SCORE - 22.2		
19. Uptake of Oxygen - Cartesian Diver.		
A 0.7	a. Most microorganisms can utilize oxygen and microorganisms are widely distributed.	1.0
	b. There are a considerable number of organisms which are strict anaerobes.	0
	c. Endogenous substrates can be utilized.	2.0
	d. Substances other than oxygen can and do serve as terminal electron acceptors.	0
	e. Many abiogenic reactions can mimic biological oxygen uptake.	0
Weighted Score 2.1	Score	3.0
B 0.7	a. The test depends upon the utilization of oxygen as a terminal acceptor in the oxidation chain and therefore the method by which the oxygen is utilized is not relative.	0
	b. In most terrestrial organisms the normal terminal acceptor in the oxidation chain is oxygen and therefore it is biologically valuable.	2.5
	c. The technique tests the terminal step in the oxidation chain.	2.5
	d. The function of terminal electron acceptor is often performed by substances other than oxygen.	0
Weighted Score 3.5	Score	5.0

Parameter	Pertinent Information or Factors Considered **	
19. Uptake of Oxygen - Cartesian Diver, Cont'd		
C 1.0	a. Many abiogenic systems take up oxygen.	0
	a. The technique measures the uptake of gases which are not soluble in alkali. Gases other than oxygen fit this description.	3
	b. The method depends upon a metabolic process which can be materially affected by small changes in the environment.	0
Weighted Score 3	Score	3
D 0.7	a. A relatively crude preparation can be employed.	4
	b. The sensitivity of the method requires 1.5×10^3 cells.	2.6
	c. A physiological change is not a requirement.	3
Weighted Score 6.72	Score	9.6
E 0.7	a. Oxygen uptake is usually a continuous function which does not depend upon a periodic variable with a long time constant.	2.5
	b. The sensitivity of the method requires 1.5×10^3 cells.	1.92
	c. The technique can be designed to be a function of the total change during a long time period.	2.5
	d. The function is related to metabolism and can not be observed in a static system.	0
Weighted Score 4.84	Score	6.92
F 0.4	a. The oxygen content of the Martian atmosphere is at the limit of detection and oxidation based on oxygen as the terminal electron acceptor would not be likely.	0
	b. The Martian temperatures are variable enough to make the selection of an incubation temperature difficult.	2
Weighted Score 0.8	Score	2

TOTAL WEIGHTED SCORE - 21.0

Parameter	Pertinent Information or Factors Considered **	
20. Uptake of Oxygen - Microrespirometry (Only questions which require answers different from those in 19 are considered.)		
D 0.7 Weighted Score 6.48	b. The sensitivity of the method requires 1.5×10^4 cells.	2.25
	Score	9.25
E 0.7 Weighted Score 4.82	b. The sensitivity of the method requires 1.5×10^4 cells.	1.88
	Score	6.88
TOTAL WEIGHTED SCORE - 20.7		
21. Uptake of Oxygen - Oxygen Electrode (Only questions which require answers different from those in 19 are considered)		
C 1.0 Weighted Score 0	a. The technique measures a change in potential which can be produced by processes other than a change in PO_2.	0
	Score	0
D	b. The sensitivity of the method requires 1.5×10^7 cells.	1.35
Weighted Score - 5.81	Score	8.3
E 0.7 Weighted Score 4.28	b. The sensitivity of the method requires 1.5×10^7 cells.	1.12
	Score	6.12
TOTAL WEIGHTED SCORE - 15.7		

Parameter	Pertinent Information or Factors Considered **	
22. Uptake of Oxygen - Magnetic susceptibility (Only questions which require answers different from those in 19 are considered.)		
C 1.0	b. The method depends upon the uptake of a gas which is magnetically susceptible. The only gas which could be confused with oxygen in this regard is nitrogen dioxide (NO)	5.0
Weighted Score 5.0	Score	5.0
D 0.7	b. The sensitivity of the method requires 6×10^5 cells.	1.95
Weighted Score - 6.1	Score	8.95
E 0.7	b. The sensitivity of the method requires 6×10^5 cells	1.62
Weighted Score 4.53	Score	6.62
TOTAL WEIGHTED SCORE - 22.0		
23. Carbon Dioxide Evolution - Detected by a change in conductivity.		
A 0.7	a. Microorganisms evolve CO_2 and they are widely distributed.	2
	b. All terrestrial life forms evolve CO_2.	2
	c. Endogenous substrates can serve as the source of CO_2.	2
	d. CO_2 evolution is a method of shortening carbon chains following dehydrogenation. It is possible that carbon chains can be shortened by liberation of CO (accumulates in macrocystis) or CN (action of mandelonitrilase).	0
	e. CO_2 can be evolved by abiogenic processes (e.g. the slow action of acids on carborates)	0
Weighted Score 4.2	Score	6

Parameter	Pertinent Information or Factors Considered **	
23. Carbon Dioxide Evolution - Detected by a change in conductivity, cont'd		
B 0.7	a. The technique detects an end-product of a biological reaction rather than an intermediate in the reaction chain.	2.5
	b. The evolution of CO_2 either aerobically or anaerobically is a method of carbon chain shortening which appears to be essential in order for the total metabolic cycle to function.	2.5
	c. The technique examines the liberation of CO_2 from all endogenous substrates.	2.5
	d. It is possible that the function could be performed by liberation of CO or CN, but the presence of CO_2 on Mars indicates that the CO_2 mechanism is probable.	2.5
Weighted Score 7.0	Score	10
C 1.0	a. The technique depends on a gas separation process followed by absorption in alkaline medium. This makes the method reasonably specific.	5
	b. The technique depends upon metabolic processes which are easily altered by changes in the environment.	0
Weighted Score 5.0	Score	5
D 0.7	a. CO_2 evolution can be monitored in relatively crude preparations.	4.0
	b. The sensitivity of the method requires 1.5×10^7 cells.	1.35
	c. The rate of CO_2 evolution may be altered by physiological change, but such changes are not requisite to its evolution.	3.0
Weighted Score 5.84	Score	8.35

Parameter	Pertinent Information or Factors Considered **	
23. Carbon Dioxide Evolution - Detected by a change in conductivity, cont'd		
E 0.7	a. CO_2 evolution depends upon a metabolic process and the metabolism may be quite slow.	1.0
	b. The sensitivity of the method requires 1.5×10^7 cells.	1.12
	c. The technique can be based on total CO_2 collected over a period of time.	2.5
	d. The system must actively metabolize.	0
Weighted Score 3.23	Score	4.62
F 0.4	a. The lack of oxygen on Mars would not be a factor in the liberation of CO_2 from endogenous substrates.	5.0
	b. The Martian temperatures are variable enough to make selection of a proper incubation temperature difficult.	2.0
Weighted Score 2.8	Score	7.0
TOTAL WEIGHTED SCORE - 28.1		
24. Carbon Dioxide Evolution - Cartesian Diver (Only questions which require answers different from those in 23 are considered).		
C 1.0	a. The technique depends upon a differential change in pressure as a result of CO_2 evolution and presumably O_2 uptake in the presence and absence of an alkaline solution. This is not a very specific method when applied to an unknown system.	0
Weighted Score 0	Score	0
D 0.7	b. The sensitivity of the method requires 1.5×10^4 organisms.	2.25
Weighted Score 6.48	Score	9.25
F 0.7	b. The sensitivity of the method requires 1.5×10^4 cells.	1.87
Weighted Score 3.76	Score	5.37
TOTAL WEIGHTED SCORE - 24.2		

Parameter	Pertinent Information or Factors Considered **		
25. Carbon Dioxide Evolution -	Microrespirometry (Only questions which require answers different from those in 24 are considered.)		
D 0.7 Weighted Score 6.16	b. The sensitivity of the method requires 1.5×10^5 cells.		1.95
		Score	8.95
E 0.7 Weighted Score 3.58	b. The sensitivity of the method requires 1.5×10^5 cells.		1.62
		Score	5.12
TOTAL WEIGHTED SCORE - 23.7			
26. Carbon Dioxide Evolution -	CO_2 Electrode (Only questions which require answers different from those in 23 are considered.)		
C 1.0 Weighted Score 0	a. The technique measures a change in potential which can be produced by processes other than a change in pCO_2.		0
		Score	0
D 0.7 Weighted Score 5.74	b. The sensitivity of the method requires 1.1×10^8 cells.		1.2
		Score	8.20
E 0.7 Weighted Score - 3.15	b. The sensitivity of the method requires 1.1×10^8 cells.		1.0
		Score	4.50
TOTAL WEIGHTED SCORE - 22.9			

Parameter	Pertinent Information or Factors Considered **		
27. Carbon Dioxide Evolution -	CO_2 sorption on piezoelectric crystal. (Only questions which require answers different from those in 23 are considered.)		
C 1.0	a. The technique depends upon selective absorption of CO_2 in materials coated on piezoelectric crystals. The specificity of the method depends upon the selectivity of the coating for CO_2. So far, coating materials which are highly selective for CO_2 have not been developed. However gas separation by molecular sieves prior to detection could be used.		1.5
Weighted Score 1.5			1.5
D 0.7	b. The sensitivity of the method requires 7.5×10^3 cells.		2.48
Weighted Score 6.64		Score	9.48
E 0.7	b. The sensitivity of the method requires 7.5×10^3 cells.		2.06
Weighted Score 3.89		Score	5.56

TOTAL WEIGHTED SCORE - 26.0

II. PRESENCE OF MOLECULAR AGGREGATES

Molecular aggregates are always associated with life as we know it on earth. These aggregates can be grouped into separate classes and specific methods employed to detect these substances or factors associated with the nature of their monomeric units. However, macromolecules can also be detected by virtue of their more general properties such as size and presence of multiple anionic and/or cationic sites. Since the methods used to detect such general properties do not depend upon a prior knowledge of the chemical composition of the macromolecular aggregate they can be successfully applied to materials which are chemically different from the molecular aggregates found in terrestrial life.

For the methods considered in this category it was assumed that a physical separation of large from small molecules prior to conducting the test would be possible. Possible sizing methods include: dialysis against carbowax or water, ultrafiltration, gel filtration and ultracentrifugation.

II. A. DETECTION OF SPECIFIC MACROMOLECULES - A physical separation of large molecules from small molecules prior to conducting the test is assumed in each case.

1. UV Absorption Spectrophotometry - Assume molecule is DNA and absorbance is measured in the 260 mμ region of the spectrum.

Parameter	Pertinent Information or Factors Considered **	
A 0.7	a. Microorganisms contain DNA and they are widely distributed.	2.0
	b. All living organisms contain DNA	2.0
	c. UV absorption is an intrinsic property of DNA.	2.0
	d. It is possible that the function of DNA could be carried out by a different substance.	0
	e. Substances which have UV spectra similar to DNA can be produced by the action of polymerase on nucleotides, but the polymerase is obtained from a living system.	2.0
Weighted Score 5.6	Score	8.0
B 0.7	a. The UV absorbance is due to the purine and pyrimidine moieties and these are essential to the function of DNA.	2.5
	b. DNA is largely responsible for information transfer and storage in living systems and this is a necessary biological property.	2.5
	c. The technique searches directly for macromolecules which give a maximum in the 260 mμ region of the spectrum.	2.5
	d. It is possible that the function of DNA could be carried out by a different substance.	0
Weighted Score 5.25	Score	7.5
C 1.0	a. The technique depends upon the detection of a maximum in the 260 mμ region of the spectrum and many polymers absorb in this region.	0
	b. Rather large changes in temperature, pH, etc. must occur before DNA is degraded to an unrecognizable product.	5
Weighted Score 5	Score	5
D 0.7	a. It appears to be necessary to at least separate large molecules from small ones.	0
	b. The sensitivity of the method requires 2×10^7 cells.	1.4
	c. A physiological change is not requisite for the detection of DNA.	3
Weighted Score 3.08	Score	4.4

Parameter	Pertinent Information or Factors Considered **	
E	a. A dynamic biological function is not involved in the detection of DNA and, therefore, a time constant is not applicable.	2.5
0.7	b. The sensitivity of the method requires 2×10^7 cells.	1.2
	c. Only if it is assumed that replication can be induced.	0
	d. DNA can be detected in a metabolically inactive system.	2.5
Weighted Score 4.3	Score	6.2
F	a. The atmospheric composition of Mars is not incompatible with the existence of DNA.	5.0
0.4	b. The Martian temperatures do not make the accumulation of DNA unreasonable.	5.0
Weighted Score 4	Score	10.0

TOTAL WEIGHTED SCORE - 30.2

2. UV Absorption Spectrophotometry - Assume molecule is protein and absorbance is measured in the 280 mμ region of the spectrum.

A	a. Microorganisms contain protein and they are widely distributed.	2.0
0.7	b. All living organisms contain protein.	2.0
	c. The detection method depends upon the absorbance of aromatic amino acids present in the protein. It is conceivable that proteins without aromatic amino acids exist on Mars.	0
	d. The proteins are essential to so many facets of life that it is difficult to see how they could be replaced in every case and still have a system which is recognized as living.	2
	e. Protein-like compounds have been produced by abiogenic methods.	0
Weighted Score 4.2	Score	6

Parameter	Pertinent Information or Factors Considered **	
2. UV Absorption Spectrophotometry (continued)	Assume molecule is protein and absorbance is measured in the 280 mμ region of the spectrum.	
B 0.7	a. The test depends upon absorption by aromatic amino acids residues - and these may not be necessary for the protein to function.	0
	b. Proteins are essential to life as structural substances, enzymes, hormones, etc.	2.5
	c. The technique tests only for proteins which contain aromatic amino acids.	0
	d. It is possible that proteins which do not contain aromatic residues could function in Martian life, but this situation (with the possible exception of rigorously purified gelatin) does not apply to terrestrial biologically formed proteins.	1.5
Weighted Score 2.8	Score	4.0
C 1.0	a. There are many polymeric substances other than protein which absorb in the 280 mμ region of the spectrum.	0
	b. Absorption at 280 mμ is not materially altered by changes induced in proteins due to small environmental changes.	5
Weighted Score 5	Score	5
D 0.7	a. It appears to be necessary to at least separate large molecules from smaller ones.	0
	b. The sensitivity of the method requires 1×10^7 cells.	1.5
	c. A physiological change is not necessary for the detection of protein.	3.0
Weighted Score 3.15	Score	4.5
E 0.7	a. A biological time constant is not a factor in the detection of protein.	2.5
	b. The sensitivity of the method requires 1×10^7 cells.	1.25
	c. Protein can accumulate in a non-dividing cell during a time period, but it also may decrease and in any event the usual change is too small to affect the probability that protein will be detected. For a time period to be advantageous it must be assumed that reproduction will occur.	0
	d. Protein can be detected in a metabolic inactive system.	2.5
Weighted Score 4.38	Score	6.25

Parameter	Pertinent Information or Factors Considered **	
F 0.4	a. The atmospheric composition of Mars is not incompatible with the existence of protein.	5.0
	b. The Martian temperatures do not make the accumulation of protein unreasonable.	5.0
Weighted Score 4.0	Score	10.0

TOTAL WEIGHTED SCORE - 23.5

3. UV Absorption Spectrophotometry - Assume molecule is protein and absorbance is measured in the 190 mμ region of the spectrum. Only questions which require answers different from 2 are considered.

A 0.7	c. The detection method depends upon the absorbance of peptide bonds present in the protein. Proteins can not exist without peptide bonds.	2.0
Weighted Score 5.6	Score	8.0
B 0.7	a. The test depends upon absorption by peptide bonds and proteins cannot exist without peptide linkages.	2.5
	c. The technique detects peptide bonds which are essential to all proteins.	2.5
	d. The proteins are essential to so many facets of life that it is difficult to see how they could be replaced in every case and still have a system which is recognised as living. The technique searches for peptide bonds which are essential to all proteins.	2.5
Weighted Score 7.0	Score	10.0
C 1.0	a. There are many polymeric substances which absorb in the 190 mμ region of the spectrum.	0
	b. Absorption at 190 mμ is not materially altered by changes induced in proteins due to small environmental changes.	5
Weighted Score 5	Score	5
D 0.7	b. The sensitivity of the method requires 1×10^6 cells.	1.8
Weighted Score 3.36	Score	4.8
F 0.7	b. The sensitivity of the method requires 1×10^6 cells.	1.5
Weighted Score 4.55	Score	6.5

TOTAL WEIGHTED SCORE - 29.5

Parameter	Pertinent Information or Factors Considered **	
4. Visible Absorption Spectrophotometry. Assume molecule is protein and absorbance is measured from 400 to 700 mμ.		
A 0.7	a. Proteins are present in microorganisms and they are widely distributed.	2.0
	b. All terrestrial life forms contain proteins.	2.0
	c. The detection of proteins by this method requires interaction with dyes.	0
	d. The proteins are essential to so many facets of life that it is difficult to see how they could be replaced in every case and still have a system which is recognized as living.	2.0
	e. Protein-like compounds which react with dyes have been produced abiogenically.	0
Weighted Score 4.2	Score	6.0
B 0.7	a. The test depends upon the presence of anionic or cationic sites and these are essential for protein functions.	2.5
	b. Proteins in their role as structural substances, enzymes, hormones, etc. are essential for life.	2.5
	c. The technique is general, rather than specific and relies only on the presence of charged sites.	2.5
	d. It does not seem possible that macromolecules which do not possess anionic or cationic sites can replace proteins.	2.5
Weighted Score 7.0	Score	10
C 1.0	a. The technique can be designed to distinguish protein-like macromolecules from other macromolecules.	3
	b. Fairly large changes in pH can alter the results.	2
Weighted Score 5	Score	5
D 0.7	a. It appears necessary to at least separate large molecules from smaller ones.	0
	b. The sensitivity of the method requires 10^7 cells.	1.5
	c. A physiological change is not necessary for the detection of macromolecules.	3
Weighted Score 3.15	Score	4.5

Parameter	Pertinent Information or Factors Considered **	
E 0.7	a. A biological time constant is not a factor in the detection of protein.	2.5
	b. The sensitivity of the method requires 10^7 cells.	1.25
	c. Protein can accumulate in a non-dividing cell during a time period, but it also may decrease and in any event the usual change is too small to affect the probability that protein will be detected. For a time period to be advantageous it must be assumed that reproduction will occur.	0
	d. Protein can be detected in a metabolically inactive system.	2.5
Weighted Score 4.38	Score	6.25
F 0.4	a. The atmospheric composition of Mars is not incompatible with the existence of protein.	5.0
	b. The Martian temperatures do not make the accumulation of protein unreasonable.	5.0
Weighted Score 4.0	Score	10.0

TOTAL WEIGHTED SCORE - 27.7

5. Visible Absorption Spectrophotometry. Assume molecule is DNA and absorbance is measured from 400 to 700 mμ. Only questions which require answers different from IIA1 are considered.

A 0.7	c. The detection of DNA by this method requires reaction with a dye.	0
	e. Substances which cause changes in dye spectra which are essentially the same as those obtained by interaction with DNA can be produced abiogenically.	0
Weighted Score 4.2	Score	6.0
B 0.7	a. The interaction of dyes with DNA is largely a function of the phosphate group which is an indispensible part of DNA.	2.5
	c. The technique searches for macromolecules which interact with specific dyes in a characteristic manner.	2.5
Weighted Score 5.25	Score	7.5

Parameter		Pertinent Information or Factors Considered**	
C 1.0		a. The technique depends upon the detection of a characteristic change in the dye spectrum. This change is related to amonic site spacings and structure of a polymer and is therefore not specific for one substance.	0
Weighted Score	6	Score	6
D 0.7		b. The sensitivity of the method requires 10^7 cells.	1.5
Weighted Score	3.15	Score	4.5
E 0.7		b. The sensitivity of the method requires 10^7 cells.	1.25
Weighted Score	4.38	Score	6.25

TOTAL WEIGHTED SCORE - 29

6. Optical Rotation - Assume macromolecule is DNA and measurement is made at 257 mμ.

A 0.7		a. Microorganisms contain DNA and they are widely distributed.	2.0
		b. All living organisms contain DNA.	2.0
		c. Optical rotation is an intrinsic property of DNA.	2.0
		d. It is possible that the function of DNA could be carried out by a different substance.	0
		e. On the average a preponderance of one optical form of a macromolecule is not produced abiogenically and in any event DNA is synthesized only by polymerases obtained from living cells.	2.0
Weighted Score	5.6	Score	8.0
B 0.7		a. The optical rotation is due to the configuration of DNA and this configuration is essential to its function.	2.5
		b. DNA is largely responsible for information transfer and storage in living systems and this is a necessary biological property.	2.5
		c. The technique searches directly for macromolecules which rotate 257 mμ light.	2.5
		d. It is possible that the function of DNA could be carried out by a different substance.	0
Weighted Score	5.25	Score	7.5

Parameter	Pertinent Information or Factors Considered **	
C 1.0	a. Optical rotation at 257 mμ can be produced by substances other than DNA and it is possible, but not very probable, that rotation could be produced by a Faraday effect.	0
	b. Acid conditions can cause degradation of DNA and racemization.	0
Weighted Score 0	Score	0
D 0.7	a. It appears necessary to at least separate large molecules from small ones.	0
	b. The sensitivity of the test requires 5×10^8 organisms.	1.0
	c. A physiological change is not required for the detection of DNA.	3.0
Weighted Score 2.8	Score	4.0
E 0.7	a. A dynamic biological function is not involved in the detection of DNA	2.5
	b. The sensitivity of the method requires 5×10^8 cells.	0.88
	c. Only if it is assumed that replication can be induced.	0
	d. DNA can be detected in a metabolically inactive system.	2.5
Weighted Score 4.12	Score	5.88
F 0.4	a. The atmospheric composition of Mars is not incompatible with the existence of DNA.	5.0
	b. The Martian temperatures no not make the accumulation of DNA unreasonable.	5.0
Weighted Score 4	Score	10.0

TOTAL WEIGHTED SCORE - 22.0

Parameter		Pertinent Information or Factors Considered **	
7. Optical Rotation. Assume macromolecule is protein and measurement is made at 233 mμ.			
A		a. Microorganisms contain protein and they are widely distributed.	2.0
0.7		b. All living organisms contain optically active proteins.	2.0
		c. Optical activity is an intrinsic property of proteins.	2.0
		d. The proteins are essential to so many facets of life that it is difficult to see how they could be replaced in every case and still have a system which is recognized as living.	2.0
		e. Protein-like compounds have been produced by abiogenic methods, but these are not optically active unless only one optical form of amino acids is selected by the experimenter for the synthesis and this amounts to biological synthesis.	2.0
Weighted Score	7.0	Score	10.0
B		a. The optical rotation is due to the configuration of the protein and configuration is essential to function.	2.5
0.7		b. Proteins are essential to life as structural substances, enzymes, hormones, etc.	2.5
		c. The technique searches directly for macromolecules which rotate 233 mμlight.	2.5
		d. The proteins are essential to so many facets of life that it is difficult to see how they could be replaced in every case and still have a system which is recognized as living.	2.5
Weighted Score	7	Score	10.0
C			
1.0		a. Optical rotation at 233mμ can be produced by substances other than protein.	0
		b. Acid conditions can cause degradation of proteins and racemization.	0
Weighted Score	0	Score	0
D		a. It appears to be necessary to separate large molecules from small ones.	0
0.7		b. The sensitivity of the test requires 7 x 10^6 cells.	1.6
		c. A physiological change is not required for the detection of protein.	3
Weighted Score	3.22	Score	4.6

Parameter	Pertinent Information or Factors Considered **		
E 0.7	a. A dynamic biological function is not involved in the detection of protein.		2.5
	b. The sensitivity of the method requires 7×10^6 cells.		1.32
	c. Only if it is assumed that replication can be induced.		0
	d. Protein can be detected in a metabolically inactive system.		2.5
Weighted Score 4.42		Score	6.32
F 0.4	a. The atmospheric composition of Mars is not incompatible with the existence of protein.	2	5.0
	b. The Martian temperatures do not make the accumulation of protein unreasonable.		5.0
Weighted Score 4		Score	10.0

TOTAL WEIGHTED SCORE - 25.6

8. Optical Rotation of Macromolecule-Dye Complex. Assume macromolecule is DNA and spectrum is scanned from 400 to 700 mμ. Only those questions which require answers different from II A-6 are considered.

A 0.7	c. The technique requires interaction with a dye.		0
Weighted Score 4.2		Score	6
C 1.0	c. Optical rotation at a specific wavelength produced by a dye - DNA complex would be more specific than optical rotation at 257 mμ because you can rely on two criteria (DNA-dye interaction and effect of wavelength) instead of relying only on wavelength.		1.5
Weighted Score 1.5		Score	1.5

TOTAL WEIGHTED SCORE - 22.1

Parameter		Pertinent Information or Factors Considered **	
9. Refractive Index. Assume macromolecule is a protein.			
A		a. Microorganisms contain proteins and they are widely distributed.	2.0
0.7		b. All organisms contain protein.	2.0
		c. The refractive index is an intrinsic property of a protein solution.	2.0
		d. The proteins are essential to so many facets of life that it is difficult to see how they could be replaced in every case and still have a system which is recognized as living.	2.0
		e. Protein-like compounds have been produced abiogenically.	0
Weighted Score	5.6	Score	8
B		a. The technique depends upon a physical measurement which results simply from the protein being in solution.	2.5
0.7		b. Proteins are essential to life as structural substances, enzymes, hormones, etc.	2.5
		c. The technique examines a result of the protein being in solution.	2.5
		d. The proteins are essential to so many facets of life that it is difficult to see how they could be replaced in every case and still have a system which is recognized as living.	2.5
Weighted Score	7	Score	10.0
C		a. Any macromolecule will alter the refractive index.	0
1.0		b. Almost any change in temperature, pH, ionic strength, etc. will alter the refractive index of a protein solution.	0
Weighted Score	0	Score	0
D		a. It is necessary to at least separate large molecules from small ones.	0
0.7		b. The sensitivity of the method requires 2×10^7 cells.	1.44
		c. A physiological change is not necessary for the detection of protein.	3
Weighted Score	3.1	Score	4.44

Parameter	Pertinent Information or Factors Considered **	
E 0.7	a. A biological time constant is not a factor in the detection of protein.	2.5
	b. The sensitivity of the method requires 2 x 10^7 cells.	1.2
	c. For a time period to be advantageous it must be assumed that reproduction will occur.	0
	d. Protein can be detected in a metabolically inactive system.	2.5
Weighted Score 4.34	Score	6.2
F 0.4	a. The atmospheric composition of Mars is not incompatible with the existence of protein.	5
	b. The Martian temperatures do not make the accumulation of protein unreasonable.	5
Weighted Score 4	Score	10

TOTAL WEIGHTED SCORE - 24.0

9. Index of Refraction - See Appendix 2, page 2-12.

10. Viscosity - See Appendix 2, page 2-12.

11. Detection of Protein by Colorimetry (Lowry Method)

A 0.7	a. Microorganisms contain protein and they are widely distributed.	2
	b. All organisms contain protein.	2
	c. The method requires reaction of the protein with phenol reagent and copper.	0
	d. The proteins are essential to so many facets of life that it is difficult to see how they could be replaced in every case and still have a system which is recognized as living.	2
	e. Protein-like compounds which will give a positive Lowry test have been produced abiogenically.	0
Weighted Score 4.2	Score	6

Parameter	Pertinent Information or Factors Considered **	
B 0.7	a. The test depends upon reaction with carbanyl groups and these are essential for the existence of proteins.	2.5
	b. Proteins are essential as structual materials, catalysts, hormones, etc.	2.5
	c. The technique examines macromolecules for adjacent carbanyl groups and these must exist in proteins.	2.5
	d. The proteins are essential to so many facets of life that it is difficult to see how they could be replaced in every case and still have a system which is recognized as living.	2.5
Weighted Score 7.0	Score	10.0
C 1.0	a. The test will give a positive result for substances which contain $-CSNH_2$, $-C(NH)NH_2$ or $-CH_2NH_2$, as wellas those containing $-CONH_2$ groups.	0
	b. Small changes in environmental parameters do not cause changes in qualitative results.	5.0
Weighted Score 5.0	Score	5.0
D 0.7	a. Large molecules must be separated from small ones.	0
	b. The sensitivity of the method requires 10^6 cells.	1.8
	c. A physiological change is not essential for the detection of protein.	3.0
Weighted Score 3.36	Score	4.8
E 0.7	a. A dynamic biological function is not necessary for the detection of protein.	2.5
	b. The sensitivity of the method requires 10^6 cells.	1.5
	c. Time can be utilized to increase the possibility of detection only if replication can be induced.	0
	d. Protein can be detected in a static system.	2.5
Weighted Score 5.25	Score	6.5
F 0.4	a. The composition of the Martian atmosphere does not make the existence of proteins unlikely.	5.0
	b. The Martian temperatures are not incompatible with the existence and accumulation of protein.	5.0
Weighted Score 4	Score	10.0

TOTAL WEIGHTED SCORE - 28.8

II. B. DETECTION OF MACROMOLECULES IN GENERAL

1. UV Spectrophotometry

Parameter	Pertinent Information or Factors Considered **	
A 0.7	a. Microorganisms contain macromolecules and they are widely distributed.	2
	b. All organisms contain macromolecules.	2
	c. UV absorption, if it occurs, is an intrinsic property of the macromolecule.	2
	d. Macromolecules which do not absorb in the UV could be present in Martian life, but the very factors which are usually related to the UV absorption are necessary for the existence of the macromolecule or for its function (e.g. peptide bonds in proteins).	2
	e. Macromolecules which absorb in the UV can be synthesized abiogenically.	0
Weighted Score 5.6	Score	8
B 0.7	a. Macromolecules which do not absorb in the UV could be present in Martian life, but the very factors which are usually related to the UV absorption are necessary for the existence of the macromolecule or for its function (e.g. peptide bonds in proteins).	1.5
	b. Macromolecules are essential to almost every attribute of life.	2.5
	c. The technique detects macromolecules by looking for an intrinsic property of the molecule.	2.5
	d. Macromolecules which do not absorb in the UV could be present in Martian life, but the very factors which are usually related to the UV absorption are necessary for the existence of the macromolecule or for its function (e.g. peptide bonds in proteins).	2.5
Weighted Score 6.3	Score	9.0
C 1.0	a. Molecules other than macromolecules absorb in the UV but the method assumes a separation as to size prior to analysis.	5
	b. The qualitative aspect of UV absorption is not altered by small environmental changes.	5
Weighted Score 10.0	Score	10

Parameter	Pertinent Information or Factors Considered **	
D 0.7	a. A separation of large from small molecules is required.	0
	b. The sensitivity of the technique should be about the same as for known terrestrial biological macromolecules. About 1×10^7 cells would be required.	1.5
	c. A physiological change is not required for the detection of macromolecules.	3.0
Weighted Score 3.15	Score	4.5
E 0.7	a. A dynamic biological function is not involved in the detection of macromolecules.	2.5
	b. The sensitivity of the technique requires 1×10^7 cells.	1.25
	c. Only if it is assumed that replication can be induced.	0
	d. Macromolecules can be detected in a metabolically inactive system.	2.5
Weighted Score 4.38	Score	6.25
F 0.4	a. The atmospheric composition of Mars is not incompatible with the existence of macromolecules.	5.0
	b. The Martian temperatures do not make the accumulation of macromolecules unreasonable.	5.0
Weighted Score 4	Score	10.0

TOTAL WEIGHTED SCORE 33.4

Parameter	Pertinent Information or Factors Considered **	
2. Optical Rotation. Only questions requiring answers different from II B1 are considered.		
A 0.7	c. Optical rotation, if it occurs, is an intrinsic property of the macromolecule.	2
	d. Macromolecules which do not have optical activity could be produced, but this does not seem likely for any biological system. One of the attributes of life is organization and without optical purity molecular organization is difficult to obtain.	2
	e. Macromolecules which are optically active cannot be synthesized by random methods. Selection of component parts must be made biologically by the organism (including an experimenter).	2
Weighted Score 7.0	Score	10
C 1.0		
	a. On the average, only biologically produced molecules show optical activity.	5.0
	b. Small changes in pH can alter the optical activity of macromolecules.	0
Weighted Score 5.0	Score	5.0
D 0.7	b. The sensitivity of the technique should be about the same as for known terrestrial biological macromolecules. Therefore, 7×10^6 cells would be required.	1.6
Weighted Score 3.2	Score	4.6
E 0.7	b. About 7×10^6 cells are required.	1.32
Weighted Score 4.4	Score	6.32

TOTAL WEIGHTED SCORE - 29.6

Parameter	Pertinent Information or Factors Considered **	
3. Visible Spectrophotometry.	Only questions requiring answers different from II B1 are considered.	
A 0.7 Weighted Score 4.2	c. The technique requires the interaction of a dye with the macromolecule. Score	0 6
B 0.7 Weighted Score 7	a. The test depends upon the macromolecule having anionic or cationic sites and all biologically indispensable macromolecules possess either or both. Score	2 10
C 1.0 Weighted Score 10.0	a. The technique assumes prior separation of large from small molecules. Consequently the test is positive only for large charged molecules. 5.0 b. The quantitative aspects of the technique are altered by changes in temperature and pH, but the detection capabilities are not seriously altered. Score	5.0 5.0 10.0
D 0.7 Weighted Score 3.68	b. The sensitivity of the method requires 5 x 10^4 cells. Score	2.25 5.25
E 0.7 Weighted Score 4.8	b. The sensitivity of the method requires 5 x 10^4 cells. Score	1.85 6,85

TOTAL WEIGHTED SCORE - 33.7

Parameter	Pertinent Information or Factors Considered **	
4. Optical Rotation - Dye-Macromolecule Complex		
A 0.7	a. Microorganisms contain optically active macromolecules which form complexes with dyes and they are widely distributed.	2.0
	b. All organisms contain optically active macromolecules which complex with dyes.	2.0
	c. The technique requires interaction with a dye.	0
	d. Macromolecules can not be replaced in total by other substances in living systems.	2.0
	e. Macromolecules having a preponderance of one optically active form are not produced abiogenically unless the experimenter selects only one optical form for the synthesis and this is then equivalent to biological synthesis.	2.0
Weighted Score 5.6	Score	8.0
B 0.7	a. The test depends upon the presence of anionic and cationic sites and optical rotation due to configuration; both specific configuration and charged sites are essential to the function of biological macromolecules.	2.5
	b. Macromolecules are the very "stuff" of life and without them life would not exist.	2.5
	c. The technique searches directly for optically active macromolecules.	2.5
	d. Life without optical purity of macromolecules is difficult to imagine since this would lead to chaotic conditions rather than organization.	2.5
Weighted Score 7	Score	10.0
C 1.0	a. Optical rotation due to a Faraday effect is possible, but not probable.	5
	b. Acid conditions can cause degradation of macromolecules and racemization.	0
Weighted Score 5	Score	5

Parameter	Pertinent Information or Factors Considered **	
D 0.7	a. A separation of small molecules from large is necessary.	0
	b. The sensitivity of the technique requires 7×10^6 cells.	1.59
	c. A physiological change is not necessary for the detection of macromolecules.	3.0
Weighted Score 3.21	Score	4.59
E 0.7	a. A dynamic biological function is not necessary for the detection of optically active macromolecules.	2.5
	b. The sensitivity of the method requires 7×10^6 cells.	1.32
	c. An accumulation of macromolecules can occur with time, but only if replication can be induced in the organism.	0
	d. Optically active macromolecules can be detected in metabolically inactive systems.	2.5
Weighted Score 4.42	Score	6.32
F 0.4	a. The composition of the Martian atmosphere is not incompatible with the existence of optically active macromolecules.	5.0
	b. The Martian temperatures do not exclude the accumulation of optically active macromolecules.	5.0
Weighted Score 4	Score	10.0

TOTAL WEIGHTED SCORE - 29.2

III. REPRODUCTION AND REPLICATION

These properties can be detected by measuring increases in complex organic materials and structures or in physiological and biochemical processes as a function of time. In this section only increases in complex materials or structures are considered, since the relative applicability of the physiological and biochemical methods can be deduced from the scores in the sections on energy transfer and conversion, catalytic activity, and organization, Paragraphs 3.1, 3.5, and 3.6 of this analysis.

III. REPRODUCTION, REPLICATION AND GROWTH

1. Turbidity Increase as a Function of Time

Parameter	Pertinent Information or Factors Considered **	
A 0.7	a. The sample must contain at least one viable organism. Microorganisms are suitable for this technique and they are widely distributed.	2.0
	b. All living systems have the capacity to reproduce in total or replicate specific structures.	2.0
	c. Reproduction or replication requires provision of adequate nutritional factors and environmental conditions.	0
	d. Reproduction and replication are essential for the continued existence of life.	2.0
	e. Swelling of substances, such as vermiculite and proteinoid spheres as well as coalescence of small particles and production of bubbles can cause changes in turbidity with time.	0
Weighted Score 4.2	Score	6.0
B 0.7	a. Test depends upon the replicated units being large enough to scatter visible light. If it is considered that a DNA molecule represents a size limit within which enough information is contained to produce a living system then the Martian replicating unit will probably be large enough to scatter light.	2.0
	b. Replication and reproduction are essential to the continued existence of living forms.	2.5
	c. The technique tests only for reproductive units which are large enough to scatter visible light. It does not detect the replication of units within a cell.	0
	d. If it is considered that a DNA molecule represents a size limit within which enough information is contained to produce a living system, then the Martian replicating unit will probably be large enough to scatter light.	2.0
Weighted Score 4.55	Score	6.5

Parameter	Pertinent Information or Factors Considered **	
C 1.0		
	a. The technique will give a positive result for many events other than an increase in number of particles. For example, the appearance of gas bubbles and an increase in particle size will alter turbidity.	0
	b. Small changes in the environment can produce gas bubbles, cause precipitation of materials and swelling of cells.	0
Weighted Score 0	Score	0
D 0.7		
	a. The test can be conducted by simply adding small quantities of Martian dust to a growth medium.	4.0
	b. The sensitivity of the technique requires of change in cell number of 10^5 cells per ml.	2.1
	c. A physiological change is essential to the test procedure.	0
Weighted Score 4.27	Score	6.1
E 0.7		
	a. The time required for cellular reproduction could be excessivle long.	0
	b. The sensitivity of the method requires an increase of 10^5 cells per ml.	1.75
	c. The experiment relies upon the accumulation of events over a time period. A greater time period could increase the likelihood that the event will be detected.	2.5
	d. This experiment cannot detect replication in the absence of reproduction and therefore it depends upon the completion of the entire cellular reproduction cycle.	0
Weighted Score 2.98	Score	4.25
F 0.4		
	a. The composition of the Martian atmosphere does not make the processes of reproduction and replication improbable.	5
	b. The Martian temperatures are variable enough to make selection of a proper incubation temperature difficult.	2
Weighted Score 2.8	Score	7

TOTAL WEIGHTED SCORE - 18.8

Parameter	Pertinent Information or Factors Considered **	
2. Increase in Mass as a Function of Time. Direct Weighing with a Balance.		
A 0.7	a. Microorganisms are suitable for this technique and they are widely distributed.	2
	b. All living systems exhibit changes in mass.	2
	c. If an increase in mass is to be obtained provision for adequate nutritional factors and environmental conditions must be made.	0
	d. An increase in mass is an essential part of reproduction and replication and these factors are necessary for the continued existence of life.	2
	e. Many abiogenic processes can cause an increase in the mass of a specific fraction. For example, co precipitation of inorganic substance with living materials.	0
Weighted Score 4.2	Score	6
B 0.7	a. The test depends upon detecting a change in the total biological system rather than in one particular portion.	2.5
	b. An increase in mass is an essential part of reproduction and replication and these factors are necessary for the continued existence of life.	2.5
	c. The technique examines a factor which is a result of the overall processes of reproduction and replication.	2.5
	d. An alternative to an increase in mass as a result of reproduction is difficult to imagine.	2.5
Weighted Score 7	Score	10.0
C 1.0	a. The technique records only changes in mass, but the changes can be due to abiogenic processes.	0
	b. Small changes in temperature, air pressure, humidity, etc. can materially affect measurements made with a balance.	0
Weighted Score 0	Score	0
D 0.7	a. The biological material must be separated from the culture medium prior to weighing.	0
	b. The sensitivity of the method requires an increase of 3.3×10^6 cells.	1.68
	c. A physiological change is essential to the test procedure.	0
Weighted Score 1.18	Score	1.68

Parameter	Pertinent Information or Factors Considered **	
E 0.7	a. The time constant involved in a change of mass could be very long.	0
	b. The sensitivity of the method requires 3.3×10^6 cells.	0.82
	c. The experiment relies upon the accumulation of events over a time period. A greater time period could increase the likelihood that the event will be detected.	2.5
	d. The experiment could conceivably detect replication in the absence of reproduction. Thus the entire cellular reproduction cycle would not be required.	1.0
Weighted Score 3.0	Score	4.32
F 0.4	a. The composition of the Martian atmosphere does not make a change in mass of a biological system unlikely.	5
	b. The Martian temperatures are variable enough to make selection of a proper incubation temperature difficult.	2
Weighted Score 2.8	Score	7

TOTAL WEIGHTED SCORE - 18.2

3. Increase in Mass as a Function of Time Change in Frequency of a Piezoelectric Crystal. Only questions which require answers different from step 2 are considered.

D 0.7	b. The sensitivity of the method requires an increase of 10^5 cells.	2.1
Weighted Score 1.47	Score	2.1
F 0.7	b. The sensitivity of the method requires an increase of 10^5 cells.	1.75
Weighted Score 3.68	Score	5.25

TOTAL WEIGHTED SCORE - 20.2

Parameter	Pertinent Information or Factors Considered **	
4. Increase in Macromolecules as a Function of Time by Change in Dye Absorbance.		
A 0.7	a. Macromolecules are present in microorganisms and they are widely dispersed.	2
	b. Macromolecules are the "stuff of life", they occur in all organisms.	2
	c. The technique requires the use of a dye and appropriate culture conditions.	0
	d. Macromolecules are indispensable to life.	2
	e. Macromolecules can be produced abiogenically.	0
Weighted Score 4.2	Score	6
B 0.7	a. The test depends upon the macromolecule having anionic or cationic sites and all biologically indispensable macromolecules possess either or both.	2.5
	b. Macromolecules in the form of nucleic acids and proteins carry on the important functions of information storage, catalysis, etc.	2.5
	c. The technique can detect both replication and reproduction.	2.5
	d. Life without changed macromolecules does not appear to be possible.	2.5
Weighted Score 7	Score	10.0
C 1.0	a. Macromolecules can be produced abiogenically.	0
	a. If large molecules are separated from small ones the results are specific.	5
	b. Extensive changes in pH or temperature can affect the dye-macromolecule interaction.	0
Weighted Score 5	Score	5
D 0.7	a. Separation of large from small molecules is necessary.	0
	b. The sensitivity of the method requires an increase of 5×10^4 cells.	2.25
	c. A physiological change is essential to the test procedure.	0
Weighted Score 1.58	Score	2.25

Parameter	Pertinent Information or Factors Considered **	
E 0.7	a. The time required to produce a detectable increase in macromolecule concentration could be very long.	0
	b. The sensitivity of the method requires an increase of 5×10^4 cells.	1.88
	c. The experiment relies upon the accumulation of events over a time period. A greater time period increases the likelihood the event will be detected.	2.5
	d. The experiment can detect replication in the absence of reproduction, thus the entire cellular reproduction cycle is not required.	1.5
Weighted Score 4.12	Score	5.88
F 0.4	a. The composition of the Martian atmosphere does not make the replication of macromolecules in a biological system unlikely.	5.0
	b. The Martian temperatures are variable enough to make selection of a proper incubation temperature difficult.	2.0
Weighted Score 2.8	Score	7.0

TOTAL WEIGHTED SCORE - 24.7

5. Increase in Macromolecules as a Function of Time. Change in Optical Rotation. Only questions requiring answers different from III-4 are considered.

A 0.7	c. The technique relies on an intrinsic property of biological macromolecules. However, appropriate culture nutrients and conditions must be supplied.	0
	e. Macromolecules having a preponderance of one optical form are not produced abiogenically unless the experimenter selects only one optical form for the synthesis and this is then equivalent to biological synthesis.	2.0
Weighted Score 5.6	Score	8.0
B 0.7	a. The technique depends upon the macromolecule being optically active and all biologically important macromolecules are optically active.	2.5
	d. Life without optical purity of macromolecules is difficult to imagine since this would lead to chaotic conditions rather than organization.	2.5
Weighted Score 7	Score	10.0

Parameter	Pertinent Information or Factors Considered **	
C 1.0		
	a. Optical rotation due to a Faraday effect is possible, but extremely unlikely.	5.0
	b. Acid conditions can cause degradation of macromolecules with racemization.	0
Weighted Score 5.0	Score	5.0
D 0.4	b. The sensitivity of the method requires an increase of 5×10^8 cells.	1.05
Weighted Score 0.4	Score	1.05
E 0.7	b. The sensitivity of the method requires an increase of 5×10^8 cells.	0.87
Weighted Score 3.4	Score	4.87

TOTAL WEIGHTED SCORE - 24.2

6. Increase in Macromolecules as a Function of Time by UV absorbance. Only questions requiring answers different from IIB1 are considered.

A 0.7	c. Appropriate nutrients and culture conditions must be supplied.	0
Weighted Score 4.2	Score	6
C 1.0	b. Increases in optical density of DNA can be obtained by denaturation due to changes in pH or temperature.	0
Weighted Score 5	Score	5
D 0.7	c. A physiological change is essential to the test procedure.	0
Weighted Score 1.0	Score	1.5

Parameter	Pertinent Information or Factors Considered **	
F 0.7	a. The time required to produce a detectable increase in macromolecule concentration could be very long.	0
	c. The experiment relies upon the accumulation of events over a time period. A greater time period increases the likelihood the event will be detected.	2.5
	d. The experiment can detect replication in the absence of reproduction, thus the entire cellular reproduction cycle is not required.	1.5
Weighted Score 3.68	Score	5.25
G 0.4	a. The composition of the Martian atmosphere does not make the replication of macromolecules in a biological system unlikely.	5.0
	b. The Martian temperatures are variable enough to make selection of a proper incubation temperature difficult.	2.0
Weighted Score 2.8	Score	7.0

TOTAL WEIGHTED SCORE - 23.7

7. Increase in Nucleic Acids as a Function of Time by UV Absorbance.

A 0.7	a. Nucleic acids are present in microorganisms and they are widely dispersed.	2
	b. All terrestrial organisms contain nucleic acids.	2
	c. The technique measures an intrinsic property of nucleic acid molecules, but it also requires that appropriate culture conditions and nutrients are supplied.	0
	d. It is conceivable that informational molecules other than nucleic acids could exist in Martian life.	0
	e. Nucleic acids are not produced abiogenically, but macromolecules which absorb in the UV can be.	0
Weighted Score 2.8	Score	4
B 0.7	a. The technique relies on an intrinsic property of the nucleic acids.	2.5
	b. The nucleic acids carry on the important biological functions of information storage and processing.	2.5
	c. The technique is based on an intrinsic property of the nucleic acids.	2.5
	d. It is possible that the function of the nucleic acids could be carried out by a different substance.	0
Weighted Score 5.25	Score	7.5

Parameter	Pertinent Information or Factors Considered **	
C 1.0	a. Many macromolecules other than the nucleic acids absorb in the 260 mμ region of the spectrum.	0
	b. Small changes in the environment can cause an increase in the UV absorption of nucleic acids.	0
	Score	0
Weighted Score 0		
D 0.7	a. It is necessary to separate large molecules from small ones.	0
	b. The sensitivity of the method requires an increase of 5×10^6 cells.	1.65
	c. A physiological change is essential to the test procedure.	0
	Score	1.65
Weighted Score 1.16		
E 0.7	a. The time required to produce a detectable increase in nucleic acid concentration could be very long.	0
	b. The sensitivity of the technique requires an increase of 5×10^6 cells.	1.38
	c. The experiment relies upon the accumulation of events over a time period. A greater time period increases the likelihood the event will be detected.	2.5
	d. The experiment can detect replication in the absence of reproduction. Thus the entire cellular reproduction cycle is not required.	1.5
	Score	5.38
Weighted Score 3.77		
F 0.4	a. The composition of the Martian atmosphere does not make the replication of nucleic acids in a biological system unlikely.	5
	b. The Martian temperatures are variable enough to make selection of a proper incubation temperature difficult.	2
	Score	7
Weighted Score 2.8		

TOTAL WEIGHTED SCORE - 15.8

Parameter	Pertinent Information or Factors Considered **	
8. Increase in Nucleic Acids as a Function of Time by Colorimetry. Only those questions requiring answers different from III-6 are considered.		
A 0.7	c. The technique analysis for the sugar moiety of nucleic acids by specific colorimetric chemical reactions. It also requires that appropriate culture conditions and nutrients are supplied.	0
	d. The sugar moiety of the nucleic acids could conceivably be replaced by a different sugar which would not respond to the chemical test.	0
	e. Macromolecules which contain ribose and deoxyribose can be produced abiogenically.	0
Weighted Score 2.8	Score	0/4
B 0.7	a. The test depends upon ribose or deoxyribose being part of the nucleic acid molecule. It is possible that other substance could replace these sugars without altering the biological function of the nucleic acids.	
Weighted Score 3.5	Score	0/5
C 1.0	a. The technique gives a positive result for any substance which contains deoxyribose or ribose and has solubility properties similar to DNA or RNA.	
Weighted Score 0	Score	0/0
D 0.7	b. The sensitivity of the method requires an increase of 2×10^8 cells.	1.14
Weighted Score 0.80	Score	1.14
E 0.7	b. The sensitivity of the method requires an increase of 2×10^8 cells.	0.95
Weighted Score 3.46	Score	4.95

TOTAL WEIGHTED SCORE - 14.6

Parameter	Pertinent Information or Factors Considered **	
9. Increase in Proteins as a Function of Time by UV Absorption at 280 mμ. Only those questions requiring answers different from II A-2 are considered.		
D 0.7 Weighted Score 1.0	c. A physiological change is essential to the test.	0
	Score	1.5
E 0.7	a. The time required to produce a detectable increase in proteins could be very long.	0
	c. The experiment relies upon the accumulation of events over a time period. A greater time period increases the likelihood the event will be detected.	2.5
	d. The experiment can detect replication in the absence of reproduction, thus the entire cellular reproduction cycle is not required.	1.5
Weighted Score 3.85	Score	5.5
F 0.4	b. The Martian temperatures are variable enough to make selection of a proper incubation temperature difficult.	2
Weighted Score 2.8	Score	7
TOTAL WEIGHTED SCORE - 19.7		
10. Increase in Proteins as a Function of Time by UV Absorption at 190 mμ. Only those questions requiring answers different from III-9 are considered.		
A 0.7	c. Nutrient substances must by supplied to induce replication and reproduction.	0
Weighted Score 4.2	Score	6
B 0.7	a. The test depends upon absorption by peptide bonds and proteins can not exist without peptide linkages.	2.5
	c. The technique detects peptide bonds which are irreplaceable in proteins.	2.5
	d. The peptide bond is essential to proteins and the proteins are necessary for so many facets of life that it is difficult to see how they could be replaced in every case and still have a system which is recognized as living.	2.5
Weighted Score 7.0	Score	10.0

Parameter	Pertinent Information or Factors Considered **	
D 0.7 Weighted Score 1.26	b. The sensitivity of the method requires an increase of 1 x 10^6 cells.	1.8
	Score	1.8
E 0.7 Weighted Score 4	b. The sensitivity of the method requires an increase of 1 x 10^6 cells.	1.5
	Score	5.75

TOTAL WEIGHTED SCORE - 24.3

11. Increase in Proteins as a Function of Time by Colorimetry (Lowry Method).

A 0.7	a. Microorganisms contain protein and they are widely distributed.	2
	b. All organisms contain protein.	2
	c. The technique relies upon the color developed with copper salts when they react with adjacent carbanyl groups. Proper nutrients and culture conditions must be supplied.	0
	d. The proteins are necessary for so many facets of life that it is difficult to see how they could be replaced in every case and still have a system which is recognized as living.	2
	e. Protein-like substances which respond to the Lowry test can be produced abiogenically.	0
Weighted Score 4.2	Score	6
B 0.7	a. The test depends upon reaction with carbanyl groups and these are essential for the existence of proteins.	2.5
	b. Proteins are essential as structural materials, catalysts, hormones, etc.	2.5
	c. The technique examines macromolecules for adjacent carbanyl groups and these must exist in proteins.	2.5
	d. The proteins are necessary for so many facets of life that it is difficult to see how they could be replaced in every case and still have a system which is recognized as living.	2.5
Weighted Score 7	Score	10.0

Parameter	Pertinent Information or Factors Considered **	
C 1.0		
	a. The test will give a positive result for substances which contain $CSNH_2$, $-C(NH)NH_2$ or $-CH_2NH_2$ as well as those containing $-CONH_2$ groups.	0
	b. Small changes in environmental parameters do not materially alter the results.	5
Weighted Score 5	Score	5
D 0.7	a. Large molecules must be separated from small ones.	0
	b. The sensitivity of the method requires an increase of 10^6 cells.	1.8
	c. A physiological change is essential to the test procedure.	0
Weighted Score 1.26	Score	1.8
E 0.7	a. The time required for replication of protein molecules could be very long.	0
	b. The sensitivity of the method requires an increase of 10^6 cells.	1.5
	c. The experiment relies upon the accumulation of protein over a time period. A greater time period increases the likelihood the event will be detected.	2.5
	d. The technique can detect protein replication in the absence of cellular reproduction.	1.5
Weighted Score 3.85	Score	5.5
F 0.4	a. The composition of the Martian atmosphere does not make the replication of proteins in a biological system unlikely.	5.0
	b. The Martian temperatures are variable enough to make selection of a proper incubation temperature difficult.	2.0
Weighted Score 2.8	Score	7.0

TOTAL WEIGHTED SCORE - 24.1

Parameter	Pertinent Information or Factors Considered **	
12. Increase in Steroids as a Function of Time by Gas Chromatography with Mass Spectrometer Detector		
A 0.7	a. Many microorganisms do not contain steroids.	0
	b. All organisms except bacteria contain steroids.	0
	c. Steroids can be analyzed by gas chromatography without prior chemical reactions. However, in order to grow the organism nutrients must be provided.	0
	d. Steroids appear to be essential constituents of cell membranes except in bacteria. Their structural and solubility characteristics are apparently uniquely fitted to this function, but their exact role is not well enough known to make their indispensability certain.	0.5
	e. Steroids are not readily produced by abiogenic processes.	2.0
Weighted Score 2.10	Score	2.5
B 0.7	a. The test depends upon the retention characteristics of steroids on appropriate columns and specific mass.	2.5
	b. Steroids are essential as constituents of membranes and hormones, but do not appear to be necessary for some bacteria.	0.5
	c. The technique analyzes directly for steroids.	2.5
	d. Steroids appear to be essential constituents of cell membranes except in bacteria. Their structural and solubility characteristics are apparently uniquely fitted to this function, but their exact role is not well enough known to make their indispensability certain.	0.5
Weighted Score 4.2	Score	6.0
C 1.0	a. If the gas chromatographic separation is carried out properly the method should be quite specific, but steroids can be synthesized without cell reproduction.	1.0
	b. Changes in the environment have virtually no effect on the test procedure.	5.0
Weighted Score 6.0	Score	6.0
D 0.7	a. A solvent extraction is required.	0
	b. The sensitivity of the method requires an increase of 5.2×10^7 cells.	1.35
	c. The test procedure relies upon reproduction of the organism.	0
Weighted Score 0.94	Score	1.35

Parameter	Pertinent Information or Factors Considered **	
E 0.7	a. The time required for reproduction could be very long.	0
	b. The sensitivity of the method requires an increase of 5.2×10^7 cells.	1.12
	c. The experiment relies upon the accumulation of cells during a time period. A greater time period may increase the likelihood that reproduction will be detected.	2.5
	d. The technique requires the operation of the complete reproductive cycle.	0
Weighted Score 2.53	Score	3.62
F 0.4	a. The composition of the Martian atmosphere does not make the biological synthesis of steroids unlikely.	5
	b. The Martian temperatures are variable enough to make selection of a proper incubation temperature difficult.	2
Weighted Score 2.8	Score	7

TOTAL WEIGHTED SCORE - 18.6

13. Increase in Steroids as a Function of Time by Infrared Spectrophotometry. Only those questions which require answers different from III-12 are considered.

B 0.7	a. The test depends upon the observation of an increase in absorbance at a specific IR band which is fundamental to the steroid nucleus.	2.0
Weighted Score 3.8	Score	5.5
C 1.0	a. There are other substances which have IR absorption bands in the same regions as the steroids.	0
Weighted Score 5.0	Score	5

Parameter	Pertinent Information or Factors Considered **	
D 0.7 Weighted Score 0.34	b. The sensitivity of the method requires an increase of 3.3×10^{10} cells.	0.48 0.48
E 0.7 Weighted Score 2.0	b. The sensitivity of the method requires an increase of 3.3×10^{10} cells. Score	0.4 2.9

TOTAL WEIGHTED SCORE - 16.0

14. Increase in Steroids as a Function of Time by Optical Rotation. Only those questions which require answers different from III-12 are considered.

B 0.7 Weighted Score 4.2	a. The test depends upon an increase in optical rotation due to the synthesis of a particular optical isomer. Score	2.5 6.0
C 1.0 Weighted Score 5.0	a. Lipid soluble substances other than steroids are optically active and steroids can be synthesized without cellular reproduction. b. Racemization of steroids at all optical centers is very difficult. Score	0 5.0 5.0
D 0.7 Weighted Score 0.1	b. The sensitivity of the method requires an increase of 5.2×10^{11} cells. Score	0.15 0.15
E 0.7 Weighted Score 1.81	b. The sensitivity of the method requires an increase of 5.2×10^{11} cells. Score	0.09 2.59

TOTAL WEIGHTED SCORE - 14.9

Parameter	Pertinent Information or Factors Considered **	
15. Increase in Steroids as a Function of Time by Colorimetry. Only those questions which require answers different from III-12 are considered.		
A 0.7 Weighted Score 1.75	c. The method requires separation of steroids by column chromatography and reaction with ferric chloride reagent.	0
	Score	2.5
B 0.7	a. The test depends upon the steroid possessing a hydroxyl group or a hydrolyzable ester. This may not be necessary in order for the steroid to function biologically.	0
	c. The technique tests only for steroids which have a hydroxyl or hydrolyzable ester group.	0
	d. It is possible that steroids in Martian organisms may not possess hydroxyl or hydroxyzable ester groups.	0
Weighted Score 0.35	Score	0.5
C 1.0 Weighted Score 10	a. If the column fractionation is carried out properly the method should be quite specific.	5.0
	Score	10.0
D 0.7 Weighted Score 0.52	b. The sensitivity of the method requires an increase of 2.5×10^9 cells.	0.74
	Score	0.74
F 0.7 Weighted Score 2.24	b. The sensitivity of the method requires an increase of 2.5×10^9 cells.	0.70
	Score	3.20

TOTAL WEIGHTED SCORE - 17.3

Parameter	Pertinent Information or Factors Considered **	
16. Increase in Steroids as a Function of Time by Mass Spectrometry. Only those questions which require answers different from III-12 are considered.		
B 0.7	a. The test depends upon the detection of characteristic mass numbers which can be identified with steroids.	2.5
Weighted Score 1.75		
C 1.0	a. The number of possible steroids (e.g., esters) makes the number of different possible masses quite large and this in turn makes it possible to confuse steroids with other substances having the same mass.	0
Weighted Score 5.0	Score	5
TOTAL WEIGHTED SCORE - 16.1		
17. Increase in Fatty Acids (esterified and free) as a Function of Time by Gas Chromatography with Mass Spectrometer Detector.		
A 0.7	a. Microorganisms contain fatty acids and they are widely distributed.	2.0
	b. All organisms contain fatty acids.	2.0
	c. Fatty acids can be analyzed by gas chromatography without prior chemical reactions, but it is essential in this case to first obtain the free fatty acids by hydrolyzing the esters. In addition, nutrients must be provided to grow the organisms.	0
	d. Fatty acids are essential constituents, in the form of phospholipids, of all cell membranes.	2.0
	e. Fatty acids can be easily produced by abiogenic processes. Even the even numbered homologs can be selectively synthesized.	0
Weighted Score 4.2	Score	6.0
B 0.7	a. The test depends upon the retention characteristics of fatty acids on appropriate columns.	2.5
	b. Fatty acids are essential components of cell membranes.	2.5
	c. The technique searches for all fatty acids which are volatile within the temperature program.	2.5
	d. It does not seem possible that a living system can function without a membrane to isolate it from the environment. However, a membrane need not include fatty acids in its structure. On the other hand, especially on Mars, a water barrier is essential and if metabolic products are utilized to construct membranes the fatty acids, because of their ability to complex (in difference to hydrocarbons) with other biological substances (e.g. proteins) make them logical candidates.	2.5
Weighted Score 7	Score	10.0

Parameter	Pertinent Information or Factors Considered **	
C 1.0		
	a. If the chromatographic conditions are reigorously selected the method should be quite specific, but fatty acids can be produced without cell division.	0.
	b. The test procedure is not materially affected by changes in the environment.	5.0
Weighted Score 5.0	Score	5.0
D 0.7	a. Solvent extraction and chemical treatment is required.	0
	b. The sensitivity of the method requires an increase of 10^6 cells.	1.8
	c. The experiment requires that reproduction take place.	0
Weighted Score 1.26	Score	1.8
E 0.7	a. The time necessary for the cell reporduction process may be very long.	0
	b. The sensitivity of the method requires an increase of 10^6 cells.	1.5
	c. The technique relies upon the accumulation of fatty acid esters and free fatty acids over a time period. A greater time period increases the likelihood the event will be detected.	2.5
	d. Reproduction of complete cells is necessary.	0
Weighted Score 2.8	Score	4.0
F 0.4	a. The composition of the Martian atmosphere appears to be favorable to fatty acid synthesis, since under anaerobic conditions fats are produced in preference to carbohydrates.	5.0
	b. The Martian temperatures are variable enough to make selection of a proper incubation temperature difficult.	2.0
Weighted Score 2.8	Score	7.0

TOTAL WEIGHTED SCORE - 23.1

Parameter	Pertinent Information or Factors Considered **	
18. Increase in Fatty Acids (esterified and free) as a Function of Time by Mass Spectrometry. Only questions requiring answers different from III-17 are considered.		
B 0.7 Weighted Score 7	a. The test depends upon the detection of substances that have specific masses which is an intrinsic property of the fatty acid.	2.5
	Score	10.0
C 1.0	a. The numbers and types of fatty acids are so great that the applicable mass numbers cover a span which contains many substances other than fatty acids. In addition, fatty acids can be produced without cell division.	0
Weighted Score 5	Score	5
TOTAL WEIGHTED SCORE - 23.1		
19. Increases in Fatty Acids (esterified and free) as a Function of Time by Infrared Spectrophotometry. Only questions requiring answers different from III-17 are considered.		
B 0.7	a. The test depends upon the detection of specific absorption bands and this is an intrinsic property of the molecule.	2.5
	c. The technique will detect all fatty acids.	2.5
Weighted Score 7	Score	10
C 1.0	a. Lipid soluble substances other than fatty acids will absorb in the IR.	0
Weighted Score	Score	5
D 0.7	b. The sensitivity of the method requires an increase of 10^9 cells.	0.9
Weighted Score 0.63	Score	0.9
E 0.7	b. The sensitivity of the method requires an increase of 10^9 cells.	0.75
Weighted Score 2.28	Score	3.25
TOTAL WEIGHTED SCORE - 22.0		

Parameter	Pertinent Information or Factors Considered **	
20. Increase in Fatty Acids (esterified and free) as a Function of Time by Colorimetry. Only questions requiring answers different from III-17 are considered.		
A 0.7	c. This technique requires extraction of fats, saponification, separation by column chromatography, methylation and reaction with hydroxylamine and ferric chloride. In addition, nutrients must be provided to grow the organisms.	0
Weighted Score 4.2	Score	6.0
B 0.7	a. The test depends upon the reactivity of carboxyl group and oil fatty acids must possess this group.	2.5
	c. The technique is applicable to all fatty acids.	2.5
Weighted Score 7	Score	10.0
D 0.7	b. The sensitivity of the method requires an increase of 2.5×10^9 cells.	0.84
Weighted Score 0.59	Score	0.84
E 0.7	b. The sensitivity of the method requires an increase of 2.5×10^9 cells.	0.70
Weighted Score 2.24	Score	3.20
TOTAL WEIGHTED SCORE - 21.8		
21. Increase in Phospholipids as a Function of Time by Colorimetric Analysis for Phosphorus.		
A 0.7	a. Microorganisms contain phospholipids and they are widely distributed.	2
	b. All organisms contain phospholipids.	2
	c. The method requires extraction with lipid solvent, oxidation and analysis for phosphate by the method of Martin and Doty. In addition, nutrients must be provided to grow the cells.	0
	d. Phospholipids are present in all cell membranes. However, it is possible that substances such as sulfolipids could carry out the same function.	0
	e. It should be possible to produce phospholipids by abiogenic methods.	0
Weighted Score 2.8	Score	4

Parameter	Pertinent Information or Factors Considered **	
B 0.7	a. The test depends upon analysis for the phosphate moiety of the phospholipid and this is a critical part of the phospholipid molecule.	2.5
	b. Phospholipids are essential constituents of biological membranes.	2.5
	c. The technique is applicable to all phospholipids.	2.5
	d. Phospholipids could conceivably be replaced by sulfolipids.	0
	Score	7.5
Weighted Score 5.25		
C 1.0	a. The technique is quite specific for inorganic phosphate, but phospholipids can be produced without cell division.	0
	b. Small changes in environmental parameters do not affect the test procedure.	5.0
	Score	5.0
Weighted Score 5.0		
D 0.7	a. A solvent extraction is required.	0
	b. The sensitivity of the technique requires an increase of 3.6×10^6 cells.	1.68
	c. Reproduction of cells is a necessary part of the experiment.	0
	Score	1.68
Weighted Score 1.18		
E 0.7	a. The time required for cell division may be quite long.	0
	b. The sensitivity of the technique requires an increase of 3.6×10^6 cells.	1.4
	c. The experiment relies upon the synthesis of phospholipids over during cell division over a period of time. A greater time period increases the likelihood the event will be detected.	2.5
	d. The experiment requires active cell division.	0
	Score	3.9
Weighted Score 2.73		
F 0.4	a. The anaerobic Martian atmosphere could favor the accumulation of phospholipids since such conditions generally favor the production of fats rather than carbohydrates.	5
	b. The Martian temperatures are variable enough to make selection of an appropriate incubation temperature difficult.	2
	Score	7
Weighted Score 2.8		

TOTAL WEIGHTED SCORE - 21.9

IV. UNIQUE SUBSTANCES ASSOCIATED WITH LIVING SYSTEMS

The substances considered in this analysis are materials which are not macromolecular constituents although they may be derived from such substances. They owe their uniqueness to the fact that they are precursor substances for the synthesis of macromolecules or essential components of enzymes or structures such as membrances and cell walls. Many of these substances (e.g., steroids, porphyrins and flavins) appear to be elaborated only by living organisms and detection of these substances is strongly suggestive of the existence or past existence of living organisms.

IV. DETECTION OF UNIQUE SUBSTANCES ASSOCIATED WITH LIVING SYSTEMS

1. Amino Acids by Gas Chromatography with Mass Spectrometer Detector

Parameter	Pertinent Information or Factors Considered **	
A	a. Microorganisms contain amino acids and they are widely distributed.	2
0.7	b. All organisms contain amino acids.	2
	c. Amino acids must be converted to volatile esters.	0
	d. No other substances can be utilized for the synthesis of proteins and proteins appear to be indispensible for life.	2
	e. Amino acids are easily produced by abiogenic processes.	0
Weighted Score 4.2	Score	6
B	a. The test requires a carboxyl group which can be esterified. The carboxyl group is a necessary part of an amino acid.	2.5
0.7	b. Amino acids are the precursor substances for protein synthesis.	2.5
	c. The technique tests for all amino acids which can be esterified and volatilized.	2.5
	d. No other substances can be utilized for the synthesis of proteins.	2.5
Weighted Score 7.0	Score	10.0
C	a. If the column is properly selected and the temperature accurately programmed the method is quite specific.	5
1.0	b. Small environmental changes do not effect the test procedure.	5
Weighted Score 10	Score	10
D	a. The amino acids must be extracted from the sample with an appropriate solvent.	0
0.7	b. The sensitivity of the method requires 1.67×10^5 cells.	2.12
	c. A physiological change is not required for the detection of amino acids.	3
Weighted Score 3.57	Score	5.1

Parameter	Pertinent Information or Factors Considered **	
E 0.7	a. A dynamic biological function is not necessary for the detection of amino acids.	2.5
	b. The sensitivity of the method requires 1.67×10^5 cells.	1.75
	c. Only if the system contains actively synthesizing organisms.	0
	d. Amino acids can be detected in biologically inactive systems.	2.5
Weighted Score 4.72	Score	6.75
F 0.4	a. The atmospheric composition of Mars does not preclude the synthesis of amino acids.	5
	b. The Martian temperatures do not make the synthesis and accumulation of amino acids unlikely.	5
Weighted Score 4	Score	10

TOTAL WEIGHTED SCORE - 33.5

2. Amino Acids by Mass Spectrometry. Only questions requiring answers different from IV-1 are considered.

C 1.0	a. The technique detects amino acids esters by their mass. The number of and types of amino acids is great enough to cover a very broad range of masses so that other compounds with the same masses could be confused with the amino acids.	0
Weighted Score 5	Score	5

TOTAL WEIGHTED SCORE - 28.3

3. Amino Acids by Colorimetry (Ninhydrin). Only questions requiring answers different from IV-1 are considered.

A 0.7	c. The amino acids must be separated from other substances by column chromatography and treated with ninhydrin.	0
Weighted Score 4.2	Score	6
B 0.7	a. The test requires an amino group and all amino acids contain this group.	2.5
	c. The technique tests for all amino acids which react with ninhydrin.	2.5
Weighted Score 7	Score	10.0
D 0.7	b. The sensitivity of the method requires 5×10^5 cells.	1.95
Weighted Score 3.46	Score	4.95

Parameter	Pertinent Information or Factors Considered **	
E 0.7	b. The sensitivity of the method requires 5×10^5 cells.	1.62
Weighted Score 4.63	Score	6.62

TOTAL WEIGHTED SCORE - 33.3

4. Purine and Pyrimidine Bases by UV Absorbance.

A 0.7	a. Microorganisms contain purine and pyrimidine bases and they are widely distributed.	2
	b. All terrestrial organisms contain purine and pyrimidine bases.	2
	c. The method requires extraction of the bases from the sample and separation by chromatography.	0
	d. The purine and pyrimidine bases are the precursor substance of the nucleic acids. It is conceivable that Martian organisms do not utilize nucleic acids.	0
	e. Purine and pyrimidine bases can be produced abiogenically.	0
Weighted Score 2.8	Score	4
B 0.7	a. The test depends upon the UV absorption of the bases and this is an intrinsic property of the molecules.	2.5
	b. Purine and pyrimidine bases are precursor substances for nucleic acid synthesis.	2.5
	c. The technique test for all the bases.	2.5
	d. The purine and pyrimidine bases are the precursor substance of the nucleic acids. It is conceivable that Martian organisms do not utilize nucleic acids.	0
Weighted Score 5.25	Score	7.5
C 1.0	a. If the chromatographic procedure is carried out carefully and the UV spectrum obtained the method is quite specific.	5
	b. Small changes in the environment do not affect the method.	5
Weighted Score 10	Score	10
D 0.7	a. The bases must be extracted from the sample and chromatographically separated.	0
	b. The sensitivity of the method requires 4×10^6 cells.	1.68
	c. A physiological change is not required to detect purines and pyrimidines.	3.0
Weighted Score 3.38	Score	4.68

Parameter	Pertinent Information or Factors Considered **	
E 0.7	a. A dynamic biological function is not necessary for the detection of purines and pyrimidines.	2.5
	b. The sensitivity of the method requires 4×10^6 cells.	1.4
	c. Only if the system contains actively synthesizing organisms.	0
	d. Purine and pyrimidine bases can be detected in biologically inactive systems.	2.5
Weighted Score 4.48	Score	6.4
F 0.4	a. The atmospheric composition of Mars is not incompatible with the synthesis of purines and pyrimidines.	5
	b. The Martian temperatures do not make the synthesis and accumulation of purines and pyrimidines unlikely.	5
Weighted Score 4	Score	10

TOTAL WEIGHTED SCORE - 29.9

5. Detection of Carbohydrates by Gas Chromatography with Mass Spectrometer Detector

A 0.7	a. Microorganisms contain carbohydrates and they are widely distributed.	2
	b. Carbohydrates are found in all terrestrial organisms.	2
	c. The method requires hydrolysis of the polysaccharide material and conversion of the monosaccharides to methyl esters.	0
	d. Carbohydrates are readily replaced by other substances both structurally and metabolically.	0
	e. Carbohydrates can be produced by abiogenic processes.	0
Weighted Score 2.8	Score	4

Parameter	Pertinent Information or Factors Considered **	
B 0.7	a. The test depends upon the presence of hydroxyl groups which can be methylated to form volatile derivatives. Hydroxyl groups are an intrinsic part of carbohydrate molecules.	2.5
	b. Carbohydrates account for a considerable amount of the metabolic fuel utilized by organisms, but other substances can be substituted.	0
	c. The technique analyzes for all volatile methylated saccharides.	2.5
	d. Carbohydrates are often replaced by other materials (e.g. proteins and fats) in biological systems.	0
Weighted Score 3.5	Score	5.0
C 1.0	a. The GL chromatographic separation of saccharides coupled with mass data is quite specific.	5
	b. Small changes in the environment do not alter the test procedure.	5
Weighted Score 10	Score	10
D 0.7	a. Hydrolysis and extraction of the carbohydrates is required.	0
	b. The sensitivity of the technique requires 1.6×10^5 cells.	2.0
	c. A physiological change is not necessary for the detection of carbohydrates.	3.0
Weighted Score 3.5	Score	5.0
E 0.7	a. A dynamic biological function is not required for the detection of carbohydrates.	2.5
	b. The sensitivity of the method requires 1.6×10^5 cells.	1.7
	c. Only if the system contains actively synthesizing organisms.	0
	d. Carbohydrates can be detected in biologically inactive systems.	2.5
Weighted Score 4.69	Score	6.7
F 0.4	a. The anaerobic conditions on Mars do not favor the biological synthesis of carbohydrate materials. On the other hand, their biosynthesis is not precluded.	2.5
	b. The Martian temperatures are not incompatible with the synthesis and accumulation of carbohydrates.	5
Weighted Score 3.0	Score	7.5

TOTAL WEIGHTED SCORE - 27.5

Parameter	Pertinent Information or Factors Considered **	
6. Detection of Carbohydrates by Mass Spectrometry. Only questions requiring answers different from IV-5 are considered.		
C 1.0 Weighted Score 5	a. Other substances can have the same mass as methylated saccharides.	0
	Score	5
D 0.7 Weighted Score 3.5	b. The sensitivity of the method requires 3.2×10^5 cells.	2.0
	Score	5.0
F 0.7 Weighted Score 4.69	b. The sensitivity of the method requires 3.2×10^5 cells.	1.7
	Score	6.7
TOTAL WEIGHTED SCORE - 22.5		
7. Detection of Carbohydrates by Colorimetry. Only questions requiring answers different from IV-5 are considered.		
A 0.7 Weighted Score 2.8	c. The method requires reaction of specific chemicals with the saccharide to produce a colored compound.	0
	Score	4
B 0.7	a. The test depends upon interaction of certain end groups with specific chemicals. These end groups are not necessarily required for the biological functioning of carbohydrates.	0
	b. The technique does not detect all types of carbohydrates.	0
Weighted Score 0	Score	0
C 1.0 Weighted Score 5.0	a. Many substances other than carbohydrates will interact to produce colors.	0
	Score	5
D 0.7 Weighted Score 2.98	b. The sensitivity of the technique requires 8×10^7 cells.	1.26
		4.26
E 0.7 Weighted Score 4.2	b. The sensitivity of the technique requires 8×10^7 cells.	1.03
	Score	6.0
TOTAL WEIGHTED SCORE - 20.0		

Parameter	Pertinent Information or Factors Considered**	
8. Detection of Porphyrins.	The detection of porphyrins is considered in I - 12 and 13	

TOTAL WEIGHTED SCORE
- a. Photosynthetic Pigments Only - 28.9
- b. Porphyrins other than Photosynthetic Pigments - 31.6

9. Detection of Fatty Acids. The fatty acids are considered in III-17, 18, 19 and 20. Of the methods considered detection by gas chromatography rated highest. Therefore, only this method and questions requiring answers different from III-17 are considered.

C
1.0
Weighted Score 10.0

a. If the chromatographic conditions and preparatory procedures are rigorously selected the method should be reasonably specific. 5.0

Score 10.0

D
0.7
Weighted Score 3.36

c. No physiological change is necessary for the detection of fatty acids. 3.0

Score 4.8

E
0.7

a. A dynamic biological function is not required for the detection of fatty acids. 2.5

c. The technique can take advantage of an increased time period only if the system contains actively synthesizing organisms. 0

d. Fatty acids can be detected in a biologically inactive system. 2.5

Weighted Score 4.55

Score 6.5

F
0.4
Weighted Score 4

b. The Martian temperatures do not make the synthesis and accumulation of fatty acids unreasonable. 5.0

Score 10.0

TOTAL WEIGHTED SCORE - 33.1

Parameter	Pertinent Information or Factors Considered **	
10. Detection of Steroids.	The steroids are considered in III - 12, 13, 14, 15 and 16. Of the methods considered, detection by gas chromatography with a mass spectrometer detector rated highest. Therefore, only this method and questions requiring answers different from III-12 are considered.	
A 0.7 Weighted Score 3.15	c. Steroids can be extracted with lipid solvents and analyzed by gas chromatography without preparing derivatives.	2
	Score	4.5
C 1.0 Weighted Score 10	a. If the extraction process and gas chromatographic separation is carried out properly the method should be reasonably specific.	5.0
	Score	10.0
D 0.7 Weighted Score 3.0	c. A physiological change is not required for the detection of steroids.	3
	Score	4.35
E 0.7	a. A dynamic biological function is not required for the detection of steroids.	2.5
	c. The technique can take advantage of an increased time period only if the system contains actively synthesizing organisms.	0
	d. Steroids can be detected in a biologically inactive system.	2.5
Weighted Score 4.28	Score	6.12
F 0.4 Weighted Score 4	b. The Martian temperatures do not make the biological synthesis and accumulation of steroids unlikely.	5
	Score	10.0
TOTAL WEIGHTED SCORE - 30.3		
11. Detection of Phospholipids.	The phospholipids are considered in III - 21. Only questions requiring answers different from III - 21 are considered.	
C 1.0 Weighted Score 10	a. The technique is quite specific for inorganic phosphate.	5.0
	Score	10.0
D 0.7 Weighted Score 3.38	c. A physiological change is not required for the detection of phospholipids.	3.0
	Score	4.68

Parameter	Pertinent Information or Factors Considered **	
E 0.7	a. A dynamic biological function is not required for the detection of phosolipids.	2.5
	c. Additional time is advantageous only if the system contains actively synthesizing organisms.	0
	d. Phospholipids can be detected in a biologically inactive system.	2.5
Weighted Score 4.48	Score	6.4
F 0.4	b. The Martian temperatures do not make the synthesis and accumulation of phospholipids unlikely.	5
Weighted Score 4	Score	10
TOTAL WEIGHTED SCORE - 29.4		
12. Detection of Total Flavins	a. Microorganisms contain flavins and they are widely distributed.	2
	b. All terrestrial organisms contain flavins.	2
A 0.7	c. The technique requires photolysis of the flavins and solvent extractions.	0
	d. The flavins are utilized as prosthetic groups in a number of enzymes and as electron transporting substances. Many of these enzymes are not essential and the electron transporting properties can be accomplished by other substances.	0
	e. The flavins are not easily produced by abiogenic methods.	2
Weighted Score 4.2	Score	6
B 0.7	a. The test depends upon the conversion of flavins to lumiflavin. All known flavins undergo this reaction and the resulting fluorescence is an intrinsic property of the lumiflavin molecule.	2.5
	b. Flavins are important enzyme components and electron transporting substances.	2.5
	c. The technique analyzes for all types of flavins.	2.5
	d. It is quite possible that other substances can replace flavins.	0
Weighted Score 5.25	Score	7.5
C 1.0	a. If the procedure is carefully carried out the technique is quite specific.	5
	b. Small environmental changes do not materially affect the procedure.	5
Weighted Score 10	Score	10

Parameter	Pertinent Information or Factors Considered **	
D 0.7	a. Several extraction procedures are required.	0
	b. The sensitivity of the method requires 2.5×10^6 cells.	1.7
	c. A physiological change is not required for the detection of flavins.	3
Weighted Score 3.29	Score	4.7
E 0.7	a. A dynamic biological function is not necessary for the detection of flavins.	2.5
	b. The sensitivity of the method requires 2.5×10^6 cells.	1.44
	c. An increase in time period is advantageous only if the system contains dividing organisms.	0
	d. Flavins can be detected in biologically inactive systems.	2.5
Weighted Score 4.5		6.44
F 0.4	a. The anaerobic nature of the Martian atmosphere would appear to be favorable for the development of flavin systems, since flavin containing enzymes can transfer hydrogen to acceptors other than oxygen.	5.0
	b. The Martian temperatures do not make the biosynthesis and accumulation of flavins unlikely.	5.0
Weighted Score 4	Score	10

TOTAL WEIGHTED SCORE - 31.2

V. DETECTION OF CATALYTIC ACTIVITY

The detection of catalytic activity is of greatest value when it can be related to a well known and essential biological catalysis. In essence, this means that the methods employed should be designed to look for specific enzymes. However, the problem immediately encountered, in this case, is that enzymes are usually detected by their action on specific substrates and therefore a substrate must be provided. The probability that Martian life will not utilize the substrate in the manner predicted can be very high. Thus, one is confronted with the paradox of wanting to search for a nonsubstrate specific biological catalyst; this is indeed a rare thing.

V. DETECTION OF CATALYTIC ACTIVITY

1. Dehydrogenases, Reduction of NAD and NADP coupled to dye reduction under anaerobic conditions.

Parameter		Pertinent Information or Factors Considered **	
A			
0.7		a. Microorganisms contain dehydrogenases and they are widely distributed.	2
		b. Every organism contains a NAD or NADP dependent apodehydrogenase.	2
		c. The technique is most effective if a substrate is supplied. However, the uncertainty involved in selecting an appropriate substrate makes use of any endogenous substrates attractive. An appropriate dye must be supplied.	0
		d. Dehydrogenases which do not transfer hydrogen to NAD or NADP and therefore may not reduce the dye are well known.	0
		e. Many abiogenically produced substances can reduce dyes.	0
		Score	4
Weighted Score	2.8		
B			
0.7		a. Reduction of the dye demonstrates hydrogen transfer and this is the biological function of the dehydrogenases.	2.5
		b. Dehydrogenases are indispensable components of the metabolic system.	2.5
		c. The technique tests only for dehydrogenase systems which can pass hydrogens to the particular dye(s) selected.	0
		d. Dehydrogenases which do not transfer hydrogen to NAD or NADP and therefore may not reduce the dye are well known.	0
		Score	5
Weighted Score	3.5		
C			
1.0		a. Factors other than reduction of the dye can cause a change in spectrophotometric absorption.	0
		b. A false-positive result is not likely because of small changes in temperature or pH and the atmospheric composition is controlled.	5
		Score	5
Weighted Score	5		
D			
0.7		a. The presence of strongly oxidizing or reducing substances would make the test unreliable. Therefore, the biological material would have to be separated from these substances.	0
		b. The sensitivity of the method requires 4.5×10^9 cells.	0.78
		c. A physiological change in the organism is not required, but fine division of the tissue of macroorganisms is desirable.	3
		Score	3.78
Weighted Score	2.65		

Parameter	Pertinent Information or Factors Considered **	
E 0.7	a. The turn over rate of an unknown dehydrogenase system is not possible to estimate, but it could be very slow.	0
	b. The sensitivity of the method requires 4.5×10^9 cells.	0.65
	c. The technique relies upon the accumulated reduction of the dye. A longer time period makes detection more likely.	2.5
	d. A dynamic enzymic system is essential for the test procedure.	0
	Score	3.15
Weighted Score 2.2		
F 0.4	a. The near anaerobic conditions on Mars indicate that Martian dehydrogenases may pass hydrogen to substances other than those which utilize oxygen as the terminal acceptor. Thus, they may also be capable of reducing dyes.	5
	b. The Martian temperatures should not prevent the operation of dehydrogenases. However, because of the great variability in the Martian temperatures, it may be difficult to select a proper incubation temperature.	2
	Score	7
Weighted Score 2.8		

TOTAL WEIGHTED SCORE - 13.9

2. Phosphatase by Fluorometry.

A 0.7	a. Microorganisms contain phosphatase and they are widely distributed.	2
	b. All organisms contain phosphatases.	2
	c. An appropriate substrate for the phosphatase must be supplied.	0
	d. Phosphatases could conceivably be replaced by sulfatases if on Mars the biologically important oxy anion is sulfate.	0
	e. Abiogenic substances can catalyze the dephosphorylation of some organic phosphates.	0
	Score	4
Weighted Score 2.8		

Parameter		Pertinent Information or Factors Considered **	
B 0.7		a. The test depends upon liberation of the phosphate group from a fluorescent substrate. Dephosphorylation is the essential function of a phosphatase.	2.5
		b. Phosphatases are necessary in order to maintain the inorganic phosphate pool.	2.5
		c. The technique tests the process of dephosphorylation.	2.5
		d. It is possible that the biologically essential oxy anion on Mars could be sulfate or that the available inorganic phosphate pool is maintained by another process, as,for example, adaptation to a much lower pH.	0
Weighted Score	5.25	Score	7.5
C 1.0		a. The techniquesgive a positive result only when the substrate is dephosphorylated, but it is possible that the Martian material itself may be fluorescent.	3
		b. Changes in pH and temperature can result in dephosphorylation of organic phosphates.	0
Weighted Score	3	Score	3
D 0.7		a. It is desirable to separate the biological material from other substances, but the test can be conducted on relatively crude materials.	2
		b. The sensitivity of the method requires 5.3×10^3 cells.	2.55
		c. A physiological change is not essential.	3.0
Weighted Score	5.28	Score	7.55

Parameter	Pertinent Information or Factors Considered **	
E 0.7	a. The turn over rate of an unknown phosphatase system is not possible to estimate, but it could be very slow.	0
	b. The sensitivity of the technique requires 5.2×10^3 cells.	2.1
	c. The technique relies upon the accumulated dephosphorylation of the substrate and a longer time period makes detection more likely.	2.5
	d. A dynamic enzymic system is essential for the test procedure.	0
	Score	4.6
Weighted Score 3.22		
F 0.4	a. The atmospheric composition of Mars does not appear to make the existence of phosphatases unlikely.	5
	b. The Martian temperatures are variable enough to make the selection of an incubation temperature difficult. However, phosphatases are not especially sensitive to temperature.	4
	Score	9
Weighted Score 3.6		

TOTAL WEIGHTED SCORE - 23.2

3. Catalase by Microrespirometry.

A 0.7	a. Aerobic microorganisms contain catalase, but these are not universally distributed.	1.0
	b. Obligate anaerobic bacteria and many facultative anaerobic bacteria do not contain catalase.	0
	c. Hydrogen peroxide must be supplied in order to detect the enzyme.	0
	d. Catalase can be replaced by peroxidases.	0
	e. Abiogenic materials (e.g. Cu and Fe salts) will cause the catalytic decomposition of H_2O_2.	0
	Score	1
Weighted Score 0.7		

Parameter	Pertinent Information or Factors Considered **	
B 0.7	a. Abiogenic materials (e.g. heavy metal salts) can cause the catalytic decompositon of H_2O_2,	0
	b. It has been claimed that catalase protects an organism from the damaging effects of H_2O_2.	2.5
	c. The technique searches for oxygen evolution which is an end product of the reaction of catalase with H_2O_2.	2.5
	d. The technique tests for the destruction of H_2O_2 only by observing oxygen evolution. H_2O_2 can also be enzymatically destroyed by peroxidases without the evolution of oxygen.	0
Weighted Score 3.5	Score	5.0
C 1.0	a. The technique measures a differential increase in pressure. Such increases can be caused by factors other than the evolution of oxygen.	0
	b. Small changes in temperature and pressure can result in positive manometric readings.	0
Weighted Score 0	Score	0
D 0.7	a. Separation of the biological entitiy from other materials is necessary if a reasonably specificity is to be obtained, but operationally the technique can be applied to crude samples.	2
	b. The sensitivity of the method requires 1.2×10^2 cells.	3
	c. Disruption of the cell membrane is required for effective action of catalase on H_2O_2.	0
Weighted Score 3.5	Score	5

Parameter	Pertient Information or Factors Considered **	
E 0.7	a. The turn over rate of catalase using H_2O_2 as the substrate is exceedingly high.	2.5
	b. The sensitivity of the method requires 1.2×10^2 cells.	2.5
	c. The technique relies upon the total evolution of oxygen and thus a longer time period makes detection more likely.	2.5
	d. A dynamic enzymic system is essential for the test procedure.	0
	Score	7.5
Weighted Score 5.25		
F 0.4	a. The near anaerobic conditions on Mars indicate that Martian organisms will be anaerobic. On Earth anaerobic organisms do not contain catalase.	0
	b. Catalase activity is especially insensitive to temperature conditions between 4 and 30°C. Thus the Martian temperatures should not make the existence of catalase unlikely.	5
	Score	5
Weighted Score 2		

TOTAL WEIGHTED SCORE - 15.0

Parameter	Pertinent Information or Factors Considered **	
4. Oxygen Exchange Between Water and Oxyanions - (Catalyzed in a manner analagous with the action of inorganic pyrophosphatase on water and phosphate)		
A 0.7	a. The function appears to occur in all microorganisms and they are widely distributed.	2
	b. All organisms appear to contain pyrophosphatase.	2
	c. Oxygen-18 as each of the potential oxyanions must be supplied.	0
	d. The biological role played by inorganic pyrophosphatase can be carried out by other phosphatases (e.g. alkaline phosphatase) which do not have the property of catalyzing oxygen exchange between water and oxyanions.	0
	e. In pure solutions without pyrophosphatase the exchange rate is negligible, but the possibility of abiogenic catalysis hasn't been completely explored.	1
Weighted Score 3.5	Score	5
B 0.7	a. The test depends upon ionization of phosphate groups.	2.5
	b. The exchange of oxygen from water with oxyanions is not a necessary biological function, but it is difficult to see how it can be avoided if the essential process of phosphorolysis occurs.	2.5
	c. The test can be used to examine oxygen exchange between any oxyanion and water.	2.5
	d. The biological role played by inorganic pyrophosphatase can be carried out by other phosphatases (e.g. alkaline phosphatase) which do not have the property of catalyzing oxygen exchange between water and oxyanions.	0
Weighted Score 5.25	Score	7.5
C 1.0	a. The technique gives a positive result for mass 19 (major peak). There appear to be few, if any materials, other than $O^{18}H$ which can be expected under the conditions employed.	5.0
	b. False-negative results can be brought about by fluoride ions which make Mg^{++} unavailable for the reaction.	0
Weighted Score 5.0	Score	5.0

Parameter	Pertinent Information or Factors Considered **	
D 0.7	a. The reaction can be carried out with a crude sample, but the water must be separated for the analysis.	2
	b. The sensitivity of the method requires 10^9 cells.	0.9
	c. A physiological change in an organism is not required.	3.0
Weighted Score 4.1	Score	5.9
E 0.7	a. The turn over rate of an unknown enzyme can not be estimated but it could be very low.	0
	b. The sensitivity of the method requires 10^9 cells.	0.75
	c. The technique relies upon the accumulation of H_2O^{18} and a longer time period makes detection more likely.	2.5
	d. A dynamic enzymic system is essential for the test procedure.	0
Weighted Score 2.28	Score	3.25
F 0.4	a. The atmospheric composition on Mars does not make the reaction unlikely.	5
	b. The Martian temperatures are variable enought to make the selection of an incubation temperature difficult. Phosphatases are not especially sensitive to temperature, but enzymes acting on other oxyanions may not behave similarly.	3
Weighted Score 3.2	Score	8

TOTAL WEIGHTED SCORE - 23.3

VI. DETECTION OF ORGANIZATION

Organization can be detected by examination of macro, micro or molecular structures. However, micro or macro examinations are often misleading in that one looks for regular structures and patterns and these can be produced by external forces (e.g., wind or water acting directionally on a lump of clay). On the other hand, biological organization is obtained from the inside out, that is, it results from molecular organization. Thus, the detection of molecular organization is preferable to that of structural organization. One of the fundamental molecular aspects of the order in biological entities is optical purity and its detection is perhaps the surest biological sign of organization.

VI. DETECTION OF ORGANIZATION

1. Optical Purity by Polarimetry

Parameter	Pertinent Information or Factors Considered **	
A 0.7	a. Microorganisms contain substances which are predominantly of one optical form and they are widely distributed.	2
	b. All terrestrial organisms contain substances which are predominantly of one optically active form.	2
	c. Optical activity is an intrinsic property of asymmetric molecules.	2
	d. Optical purity is essential to life since an ordered structure would otherwise be impossible. Organization at the molecular level is equivalent to the necessary life-information which makes it possible to derive order from order as distinct from non-living processes in which order leads to disorder.	2
	e. An over-all asymmetry in which one optical form predominates is not produced and maintained by abiogenic processes.	2
Weighted Score 7	Score	10
B 0.7	a. The test is based upon an intrinsic property of asymmetric molecules.	2.5
	b. Optical purity is valuable since it permits order in structures.	2.5
	c. The technique tests for rotation of polarized light and this is a direct function of optical purity.	2.5
	d. Optical purity is essential to life since an ordered structure would otherwise be impossible. Organization at the molecular level is equivalent to the necessary life-information which makes it possible to derive order from order as distinct from non-living processes in which order leads to disorder.	2.5
Weighted Score 7	Score	10.0
C 1.0	a. Only a perponderance of one optical from should cause rotation of polarized light. It is possible that a Faraday effect could also cause such rotation, but the possibility of confusing such rotation with that due to asymmetric molecules is remote.	5
	b. Small changes in pH can cause racemization, but this would result in no response rather than a false positive.	5
Weighted Score 10	Score	10

Parameter	Pertinent Information or Factors Considered **	
D 0.7	a. It is necessary to dissolve the asymmetric substances in a suitable solvent.	0
	b. The sensitivity of the technique requires about 10^7 cells.	1.5
	c. A physiological change is not required for the detection of optical purity.	3.0
Weighted Score 3.15	Score	4.5
E 0.7	a. A dynamic biological function is not required for the detection of optical purity.	2.5
	b. The sensitivity of the method requires about 10^7 cells.	1.25
	c. An increased time period is advantageous only if the sample contains an actively proliferating organism.	0
	d. Detection of optical purity does not require a biologically active system at the time the detection is made.	2.5
Weighted Score 4.38	Score	6.25
F 0.4	a. The composition of the Martian atmosphere is not incompatible with the existence of optical purity.	5
	b. The Martian temperatures do not make the existence of optical purity unlikely.	5
Weighted Score 4	Score	10

TOTAL WEIGHTED SCORE - 35.5

Parameter	Pertinent Information or Factors Considered **	
2. Macroscopic Observation of Macrostructues with a Camera.		
A 0.7	a. Only macroorganisms (about 0.5 mm or greater in diameter) or macrostructures produced by microorganisms can be detected.	0
	b. Macroorganisms occur very irregularly in certain environments.	0
	c. No reaction with preselected chemicals is required.	2
	d. Life can be restricted to microorganisms.	0
	e. Macroscopic biological forms are easily confused with abiogenically produced structures.	0
Weighted Score 1.4	Score	2
B 0.7	a. The test depends only upon acquiring the object in the visual scan.	2.5
	b. A macroscopic structure can be advantageous to an organism under certain environments and conditions. For example, water retention per g or organism is increased by an increase in size which results in a lower external surface to mass ratio. Also in a macrostructure specialized tissues are developed, whereas this is generally not the case with microorganisms.	2.5
	c. The technique looks for any structure which has attributes that indicate organization. For example, structures constructed by organisms would be included.	2.5
	d. It is possible that no biologically formed macrostructure exist on Mars, but this is doubtful since even microorganisms produce macrostructures which can be recognized (e.g. Coral structures or colonies of microorganisms).	2.5
Weighted Score 7	Score	10.0
C 1.0	a. The property sought is visual evidence of macroscopic organization. It is possible that due to the perspective a substance could appear to be highly organized when it in fact is not.	0
	b. Small changes in the environment such as a change in lighting could cause false positive results.	0
Weighted Score 0	Score	0

Parameter	Pertinent Information or Factors Considered **	
D 0.7	a. The object can be viewed <u>in situ</u>.	4
	b. The sensitivity of the method requires 10^6 cells.	1.8
	c. No physiological change is required, but motion of the object would be very valuable.	3
	Score	8.8
Weighted Score 6.2		
E 0.7	a. A dynamic biological function is not required, but movement would be very informative. The rate of movement could however be very slow.	2.5
	b. The sensitivity of the method requires 10^6 cells.	1.5
	c. A long time period is advantageous only if the organism is reproducing.	0
	d. A dynamic function is not necessary for the detection of organization.	2.5
	Score	6.5
Weighted Score 4.55		
F 0.4	a. The composition of the Martian atmosphere does not make the existence of biologically organized macrostructures unlikely.	5
	b. The Martian temperatures are not incompatible with the existence of biologically organized macrostructures.	5
	Score	10
Weighted Score 4		

TOTAL WEIGHTED SCORE - 23.1

Parameter		Pertinent Information or Factors Considered **	
3. Microscopic Observation of Microstructures with a Light Microscope and Camera.			
A 0.7		a. Microorganisms are widely distributed.	2
		b. All organisms can be examined for microscopic organization.	2
		c. Reaction with stains could be very helpful.	0
		d. All living forms are microscopically organized.	2
		e. Many abiogenic substances are microscopically organized.	0
Weighted Score 4.2		Score	6
B 0.7		a. The test depends only upon acquiring the object in the visual scan.	2.5
		b. Microscopic organization is a reflection of molecular organization and this is essential to all living systems.	2.5
		c. The technique looks for any microscopic structure, including pieces of a macrostructures, which has attributes indicative of organization.	2.5
		d. It is possible that the unit Martian cell could be too small to be resolved by a light microscope.	0
Weighted Score 5.25		Score	7.5
C 1.0		a. The property sought is visual evidence of microscopic organization. It is possible that due to the perspective a substance could appear to be organized when it is not.	0
		b. Small changes in lighting can cause unorganized substances to appear to be organized and changes in temperature gradients can cause apparent uniform motion.	0
Weighted Score 0		Score	0
D 0.7		a. If there is to be any hope of finding a biological structure in the field of a microscope it must be separated from non-biological materials.	0
		b. The sensitivity of the method requires 3×10^4 cells per g of soil.	2.2
		c. A physiological change is not required, but observation of such a phenomenon (e.g. reproduction) would be invaluable.	3
Weighted Score 3.6		Score	5.2

Parameter	Pertinent Information or Factors Considered **	
E 0.7	a. A dynamic biological function is not required, but observation of streaming, reproduction, movement, etc. would be invaluable.	2.5
	b. The sensitivity of the method requires 3×10^4 cells per gram of non-living material.	1.92
	c. Only if the preparation contains organisms which are undergoing reproduction.	0
	d. Biologically inactive cells could be distinguished from other materials by their microscopic appearance, but such a state makes the task more difficult.	1.5
Weighted Score 4.14	Score	5.92
F 0.4	a. The composition of the Martian atmosphere does not make the existence of organized microscopic units of life unlikely.	5
	b. The Martian temperatures are not incompatible with the existence of organized microscopic units of life.	5
Weighted Score 4	Score	10

TOTAL WEIGHTED SCORE - 21.2

SELECTED REPRESENTATIVE EXPERIMENTS FROM THE RANKED LIST

I. ENERGY TRANSFER AND CONVERSION

A. Carbon Dioxide Evolution and Fixation - All of the CO_2 evolution and fixation experiments considered received about the same rank. These techniques utilize essentially the same equipment. Therefore, it seemed reasonable to include all of these techniques under this general heading.

1. Light-stimulated $C^{14}O_2$ fixation and dark $C^{14}O_2$ fixation as function of temperature.

a. Collect surface scraping of Martian soil.
b. Sieve, 20-40 mesh, to obtain sample uniformity.
c. Distribute 1-g portions to each of six incubation chambers.

1. Chamber dimensions: 2-3 cm diameter, 5-10 ml volume.
2. Two chambers with illumination facilities; 4 chambers dark.
3. All chambers equipped for temperature programming from 0°-1000°C. Requisite for incubation at specified temperatures and for pyrolysis of organic material to CO_2 at the end of incubation for gas phase counting of fixed activity.

d. Sterilize two chambers with ethylene oxide for four hours. (Complete sterilization is not essential. It is only necessary to obtain a significant reduction in biological activity by a reduction in population.) Meanwhile, remaining chambers may be exposed to ambient atmosphere.

e. Flush all chambers with N_2-argon-O_2 (low O_2) mixture while equilibrating three chambers at 0-1°C (one light, one dark, and one sterile) and remaining three at 25°C.
f. Inject $C^{14}O_2$ into all chambers to give CO_2 at about ambient partial pressures (ca. 8 mb).
g. Incubate at 0 and 25 degrees for two hours.
h. Acidify all samples with 10 N HCl and flush out $C^{14}O_2$ with N_2-argon while evaporating HCl by elevating temperature to boiling point at ambient pressure. Exhaust $C^{14}O_2$ to outside.
i. Pressurize dried sample chambers with O_2; heat to maximum temperature. (~1000°C)
j. Transfer evolved gases to β-counting chamber (ionization chamber or GM counter).
k. If negative (no detectable difference in total activity between samples in light, dark, and sterile controls or between samples at two different temperatures), recycle with new samples for 24-48 hours.
l. If still negative, recycle, but add aqueous medium to soil prior to incubation.

2. Evolution of CO_2 by <u>In Situ</u> Metabolism

a. Collect samples - may be surface or subsurface.
b. Obtain uniformity by sieving to 20-40 mesh.
c. Distribute 1-g portions (volume measurement) to each of four sample chambers.

    1. Chambers may be of any dimensions sufficient to hold samples, but with provisions for rapid diffusion to conductivity-CO_2 gage.

d. Sterilize two samples by four-hour incubation with ethylene oxide mixture (Note: complete sterilization is not essential).
e. Flush all chambers with CO_2-free, N_2-argon-O_2 (low O_2) mixture.
f. Equilibrate (while flushing) two chambers (one sterile, one control) at 0-1°C others at 30-35 degrees.
g. Stop flow of gas and observe change in CO_2 composition of chamber atmospheres by change in conductivity of $Ba(OH)_2$ solution (or, CO_2 specific coating on piezoelectric crystal device).
h. Positive results indicated by significantly higher CO_2 evolution (in 24-hour period) in samples over sterile controls and in elevated temperature sample over low temperature sample. If negative, continue for up to 72 hours. If still negative, repeat, but add aqueous medium to soil prior to incubation.

3. $C^{14}O_2$ Evolution from labeled substrates.

a. Obtain sample, may be either surface or subsurface.
b. Obtain uniformity in sample by sieving to 20-40 mesh.
c. During collection of sample and sieving, prepare substrates by flushing with CO_2 to remove $C^{14}O_2$ accumulated from radioactive, self-decomposition during storage. Substrate solution should be 0.01 M, C^{14} formaldehyde and glucose-U-C^{14} specific activities of 5 mc/m mole and 30 mc/m mole, respectively.
d. Distribute portions of about 0.2 g (measured by volumes) to each of four thermostated chambers with control for 0-150°C and with β counting systems arranged for measurement of diffusing gas from chambers. Sample chamber should be about 2 cm^2 in area of bottom. (See Gulliver descriptions for reasonable arrangement of chambers and counters.)
e. Heat two samples (controls) at 120 degrees for 15 minutes.
f. Equilibrate two samples at (one sterilized, one native) at 0-1°C and remaining two at 30°C.
g. Inject 0.2 ml substrate solution into each sample.
h. Incubate up to 24 hours, comparing rates of evolution of β-activity from experimental and control samples at two temperatures.
i. If results are negative (little or no difference in controls and experimental at a single temperature or between two experimental samples at two temperatures) repeat with larger samples for 48 hours.

4. $C^{14}O_2$ uptake in light and dark and subsequent evolution by metabolism (This system may not be as sensitive, but will probably be more definitive in a positive result and has more chance of success than the metabolic experiments relying upon more complex substrates).

a. Collect sample, surface scrapings.
b. Sieve to 20-40 mesh for sampling uniformity.
c. Transfer 1-g portions (volume measurement) to each of six sample chambers (See A1 procedure for description of sample chambers).

1. Three chambers are associated with β-counting devices for measuring β activity in gases diffusing from the chambers. (A series of samples.) Other three equipped with gas transfer facilities for eventual transfer to β-counter (B series of samples).
2. One A and one B chamber equipped with lighting facilities.

d. Two chambers sterilized (one A and one B series) by exposure to ethylene oxide for four hours. (Note: Complete inactivation is not essential, only a significant reduction of biological activity). Other chambers exposed to ambient atmosphere during sterilization.
e. With chambers equilibrated to 25°C, flush all chambers with N_2-argon-O_2 (low O_2) mixture. Turn on light (or open light port) on light samples.
f. Inject $C^{14}O_2$ to provide CO_2 at ambient CO_2 pressure.
g. Incubate all samples 2 hours.
h. Flush $C^{14}O_2$ from all chambers first with $C^{12}O_2$, then with Martian atmosphere or equivalent synthetic mixture.
i. Treat series B samples (one light, one dark, one sterile control) as in steps h, i, and j in procedure 1A to determine radioactivity fixed in each sample.
j. Meanwhile, follow rate of re-evolution of $C^{14}O_2$ from A series (one light, one dark, one sterile) in chambers. Determine differences in rates as a function of time. Positive results indicated by greater continued rate of evolution from light than dark sample and greater evolution from dark than sterile sample. Continue observation 24 hours.
k. If results are negative (indicated by failure to find difference in fixation in light, dark, and control samples in B series, failure to observe differences in evolution rates in A series, particularly in rates observed after several hours) repeat with longer incubation in $C^{14}O_2$, 4 to 24 hours.

II. Macromolecule Detection

A. <u>UV Spectrophotometry</u>

1. Acquire sample.
2. Homogenize sample in five volumes of 0.5 N NaOH.
3. Filter through sintered glass.
4. Dialyze against carbowax or water.
5. If dialyzed against carbowax, reconstitute solution with water.
6. Transfer solution to spectrophotometer and determine UV absorption against a dialyzed 0.5 N NaOH solution.

B. <u>Visible Spectrophotometry</u> (See Philco Aeronutronic Division Publication No. U-2769)

1. Add thiocarbocyanine dye to part of preparation obtained in step A-5 above.
2. Transfer solution to spectrophotometer and determine visible absorption against dialyzed 0.5 N NaOH solution plus dye.

h. Partition with 2 ml petroleum ether, two times.
i. Evaporate ether with N_2 stream.
j. Redissolve in 0.1 ml petroleum ether.
k. Transfer 0.1 to 10 μl to gas chromatogram (¼ in. x 60 cm, 200μ glass beads (acid washed) coated with 0.25-percent carbowax and 0.4-percent isophthalic acid (in volatile solvent). Temperature programmed from 90° to 190°, 3.3°C/min. Gas flow rate 80 ml/min. Ionization detector can be used.
l. If negative, repeat GLC with larger aliquot (to 10 μl) of final preparation.

3. Amino Acid Analysis as N-trifluoracetylamino acid methylesters.

a. Collect soil sample, transfer 2 to 5 g to extraction system.
b. Extract by homogenizing in 10 ml water.
c. Filter through diatomaceous earth, sintered glass.
d. Add equal volume of 10 N HCl and heat for one hour at 760 mm Hg. Reduce pressure and evaporate to dryness. Take up in 1-5 ml H_2O.
e. Pass through bed of carboxylic ion exchange resin (preconditioned to hydrogen ion form, washed free of acid) such as Duolite CS-101. About 20 ml bed volume, 100-200 mesh.
f. Wash column with 40 ml water.
g. Elute amino acids with 40 ml of 1N HCl.
h. Evaporate amino acid fraction to dryness under reduced pressure (Martian ambient may be sufficient) at T = 50° - 70°C.
i. Add 1 ml Trifluoroacetic anhydride, heat 10 minutes at 70 - 72°. (Enclosed to prevent evaporation.)
j. Evaporate reagent at 70 - 72° with stream of N_2.
k. Add 2 ml methanol and 2 ml of 5-percent diazo methane in ether and incubate 10 minutes. Add 0.1 ml glacial acetic acid.
l. Remove insoluble material by filtration through sintered glass.
m. Evaporate to dryness at 35 to 50°, with N_2 stream.
n. Dissolve in acetonitrile, 0.1 ml.
o. Transfer 2 μl to gas chromatograph with 2-foot column, 1.5 mm i.d. packed with Gas Chrom. P, coated with 5-percent neopentyl glycol succinate, temperature equilibrated at 65°, programmed for 1.5°C per minute increase for 20 minutes; 20 C/per minute for next 22.5 minutes and 4°C to 70 minutes.

p. If positive results not obtained, repeat GLC with larger aliquots.

4. Amino Acid Analysis by Pyrolysis - Gas Chromatography

a. Acquire sample.
b. Transfer to pyrolysis oven.
c. Initiate pre-set pyrolysis cycle.
d. Transfer vapors to chromatograph.
e. Do gas chromatography on 12' x (¼"); 15-percent Apiezon L, 4.5-percent carbowax 20 M and 3-percent phospheric acid on Chromosorb. Flow 10 ml/min 105-107°C. (See V.I. Oyama, North American Lunar and Planetary Colloquim, May 23, 1962.)

B. Fluorometric Analysis

1. Porphyrins

a. Homogenize sample with 2-3 volumes of ethyl acetate-acetic acid (4:1).
b. Filter through sintered glass or equivalent.
c. Treat sample to remove heavy metals from porphyrin structures (add chelating agents EDTA + acid).
d. Oxidize porphyrinogens (oxygen or aqueous iodine treatment).
e. Place preparation in fluorometer.
f. Excite with 400 mμ light (Soret absorption band) or 405 mμ Hg line.
g. Record fluorescence in 600-750 mμ region of spectrum.
h. Make sample 2N with HCl and record fluorescence in 600-750 mμ region.

2. Flavins

a. Acquire powdered sample.
b. Determine appropriate amount of water, 2-3 volumes.
c. Acquire measured amount of water.
d. Add to sample and mix.
e. Transfer to oven.
f. Heat at 80°C for 15 minutes.
g. Remove from oven.
h. Wait pre-set time for cooling.
i. Homogenize.
j. Add 1N H_2SO_4 to give concentration of 0.25 N H_2SO_4 and mix.
k. Transfer sample to oven.
l. Heat to 80°C for 15 minutes.

m. Remove from oven.
n. Wait pre-set time for cooling.
o. Add pre-set amount of chloroform and separate phases.
p. Inspect $CHCl_3$ with spectrofluorometer and discard $CHCl_3$.
q. If fluorescence is detected, go to r. If fluorescence is not detected, go to u.
r. Add pre-set quantity of chloroform.
s. Mix.
t. Go to p.
u. Measure volume, V_1 (aqueous).
v. Determine volume of NaOH to be added (from u).
w. Acquire volume of NaOH.
x. Add to sample.
y. Mix.
z. Filter.
aa. Remove residue.
bb. Transfer filtrate to light chamber.
cc. Irradiate sample for pre-set time at pre-set intensity.
dd. Determine amount of chloroform (3 x Vcc).
ee. Acquire measured amount of chloroform.
ff. Add to sample.
gg. Mix.
hh. Fill fluorometer cell with and direct light path through lower $CHCl_3$ layer.
ii. Measure fluorescence.
jj. Remove cell from fluorometer.
kk. Remove liquid from cell.
ll. Clean and sterilize as required.
mm. Recycle to ready.

V. Specific Enzymic Function

A. Exhange of O^{18} from Oxyanious with H_2O

Equipment and Materials

Mass Spectrometer
Sample collector and sieve or homogenizer
Sample measuring systems
Reaction cells (2)
Sterilizing chamber, 125^{o}, sealed
Water transfer system, with filter, from reaction cells to MS
Media (7), containing O^{18}-oxyanions as follows:

a. Complete medium with all oxyanions labeled, to include phosphate-O^{18}, nitrate-O^{18}, nitrite-O^{18}, sulfate-O^{18}, sulfite-O^{18}, acetate-O^{18}.
b. phosphate-O^{18} only

c. nitrate-O^{18} only
d. nitrite-O^{18} only
e. sulfate-O^{18} only
f. sulfite-O^{18} only
g. acetate-O^{18} only

The water and any other oxygen containing materials in each medium should be free of or very low in O^{18}.

Procedure

1. Acquire sample.
2. Make uniform by sieving or homogenizing.
3. Measure 5 g (or volume equivalent) into sterilizing chamber.
4. Heat at 125°C, sealed, one hour.
5. Cool and release seal.
6. Transfer 0.5 g (volume measure) original sample into reaction cell 1.
7. Transfer 0.5 g sterilized sample into reaction cell 2.
8. Add medium (a) above, x ml.
9. Incubate four hours.
10. Transfer y ml water from cell 1 to mass spectrometer.
11. Determine O^{18} content of water.
12. Transfer y ml water from cell 2 to mass spectrometer.
13. Determine O^{18} content of water.
14. Compare O^{18} content of water in cell 1 and 2. If O^{18} of cell 1 is significantly higher than that of cell 2, proceed to step 15. If no difference, continue incubation 24 hours and repeat steps 10 to 14. If still no difference go to step 15.
15. Discard samples.
16. Clean cells.
17. Repeat steps 6 to 14, substituting medium b for medium a.
18. Continue repetitions until all media have been tested.

Note: This procedure could also be carried out with all media simultaneously using 14 cells with sample and sterile control for each oxyanion system.

VI. Evidence for Organization

A. Visual Inspection

a. Locate sample of suspected biological material by HRF searching mode.
b. Position for close-up.
c. Inspect sample by HRF.
d. Inspect sample under X10 magnification.

B. Analysis for Optical Purity

a. Acquire sample.
b. Weigh.
c. Determine solvent type.
d. Determine amount of solvent.
e. Acquire measured amount of solvent and add to sample.
f. Homogenize.
g. Filter
h. Remove residue.
i. Transfer filtrate to ORD cell.
j. Transfer cell to ORD apparatus.
k. Measure optical rotatory dispersion.
l. Remove cell.
m. Remove sample.
n. Clean and sterilize as required.
o. Recycle to ready.

APPENDIX 4

RATING OF ENVIRONMENTAL EXPERIMENTS

The essentiality criteria given in Section 4 of Volume II have been restated in terms of simple questions to facilitate the actual evaluation process for the environmental experiments and weighting factors are applied to each question, since not all are equally important. The questions are, in order of assigned importance:

(1) Does the experiment support the biology experiments?

(2) Will the experiment detect or locate fossils?

(3) Will the experiment aid sample collection?

(4) Does the experiment provide ecological data or characterize environmental interactions of life?

(5) Will the experiment indicate the reasons for extinction or preclusion of life?

Each suggested experiment was rated in terms of these criteria on a scale from one to ten (low experimental value to high value). The score was then multiplied by the appropriate weighting factor for each criterion. Weighting factors are summarized in Table I.

TABLE I

WEIGHTING FACTORS FOR ENVIRONMENT MEASUREMENT
ESSENTIALITY CRITERIA

	Criteria	Weighting Factor
(1)	Biology	10
(2)	Fossils	8
(3)	Samples	8
(4)	Ecology	5
(5)	Extinction or Preclusion	2

The rationale for the assignment of weighting factors is as follows: the primary scientific goal of the ABL is to detect life if it exists on Mars. Therefore, criteria (1) must be heavily weighted. Collection of possible life containing samples is essential to many of the life detection experiments so that criteria (3), sample collection, must be given a high value. If previously life did exist on Mars, then life detection becomes a search for remnants of dead life forms. Thus, criteria (2), fossils, are given an equal weight as sample collection. If existent life forms are detected, the secondary goal of the ABL is to characterize such forms to the extent possible with available equipment and supplies. Finally, least important of these criteria is the study of factors which prevent life from developing on Mars or which caused extinction of previous life.

It should be pointed out that these criteria apply to the first biology laboratory landed upon Mars. The scientific goals of subsequent missions may change to emphasize other kinds of experiments.

Tables II and III contain the evaluation (rating and ranking based on the essentiality criteria) of suggested environmental measurements. The ratings in many cases are of course subjective. Nevertheless, the results are felt to be quite reasonable. Emphasis is definitely placed on those experiments which are most germane to the biological experiments.

TABLE II

RATING OF SUGGESTED ENVIRONMENT EXPERIMENTS

Experiment	Criteria	Score
4.3.1 a.* Static and Dynamic Atmospheric Pressure at the Surface	1. Biology Information useful for growth experiments but not essential. Backup for measurement of water vapor.	30
	2. Fossils No information is provided on fossils.	0
	3. Samples Aid interpretation of aerosol sampling.	10
	4. Ecology Atmospheric pressure is a factor in bioenvironment.	25
	5. Extinction Thin atmosphere may allow damaging radiation to reach surface. May indicate poor liquid water availability.	6
	Total	71
4.3.1 b. Wind Velocity and Direction	1. Biology May indicate occurrence of sand storms and surface damage.	10
	2. Fossils May be responsible for covering or destroying fossils.	8
	3. Samples To interpret aerosol sampling.	32
	4. Ecology Important environmental factor for life, transport, etc.	35
	5. Extinction Little or no pertinent information can be derived concerning extinction.	0
	Total	85

*Numbers correspond to those in Section in Volume II in which experiments are discussed.

Experiment		Criteria	Score
4.3.1 c. Temperature of Near Surface Air	1.	Biology A parameter in determining water availability.	30
	2.	Fossils No applicable information.	0
	3.	Samples No applicable information.	0
	4.	Ecology Indicates thermal environment of surface and meteorological events.	45
	5.	Extinction Little or no applicable information.	0
		Total	75
4.3.1 d. Atmospheric Gas Composition	1.	Biology Indicates chances of finding photosynthesis and other life processes. Aids selection of atmosphere for growth experiments. Detects biologically significant gases.	80
	2.	Fossils If hydrocarbon vapors found, perhaps fossil deposits may exist.	16
	3.	Samples Gas composition at surface of collected sample might be used as sample characterization.	8
	4.	Ecology Atmospheric composition is an important ecological factor.	50
	5.	Extinction Absence of water vapor, or other properties of the atmosphere may have bearing on extinction.	12
		Total	166
4.3.1 e. Atmospheric Humidity	1.	Biology Indicates water availability.	40
	2.	Fossils No pertinent information derived.	0
	3.	Samples Needed for interpretation of aerosol sampling but not essential.	16
	4.	Ecology Atmospheric moisture is an important ecological parameter.	35
	5.	Extinction Absnece or scarcity of water may prevent life.	12
		Total	103

Experiment		Criteria	Score
4.3.1 f. Windborne Materials (both coarse and fine)	1.	Biology Provides a sampling mechanism. Indicates occurrence of possible erosion damage to ABL. Indicates possible interference with surface life (coverage, blowing).	30
	2.	Fossils Wind materials may contain small fossil forms. Erosion may destroy exposed macroscopic life residues.	24
	3.	Samples A method of sample collection. Impaction of fine airborne materials into culture media or onto microscopic slides can be part of life detection experiments.	80
	4.	Ecology Mechanism for transport of spores, pollen, etc. Possible hazard to surface life.	35
	5.	Extinction Little or no direct evidence provided pertaining to extinction.	0
		Total	169
4.3.1 g. Visual Scan of Sky	1.	Biology Slim chance of observing airborne life.	10
	2.	Fossils Little or no applicable data.	0
	3.	Samples Indicates utility of airborne material sampling.	16
	4.	Ecology Nature of cloud cover, haze, etc. affects bioenvironment.	20
	5.	Extinction Little or no applicable data.	0
		Total	46
4.3.1 h. Infrared Scan of Sky	1.	Biology No pertinent information derived.	0
	2.	Fossils No pertinent information.	0
	3.	Samples May detect clouds of dust.	8
	4.	Ecology May illucidate atmospheric mechanisms of dust transport. Indicates nature of cloud cover.	20
	5.	Extinction Some data derived from this experiment concerning possible water vapor.	2
		Total	30

Experiment	Criteria	Score
4.3.1.i. Cloud Composition	1. Biology May indicate atmospheric water.	10
	2. Fossils No pertinent data derived.	0
	3. Samples May detect dust clouds.	8
	4. Ecology Defines an important meteorological factor of the environment.	20
	5. Extinction Will facilitate understanding of hydrospheric phenomena.	14
	Total	34
4.3.2 Solar Radiation Experiments	1. Biology Can indicate possible damaging ultraviolet flux. Solar spectrum useful in studying photosynthesis. Some information on atmospheric composition is obtained.	50
	2. Fossils No data.	0
	3. Samples Provides no useful data for sample gathering.	0
	4. Ecology Important environmental factor is the light intensity over the visible and UV ranges. Solar heating of the surface is measured in the infrared region.	50
	5. Extinction The thin atmosphere may permit damaging radiation intensities to reach the surface.	12
	Total	112
4.3.3 Ionizing Radiation Experiments	1. Biology Radiation can result in adaptive mechanisms affecting the gross nature of life.	10
	2. Fossils No information is derived unless a traversing radiation survey of the surface is performed. Then anomalties may indicate fossil remains.	0
	3. Samples Radiation levels of surface magerials could be measured to characterize samples. Radioactive material content of samples would be indicated.	8

Experiment		Criteria	Score
4.3.3 (continued)	4.	Ecology Radiation environment is probably higher than on Earth and can have possible effects.	15
	5.	Extinction Probably not important factor in preventing life.	0
		Total	33
4.3.4 a. Surface and Subsurface Temperature	1.	Biology Indicates severity of thermal conditions, freeze/thaw cycles. Together with water vapor measurement can provide indication of state of soil moisture.	40
	2.	Fossils Probably provides no information on fossils.	0
	3.	Samples Characterizes important parameter of samples used in biology experiments. If a thermal survey is made, will locate warm spots for sample sites.	72
	4.	Ecology Defines thermal environment of surface and subsurface.	45
	5.	Extinction Present temperatures probably will not show cause of possible previous extinction. However, may be a factor preventing development of life now.	10
		Total	167
4.3.4 b. Surface Material Electrical Conductivity	1.	Biology Information on electrolyte and water concentration in soil may be derived which would help select appropriate growth media.	40
	2.	Fossils No information.	0
	3.	Samples May help select sample sites having optimum water and salt concentrations. Characterizes sample properties.	48
	4.	Ecology Important ecological factors for life in soil are water and soluble salt concentrations.	35
	5.	Extinction Extreme electrical conductivity may have bearing on conditions preventing life.	8
		Total	131

Experiment		Criteria	Score
4.3.4 c. Density of Surface Material	1.	Biology Density may indicate presence of organic matter.	60
	2.	Fossils Presence of organic matter, carbonates, porous rock.	32
	3.	Samples For above reasons to select sample. To characterize and label sample.	48
	4.	Ecology Ecological information derivable from density measurement is limited.	10
	5.	Extinction Probably no pertinent data is derived.	0
		Total	150
4.3.4 d. Soil Mechanics	1.	Biology Soil mechanics experiments might be performed on a macroscopic (large) sample of suspected biological material. Unusual properties may indicate organic or life structure.	20
	2.	Fossils Results may be correlated with soils containing organic material.	16
	3.	Samples Knowledge of mechanical properties useful to select sampling technique.	80
	4.	Ecology Soil mechanical structure is determined and is useful ecological data.	10
	5.	Extinction No applicable information.	0
		Total	126

Experiment		Criteria	Score
4.3.4 e. Soil Particle Size and Particle Density Distribution	1.	**Biology** Low density fractions, especially if in discrete size ranges, may indicate organic material, small organisms.	40
	2.	**Fossils** Occurrence of peaks on an otherwise uniform distribution may indicate skeletons (diatoms, etc.).	32
	3.	**Samples** Indicates sample mechanical processing (separation) utility in concentrating organic materials. May influence subsequent sample gathering operations. Characterizes division of sample.	48
	4.	**Ecology** Has some value in defining soil ecology.	10
	5.	**Extinction** Probably no information derived.	0
		Total	130
4.3.4 f. Binding Capacity of Soil for Water	1.	**Biology** The ability of Martian soil to retain liquid water may have some bearing on Martian biology and life detection experiments.	10
	2.	**Fossils** No useful data directly derivable pertaining to fossils.	0
	3.	**Samples** Soils with high relative binding capacities should be sampled in preference to others.	32
	4.	**Ecology** Of some value for ecological characterization of the soil microenvironment.	25
	5.	**Extinction** Inability of soil to hold significant moisture may be a factor in extinction.	12
		Total	79

Experiments		Criteria	Score
4.3.4 g. Density of Objects	1.	Biology Density of object suspected to be biological material may confirm the supposition.	70
	2.	Fossils Suspected fossil and carbonaceous material could be examined.	56
	3.	Samples Excellent criteria for selecting macroscopic sample.	80
	4.	Ecology Little or no information.	0
	5.	Extinction Little or no information.	0
		Total	206
4.3.4 h. Acoustical Monitoring	1.	Biology Detects life noises.	40
	2.	Fossils No information.	0
	3.	Samples Indicates level of wind borne material by impacts. Indicates when winds are prevelent.	16
	4.	Ecology Wind and dust environment is determined approximately.	10
	5.	Extinction Little or no information.	0
		Total	66
4.3.5 a. Elemental Analysis of Soil	1.	Biology Indicates life related elements and abundances.	80
	2.	Fossils May indicate fossil materials.	48
	3.	Samples May provide criteria for sample selection.	40
	4.	Ecology Valuable ecological information.	40
	5.	Extinction May provide some interpretable data concerning extinction.	8
		Total	216

Experiment		Criteria	Score
4.3.5 b. Water Content and Type in Soil Samples	1.	**Biology** Indicates available water and type of binding.	50
	2.	**Fossils** No information derived to enable fossil detection.	0
	3.	**Samples** Indication of water in soil sample. Samples high in water should be collected.	56
	4.	**Ecology** Water availability and type is a very important environmental factor.	50
	5.	**Extinction** Lack of water may have strong bearing on absence of life.	16
		Total	172
4.3.5 c. Soil Gas Analysis	1.	**Biology** Identity of gases evolved by soils may indicate microbial action.	100
	2.	**Fossils** Soil gases may indicate carbonaceous material deposits.	80
	3.	**Samples** May indicate location of microbial activity.	64
	4.	**Ecology** Helps identify subsoil ecology and chemistry.	40
	5.	**Extinction** May provide data on factors preventing life.	6
		Total	290
4.3.5 d. Soil pH	1.	**Biology** Aids selection of culture media pH.	50
	2.	**Fossils** Soil pH may reflect presence of fossil material.	24
	3.	**Samples** Characterizes samples with regard to pH.	56
	4.	**Ecology** Important factor for soil microbes.	40
	5.	**Extinction** Extreme pH may have some bearing on extinction.	6
		Total	176

Experiment	Criteria	Score
4.3.5 e. Soluble Inorganic Ions	1. Biology Indicates soil salinity and ionic composition which may be important to life. Aids selection of culturing conditions. May detect essential trace elements.	90
	2. Fossils May indicate fossilization mechanisms and fossil material or their absence.	48
	3. Samples Constitutes an important sample characterization experiment.	40
	4. Ecology Defines soil ecology parameters of significance.	40
	5. Extinction Perhaps some information affecting extinction would be derived.	6
	Total	224
4.3.5 f. Organic Carbon in Soil	1. Biology Provides indication of presence and amount of soil organic matter. Qualifies probability of existence of life.	90
	2. Fossils Organic residues of life may be implied.	32
	3. Samples Subsequent sampling would be encouraged where organic carbon content was found high.	40
	4. Ecology Indicates availability of organic material to life.	30
	5. Extinction Little or no pertinent information.	0
	Total	192

Experiment		Criteria	Score
4.3.5 g. Fractional Solubility	1.	Biology Indicates both gross soil salinity and classes of organic materials in soil.	90
	2.	Fossils May indicate fossil material.	48
	3.	Samples Characterizes sample chemistry and solubility properties.	40
	4.	Ecology Some information on soil environment is obtained.	25
	5.	Extinction Little information derivable.	2
		Total	205
4.3.5 h. Fractional Volatility	1.	Biology Provides indication of soil organic matter and water.	90
	2.	Fossils Fossil organic matter may be detected.	32
	3.	Samples Subsequent sampling would be encouraged where organic carbon is high.	40
	4.	Ecology Organic and water content of soil is indicated.	35
	5.	Extinction May provide some data.	6
		Total	203
4.3.6 a. Visual Scan	1.	Biology May detect life by visual form (organization structure, movement).	100
	2.	Fossils Probably of intermediate value in detecting or locating fossils.	24
	3.	Samples Highly useful in selecting sampling sites.	80
	4.	Ecology Provides visual description of environment.	40
	5.	Extinction Causes of extinction may be subtle and not obvious in visual scan.	4
		Total	248

Experiment		Criteria	Score
4.3.6 b. Radiometer Scans of Surface to just above Horizon (Water Band)	1.	Biology Water availability important to life. Indicates opportunity for life, not existence of life.	40
	2.	Fossils Probably no pertinent information derived from radiometry scan.	0
	3.	Samples If a water anomaly is indeed detected, indicates likely spot for sampling.	72
	4.	Ecology Indicates water availability on surface.	35
	5.	Extinction Absence of water may be important factor preventing life.	12
		Total	159
4.3.6 c. Radiometer Scan of Surface to Just above Horizon (Thermal Mapping)	1.	Biology Presence of warm spots supports possibility for life. Less important than water availability.	30
	2.	Fossils Provides no pertinent information.	0
	3.	Samples A thermal anomaly indicates likely sampling site.	72
	4.	Ecology Indicates thermal environment.	45
	5.	Extinction Present temperature on Mars does not indicate cause of previous extinction if any. May be a factor preventing development of life now.	4
		Total	151

TABLE III

RANKING OF ENVIRONMENTAL EXPERIMENTS

	Experiment	Score
4.3.5 c.	**Soil Gas Analysis**	290
4.3.6 a.	**Visual Scan**	248
4.3.5 e.	**Soluble Inorganic Ions**	224
4.3.5 a.	**Elemental Analysis**	216
4.3.4 g.	**Density of Objects**	206
4.3.5 g.	**Fractional Solubility**	205
4.3.5 h.	**Fractional Volatility**	203
4.3.5 f.	**Organic Carbon**	192
4.3.5 d.	**Soil pH**	176
4.3.5 b.	**Water Content**	172
4.3.1 f.	**Windborne Materials**	169
4.3.4 a.	**Surface Temperature**	167
4.3.1 d.	Atmospheric Composition	166
4.3.6 b.	Radiometer Scan (Water)	159
4.3.6 c.	Radiometer Scan (Thermal)	151
4.3.4 c.	Density of Surface Material	150
4.3.4 b.	Electrical Conductivity	131
4.3.4 e.	**Particle Sizes and Densities**	130
4.3.4 d.	**Soil Mechanics**	126
4.3.2	Solar Radiation Experiments	112
4.3.1 e.	Atmospheric Humidity	103
4.3.1 b.	Wind	85
4.3.4 f.	Binding Capacity	79
4.3.1 c.	Air temperature	75
4.3.1 a.	Atmospheric Pressure	71
4.3.4 h.	Acoustical Monitoring	66
4.3.1 g.	Visual Scan of Sky	46
4.3.1 i	Cloud Composition	34
4.3.3	Ionizing Radiation Experiments	33
4.3.1 h.	Infrared Scan of Sky	30

APPENDIX 5

EXPERIMENT PROCEDURES

This appendix contains a description of each experiment employed in the ABL design point payload, the apparatus used, and stey-by-step experimental procedures. A time-line diagram has been prepared for each experiment showing event time sequencing of each procedural step. The appendix is organized by experiment, and the experiment numbers (1 through 35) correspond with those given in Tables 5.1-I and 5.3-I of Volume II. The last three paragraphs (5.36, 5.37, and 5.38) present descriptions of the Gas Chromatograph Subroutine, the Spectral Analyzer Subroutine, and the General Growth Culture Routine.

5.1 EXPERIMENT NO. 1: ATMOSPHERIC PRESSURE, TEMPERATURE, AND WIND

5.1.1 DESCRIPTION

This experiment is designed to measure atmosphere static pressure, dynamic pressure and direction of the wind, and ambient atmospheric temperature.

5.1.2 APPARATUS USED

Static and dynamic pressure will be measured with a pitot tube connected to an aneroid bellows. The unit will have the capability of traversing 360 degrees to determine peak dynamic pressure. The pitot head is servo-driven to point in the direction controlled by a wind vane. The wind vane will incorporate a thermocouple or thermistor to measure ambient atmospheric temperature and a "hot-wire" anemometer to provide sensitivity at low wind velocities. A resistance-heated ceramic mass controlled by a bimetallic switch will provide a reference temperature source.

5.1.3 RANGE OF PARAMETERS

Static Pressure:	2 to 100 mb $\pm$ 1%
Dynamic Pressure:	0.001 to 200 mb $\pm$ 10%
Ambient Temperature:	-238°F (-150°C) to 100°F (38°C) $\pm$ 1%
Wind Velocity (Hot-Wire):	0 to 15 fps $\pm$ 1 fps
Wind Direction:	To nearest 5 deg

5.1.4 PROCEDURE

(1) Uncage and deploy wind vane, hot-wire anemometer, and temperature thermistor.

(2) Close pitot head dynamic pressure port.

(3) Introduce fixed quantity of N_2 into dynamic pressure line.

(4) Read total pressure converted to dynamic pressure and check calibration. DATA OUT

(5) Close pitot head static pressure port.

(6) Introduce fixed quantity of N_2 into static pressure line.

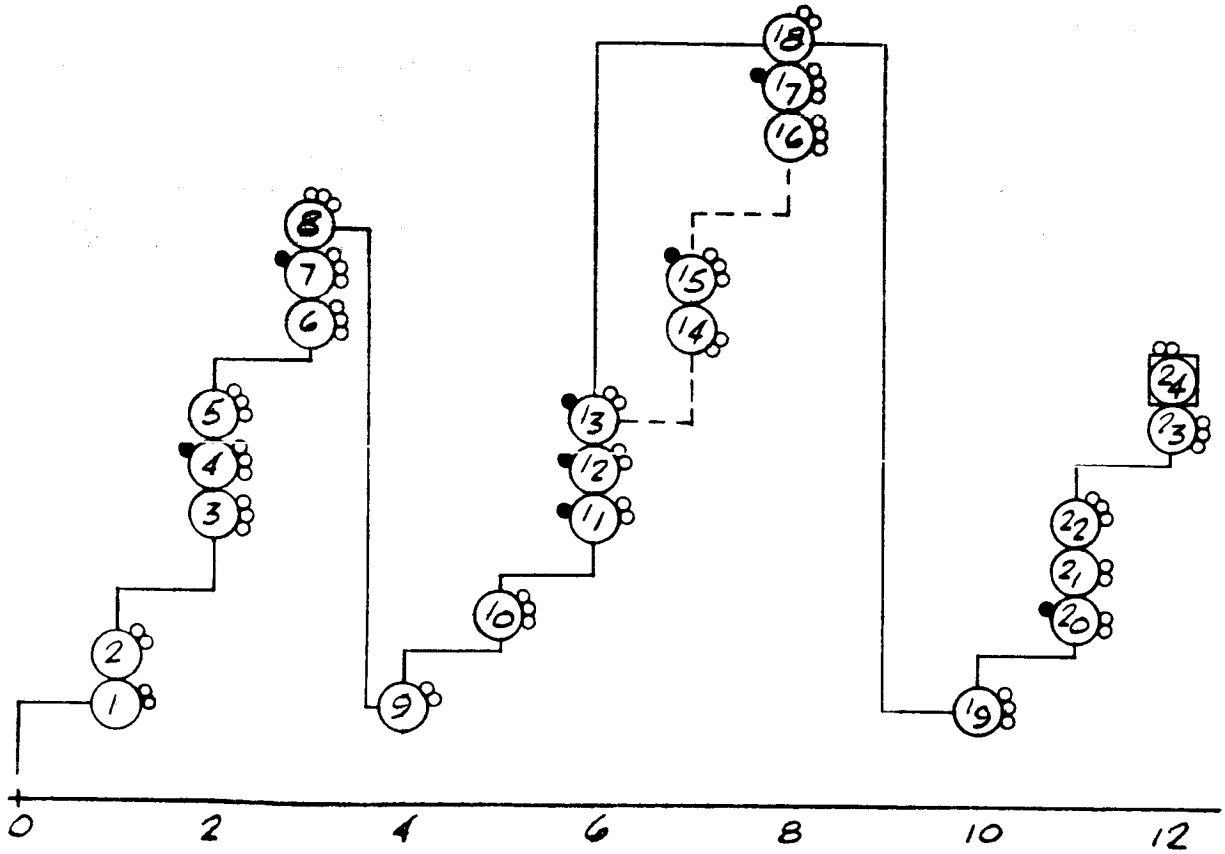
(7)	Read static pressure and check calibration.	DATA OUT
(8)	Open static and dynamic pressure ports.	
(9)	Uncage and extend pitot head.	
(10)	Activate pitot head drive servo with direction signal from wind vane.	
(11)	Read static pressure.	DATA OUT
(12)	Read dynamic pressure.	DATA OUT
(13)	Read direction.	DATA OUT
(14)	If low reading is obtained in Step (12), shield hot-wire anemometer from wind.	
(15)	Read anemometer output.	DATA OUT
(16)	Expose anemometer.	
(17)	Read wind velocity.	DATA OUT
(18)	Initiate heating of reference temperature source.	
(19)	Monitor heating until thermal equilibrium is attained.	
(20)	Record atmospheric temperature.	DATA OUT
(21)	Terminate power to reference temperature source, anemometer, and pitot head drive servo.	
(22)	Close pitot head static and dynamic ports.	
(23)	Erect shields over critical sensors and/or components.	
(24)	Recycle to ready.	

EXPERIMENT 1- ATMOSPHERIC PRESSURE, TEMPERATURE, & WIND VELOCITY & DIRECTION

SYMBOLS

- ABL COMMAND
- SELF-CONTROLLED FUNCTION
- DATA OUTPUT
- CYCLE COMPLETION

NUMBERED CIRCLES REFER TO EXPERIMENT PROCEDURES



5.2 EXPERIMENT NO. 2: DETERMINATION OF ATMOSPHERIC HUMIDITY

5.2.1 DESCRIPTION

This experiment is designed to measure the water vapor content of the atmosphere.

5.2.2 APPARATUS USED

A gold-film/aluminum-oxide element[1] is used to determine humidity by measuring the change in resistance caused by water absorbed in the aluminum oxide. Determination of resistance change can be made by referring to a constant resistance in a bridge circuit.

5.2.3 RANGE OF PARAMETERS

Sensitivity down to 10^{-5} mm Hg partial pressure of water vapor.

5.2.4 PROCEDURE

(1) Uncage and deploy wind vane head.

(2) Switch reference resistor into bridge circuit.

(3) Read ΔR required for balance and check against calibration. DATA OUT

(4) Switch reference resistor out of circuit.

(5) Read ΔR required to balance sensor output. DATA OUT

(6) Erect cover, or shield, over sensor, if required.

---

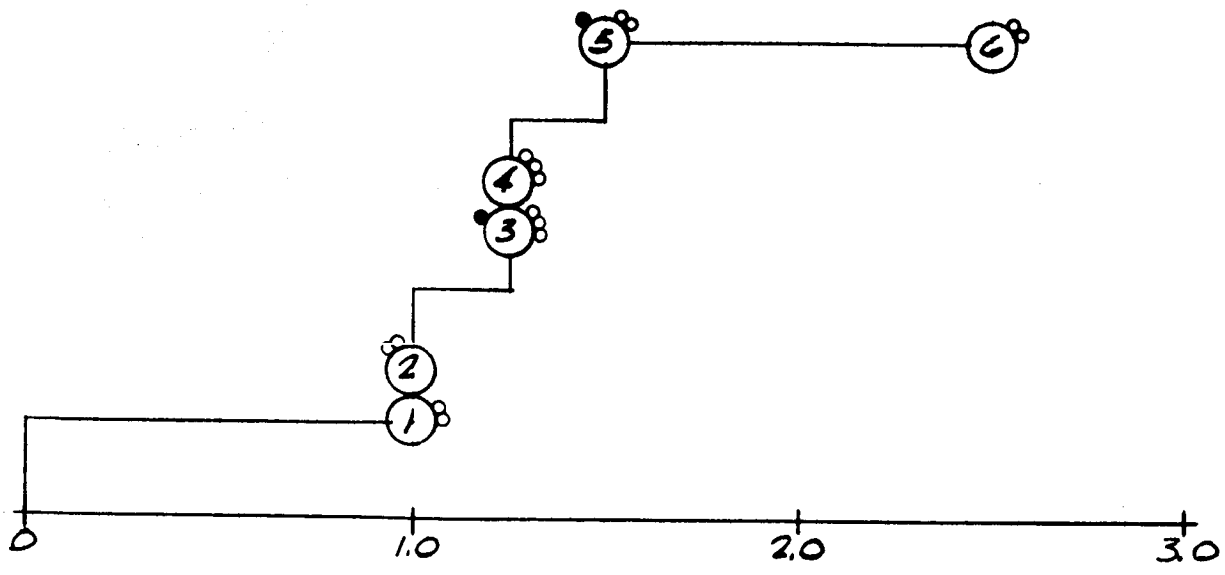
[1] To be based on unit developed by Parametrics, Waltham, Massachusetts, under JPL Contract 950684, 31 December 1964.

EXPERIMENT 2- DETERMINATION OF ATMOSPHERIC HUMIDITY

SYMBOLS

- ABL COMMAND
- SELF CONTROLLED FUNCTION
- DATA OUTPUT
- CYCLE COMPLETION

NUMBERED CIRCLES REFER TO EXPERIMENT PROCEDURES



TIME IN MINUTES

5.3 EXPERIMENT NO. 3: WIND TRANSPORTED PARTICULATE MATTER

5.3.1 DESCRIPTION

To detect particulate matter carried in the atmosphere and to obtain an estimate of the concentration and particle size.

5.3.2 APPARATUS USED

Counts of impingement of atmospheric particles on a resonator with a microphone pickup will detect the gross transport of relatively large particles. For finer particles, and to determine concentration, miniaturized separators will be used.

5.3.3 PROCEDURE

(1) Deploy resonator head and vertical sampler mechanism.

(2) Monitor auditory signal from microphone enclosed in resonator head.

(3) Record signal strength and impact rate. (Used to initiate protective measures.) DATA OUT

(4) When predetermined threshold level is reached, initiate quantitative sampling routine.

(5) Transport collector head to lowest position in collector tube.

(6) Open collector head port.

(7) Monitor collection rate. DATA OUT

(8) Close collector head port.

(9) Read amount of particles collected. DATA OUT

(10) Transport sampler collector to laboratory.

(11) Remove and store soil sample.

(12) Transport collector head to intermediate elevation.

(13) Repeat Steps (6) through (11). DATA OUT

(14) Transport collector head to upper elevation.

(15) Repeat Steps (6) through (11). DATA OUT

(16) Recycle to ready.

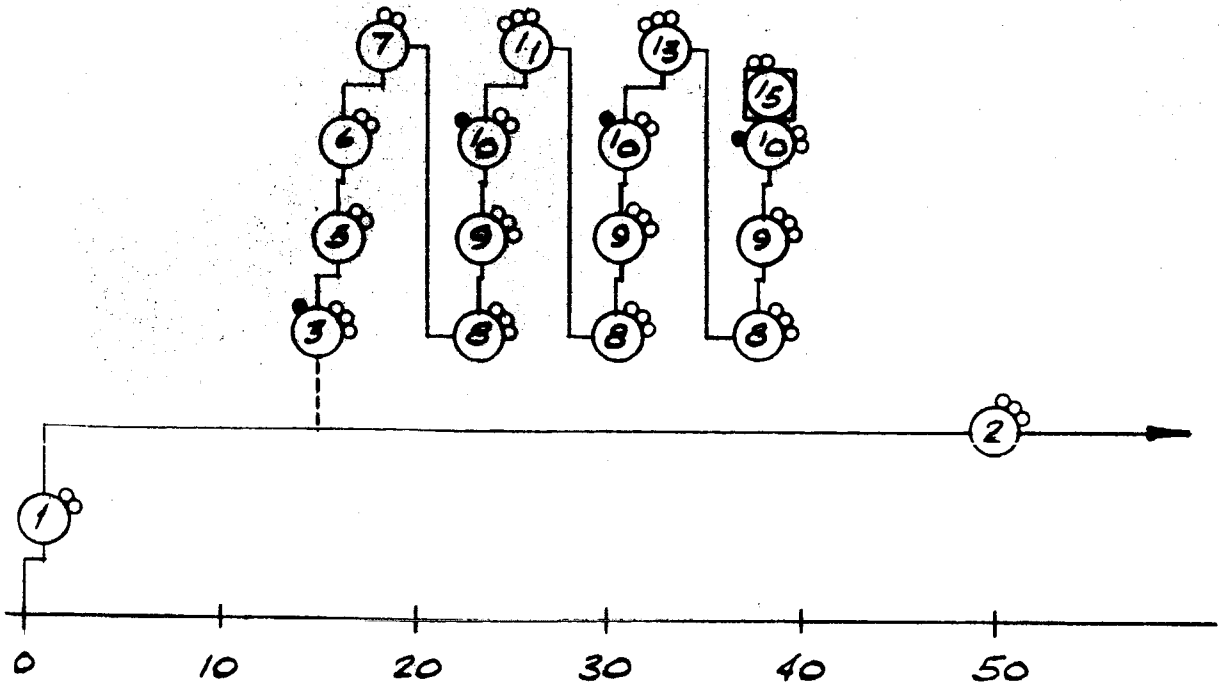
EXPERIMENT 3 - WIND-TRANSPORTED PARTICULATE MATTER

SYMBOLS

- ABL COMMAND
- SELF CONTROLLED FUNCTION
- DATA OUTPUT
- CYCLE COMPLETION

NUMBERED CIRCLES REFER TO EXPERIMENT PROCEDURES

NOTE. THE INITIATION OF THE SEQUENCE BEGINNING WITH STEP 3 IS NOT TIME DEPENDENT, BUT IS EVENT DEPENDENT.



TIME IN MINUTES

5.4 EXPERIMENT NO. 4: ACOUSTICAL MONITOR

5.4.1 DESCRIPTION

Detection of surface noises such as might be produced by wind in large plants, by animals, or by atmospheric disturbances.

5.4.2 APPARATUS USED

A microphone located at the focus of an acoustical collector covering a 360 degree lateral field of view will be employed.

5.4.3 RANGE OF PARAMETERS

Sound intensity in db above a reference sound pressure level of 0.0002 microbar and up to 150 db.

Frequency - 20 to 20,000 cps.

5.4.4 PROCEDURE

(1) Deploy collector and retract cover.

(2) Monitor sound intensity.

(3) When intensity exceeds reference value, record signal and correlate with motion signals from Experiment 34 and to Step (4). DATA OUT

(4) Transmit microphone output to reed bank frequency analyzer.

(5) Record frequency spectrum from reed bank response. DATA OUT

EXPERIMENT 4- ACOUSTICAL MONITOR

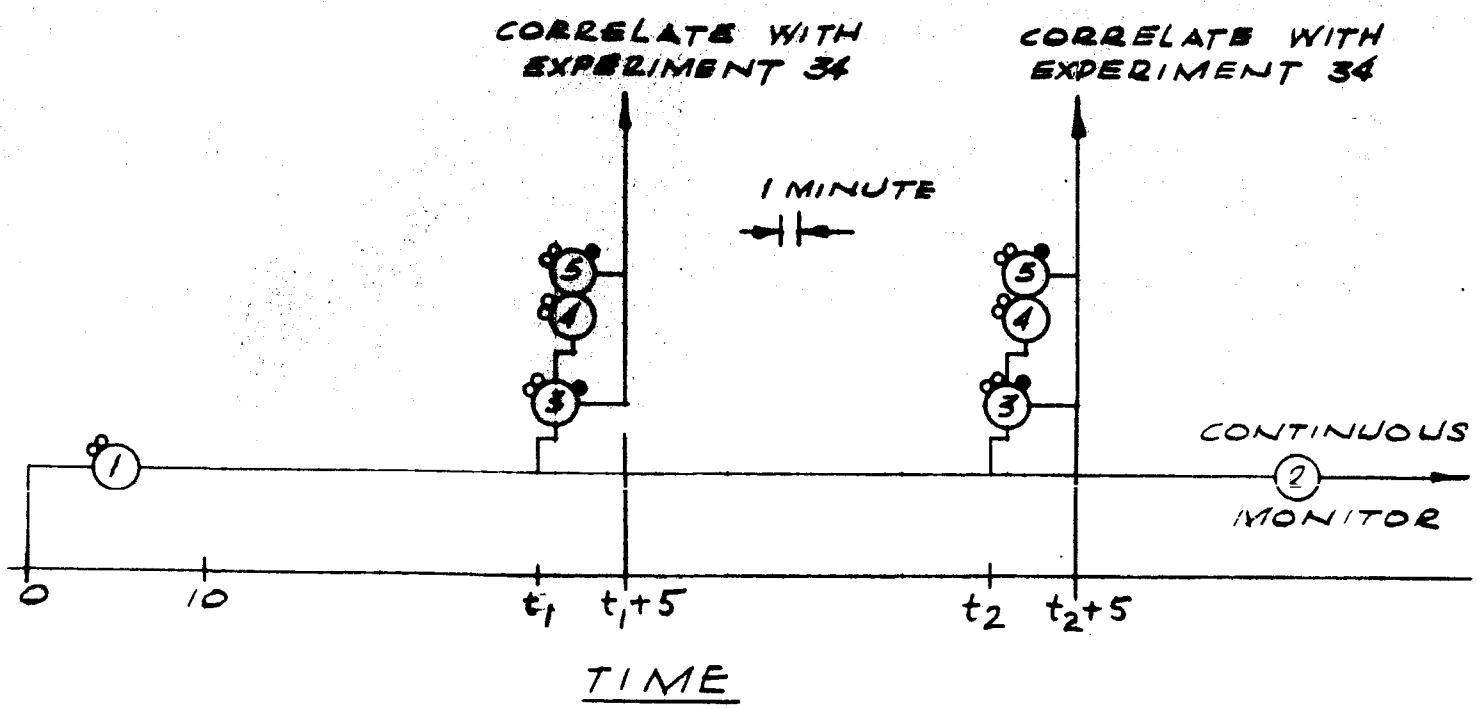
SYMBOLS

ABL COMMAND

DATA OUTPUT

NUMBERED CIRCLES REFER TO EXPERIMENT PROCEDURES

NOTE: NO EXTENDED TIME SCALE IS SHOWN. THIS EXPERIMENT IS EVENT, NOT TIME, DEPENDENT.



5.5 EXPERIMENT NO. 5: ULTRAVIOLET AND VISIBLE INSOLATION

5.5.1 DESCRIPTION

This experiment is designed to measure the total flux incident at the surface of Mars and determine the portion of the total flux occurring in the ultraviolet region of the spectrum from 200 to 350 mμ and in the visible from 350 to 700 mμ, and in the infrared from 700 mμ to 2.5μ.

5.5.2 APPARATUS USED

A simple integrating spectrophotometer capable of measuring the incident flux over fixed bandwidths. An objective lens with an approximate field of view of 2π steradians is used to give an integrated value of direct and scattered flux including the sun. IR range is determined by the limits of fused silica optics.

5.5.3 RANGE OF PARAMETERS

Ultraviolet Flux:	200-350 mμ	3 bandwidths
Visible Flux:	350-700 mμ	4 bandwidths
Infrared:	700 mμ - 2.5μ	2 bandwidths

5.5.4 PROCEDURE

(1) Initiate power to reference source/filter combination.

(2) Compare output wavelength with calibrated value. DATA OUT

(3) Terminate reference source power.

(4) Expose objective lens.

(5) Cycle through frequency bandwidth.

(6) Record I = f (λ). DATA OUT

(7) Cover objective lens and terminate power.

EXPERIMENT 5- ULTRAVIOLET INSOLATION

SYMBOLS

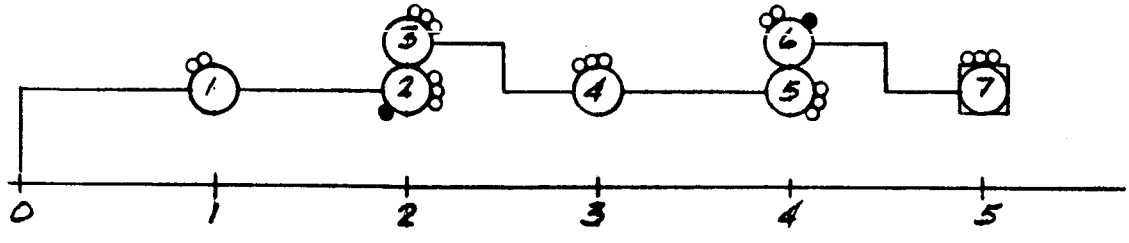
ABL COMMAND

SELF CONTROLLED FUNCTION

DATA OUTPUT

CYCLE COMPLETION

NUMBERED CIRCLES REFER TO EXPERIMENT PROCEDURES



TIME IN MINUTES

5.6 EXPERIMENT NO. 6: β AND γ RADIATION BACKGROUND

5.6.1 DESCRIPTION

This experiment is designed to obtain information on the β and γ radiation at the surface and to provide correlation backup data needed to evaluate those experiments using radiation counting devices as sensors. Selected background radiation level at the surface of Mars is determined.

5.6.2 APPARATUS USED

Count rate is measured using two proportional counters each having 2π - steradian field of view external to the laboratory. Pulse height discrimination is incorporated.

5.6.3 RANGE OF PARAMETERS

Count Rate:	Up to 100 counts per second

5.6.4 PROCEDURE

(1) Deploy counter head to sampling location.

(2) Orient the counter head vertically upward.

(3) Energize counter tubes with voltage potential.

(4) Count pulses and pulse heights.

(5) Reduce and condition data. DATA OUT

(6) Orient the counter head vertically downward.

(7) Count pulses and pulse heights.

(8) Reduce and condition data. DATA OUT

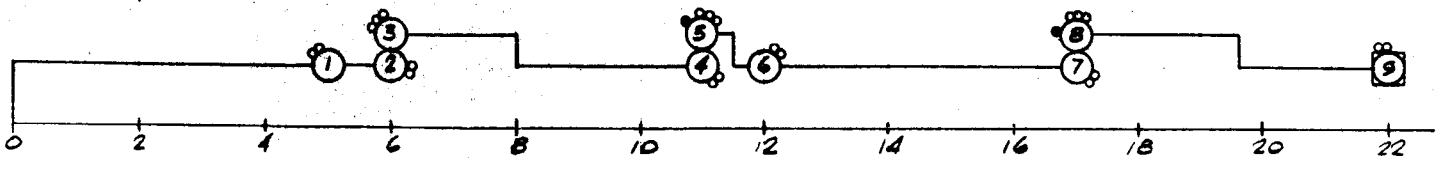
(9) Turn off power and return instrument to stowed position.

EXPERIMENT 6 - β AND γ RADIATION BACKGROUND

SYMBOLS

- ABL COMMAND
- SELF CONTROLLED FUNCTION
- DATA OUTPUT
- CYCLE COMPLETION

NUMBERED CIRCLES REFER TO EXPERIMENT PROCEDURES



TIME IN MINUTES

5.7 EXPERIMENT NO. 7: DETERMINATION OF ATMOSPHERIC CONSTITUENTS

5.7.1 DESCRIPTION

This experiment is designed to detect and determine the concentration of H_2O vapor, O_2, N_2, CO_2, A, CO, NO, NO_2, and O_3 in the atmosphere.

5.7.2 APPARATUS USED

An atmospheric gas chromatograph, using three columns, and a mass spectrometer detector will be employed. Column dimensions are 1.5 mm in diameter by 12 inches long. A glow discharge detector and helium carrier gas will be used.

5.7.3 RANGE OF PARAMETERS

Molecular Weight:	Up to 50
Concentration (Partial Pressure):	Up to 10^{-2} mm Hg

5.7.4 PROCEDURE

(1) Collect atmospheric sample with gas pump.

(2) Record downstream pressure rise. DATA OUT

(3) Feed atmospheric gas sample slug into gas chromatograph column carrier gas.

(4) Record detector output from each column at a rate of one point per second for one minute. DATA OUT

(5) Divert gas samples of interest to transfer ampule.

(6) Transport gas samples from Step (5) to mass spectrometer.

(7) Inject sample into mass spectrometer.

(8) Record spectrometer detector output. DATA OUT

(9) Release stored atmospheric gas sample.

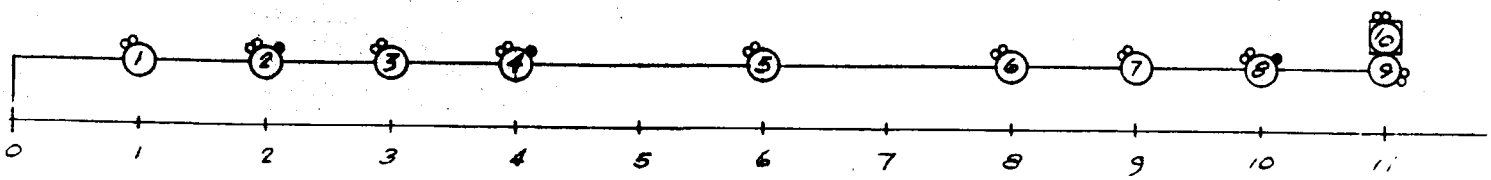
(10) Recycle equipment to ready.

EXPERIMENT 7. DETERMINATION OF ATMOSPHERIC CONSTITUENTS

SYMBOLS

- ABL COMMAND
- DATA OUTPUT
- CYCLE COMPLETION

NUMBERED CIRCLES REFER TO EXPERIMENT PROCEDURES



TIME IN MINUTES

5.8 EXPERIMENT NO. 8: SOIL TEMPERATURE AND WATER CONTENT AS A FUNCTION OF DEPTH

5.8.1 DESCRIPTION

This experiment is designed to determine the temperature of the soil from the surface to a depth of 100 cm at 5-cm intervals and the water content of the soil at these same depth intervals.

5.8.2 APPARATUS USED

A soil probe which is injected into the soil to a depth of 100 cm or as deep as mechanically possible. Thermocouples or resistance-wire thermometer and gold-film/aluminum-oxide detectors are integrally incorporated in the probe at 5-cm intervals. Heating elements are incorporated to free any frozen water as vapor after initial temperature profiles and water vapor content have been determined. A soil gas collection system can be incorporated to serve as a soil gas sample collector for Experiment No. 14. Soil probes will be inserted at each sample site from which soil samples are taken.

5.8.3 RANGE OF PARAMETERS

Water Content Sensitivity:	Down to 10^{-5} mm Hg partial pressure
Temperature:	-238°F (-150°C) to 100°F (38°C) $\pm$ 1%

5.8.4 PROCEDURE

(1) Initiate heating of reference temperature source.

(2) Record probe response and compare with calibration curve. DATA OUT

(3) Terminate reference heat source.

(4) Deploy probe.

(5) Insert probe into surface to desired depth, or until maximum axial force is detected. DATA OUT

(6) Read output of temperature and moisture detectors. DATA OUT

(7) Collect soil gases

(8) Initiate heating of heating elements.

(9) Collect soil gases.

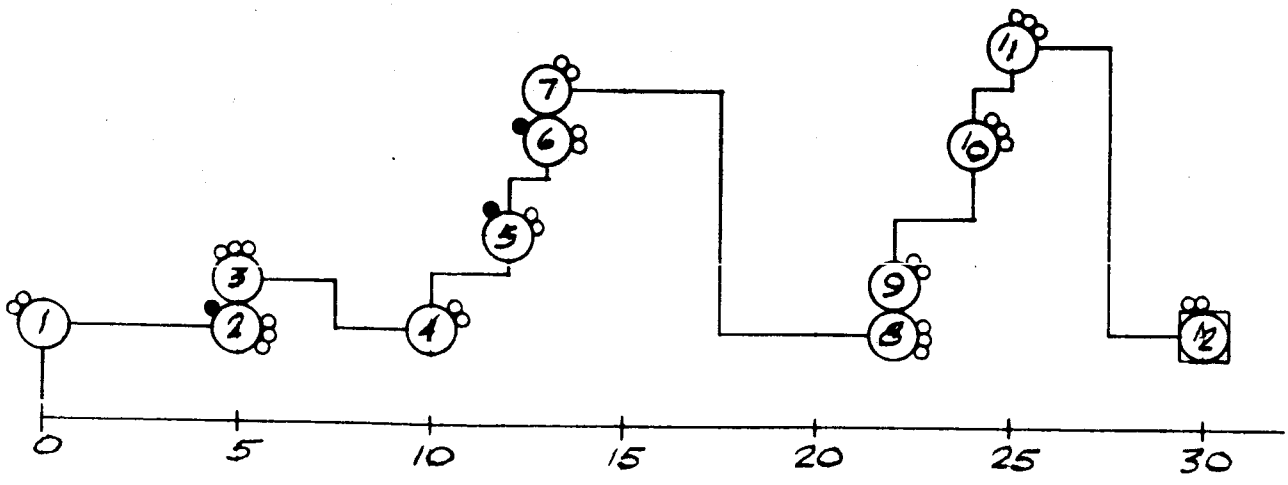
(10) Terminate heating.

EXPERIMENT 8 - SOIL TEMPERATURE & WATER CONTENT AS A FUNCTION OF DEPTH

SYMBOLS

- ABL COMMAND
- SELF CONTAINED FUNCTION
- DATA OUTPUT
- CYCLE COMPLETION

NUMBERED CIRCLES REFER TO EXPERIMENT PROCEDURES.



TIME IN MINUTES

5.9 EXPERIMENT NO. 9: SOIL ELECTRICAL CONDUCTIVITY

5.9.1 DESCRIPTION

This experiment is designed to determine the electrical conductivity as a function of depth in the Martian soil. These data are used as backup to the other environmental and life detection experiments, giving an indication of soil moisture content.

5.9.2 APPARATUS USED

Instrumentation on the core hole traversing sonde is used. Two types of instrumentation are possible. A direct measure will be made of electrical potential between two points in contact with the soil. This is simple to incorporate on the probe bow spring and will be used as a cross reference to the second method using a resonant tank circuit. Changes in the Q of the circuit are determined when the inductance is placed near the soil.

5.9.3 PROCEDURE

(1) Activate resonant tank circuit in sonde.

(2) Run calibration and check on instrument.

(3) Position core hole sonde in core hole.

(4) Measure potential drop between low spring contacts. DATA OUT

(5) Determine change in Q in tank circuit. DATA OUT

(6) Reposition sonde at next location and repeat Steps (3) through (5).

(7) Repeat Step (6) until complete core hole traverse is made.

(8) Remove sonde and recheck calibration.

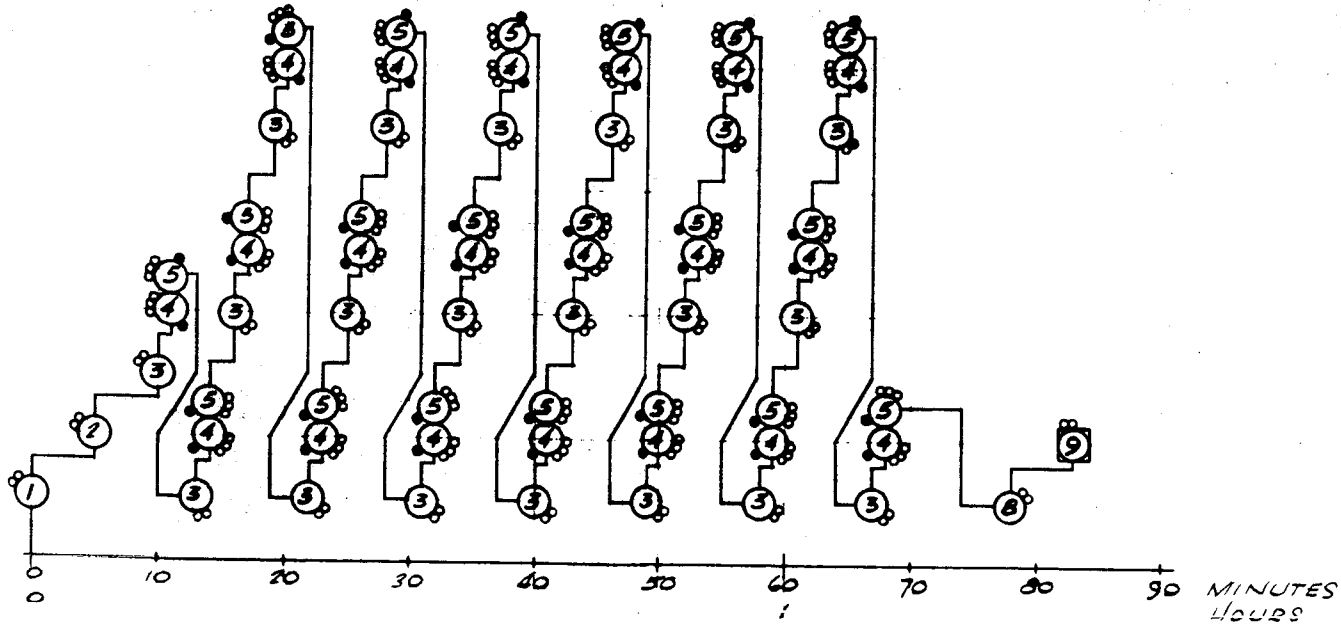
(9) Turn off power and stow.

EXPERIMENT 9- SOIL ELECTRICAL CONDUCTIVITY

SYMBOLS

- ABL COMMAND
- SELF CONTROLLED FUNCTION
- DATA OUTPUT
- CYCLE COMPLETION

NUMBERED CIRCLES REFER TO EXPERIMENT PROCEDURES



5.10 EXPERIMENT NO. 10: SOIL DENSITY BY γ-RAY SONDE

5.10.1 DESCRIPTION

This experiment is designed to provide a measurement of soil density as a function of depth, using a traversing sonde carrying instrumentation in the core hole from which the core drill sample is taken. In deep loose sand where no core hole can be made, the sonde will be capable of being driven into the soil without a hold. Supplemental measurements of soil temperature and humidity are made. Surface data from this experiment will be correlated with results of Experiment No. 11.

5.10.2 APPARATUS USED

A sonde similar in design to that of Texaco Experiment, Inc., but with less instrumentation. Soil density is determined using a gamma source and a Geiger-Mueller counter to detect scattered radiation. Subsurface temperature is determined from an external resistance thermometer mounted on the bow spring and by means of a modified Michelson interferometer. Water vapor or humidity will be detected using the aluminum-oxide/gold-film water vapor detector.

5.10.3 RANGE OF PARAMETERS

Temperature:	-160° to 50°C
Soil Density:	$0.5 < \text{sp. gr.} < 5$
Depth of Traverse:	3 meters

5.10.4 PROCEDURE

(1) Activate modified Michelson interferometer and check calibration with blackbody source.

(2) Check continuity and calibration of external resistance thermometer and water vapor detector.

(3) Check Geiger-Mueller tube output with source shielded and extended.

(4) Position sonde in core hole.

(5) Measure soil temperature with external resistance thermometer. DATA OUT

(6) Activate modified Michelson interferometer and stabilize bolometer sensor temperature.

(7) Record bolometer output and condition signal. DATA OUT

(8) Count scattered background radiation with gamma ray source retracted in shield. DATA OUT

(9) Position gamma ray source at the collimating window in shield.

(10) Count scattered radiation. DATA OUT

(11) Retract gamma ray source into shield.

(12) Advance subsurface sonde to next survey position in core hole.

(13) Repeat Steps (5) through (12) until core hole traverse is complete.

(14) Retract subsurface sonde to stowed position in the laboratory and secure for future use.

EXPERIMENT 10. SOIL DENSITY BY X-RAY SONDE

SYMBOLS

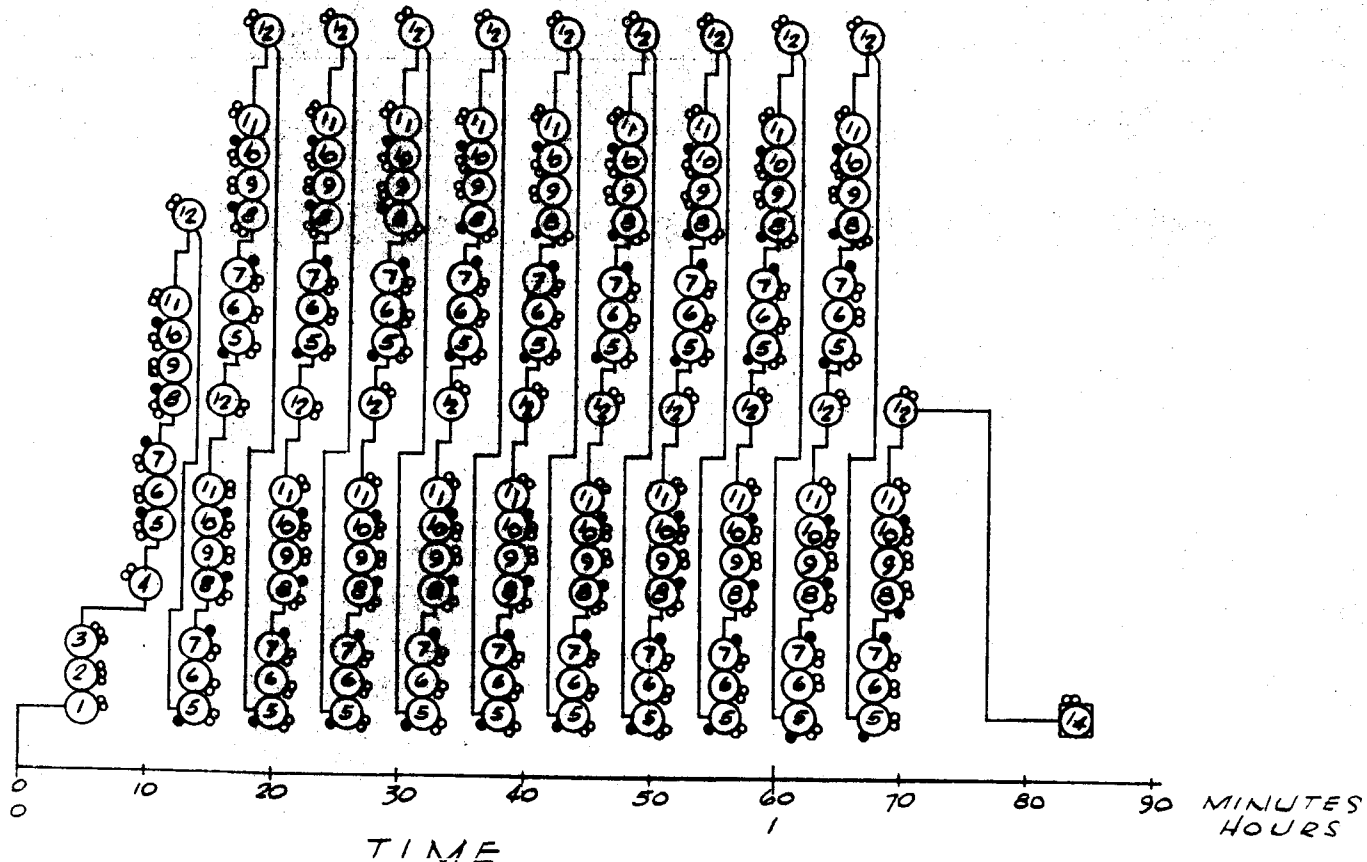
ABL COMMAND

SELF CONTROLLED FUNCTION

DATA OUTPUT

CYCLE COMPLETION

NUMBERED CIRCLES REFER TO EXPERIMENT PROCEDURES



5.11 EXPERIMENT NO. 11: SOIL MECHANICS DETERMINATION

5.11.1 DESCRIPTION

This experiment provides the information necessary to reliably select and use the proper sample collection equipment and provides environmental background to support the results of the lift detection experiments. It will provide soil strength data (bearing and shear), porosity or permeability data, and some estimates of soil particle size distribution.

5.11.2 APPARATUS USED

Bearing strength is determined from load-versus-sinkage relations. This relationship is most accurately described with the relationship

$$p = \left(\frac{k_c}{b} + k_\phi \right) z^n,$$

in which p is bearing pressure, z is sinkage, k_c is a cohesive constant, k_ϕ is the internal friction constant, b is a characteristic dimension of loading area, and n is an exponent determining the shape of the sinkage curve. This data is obtained by two annular rings of widths b_1 and b_2. The wide ring (b_1) is made integral with the housing of the pneumatic sample collector, with contact switches located circumferentially to indicate when complete bearing contact is achieved. These switches, coupled with load-sinkage data, give bearing strength and invormation on the nature of the surface; i.e., hard and uneven or yielding and smooth surface. A smaller-diameter tube with a narrow width (b_2) mounted concentric to the outer will obtain another set of load-sinkage data which is necessary to determine k_c and k_ϕ. After the load-sinkage data is obtained, the central tube is pressurized with Martian atmosphere and either pressure decay or flow rate used to determine soil permeability or porosity. Additional permeability data is obtained by measuring the rate of change in pressure as a function of time in the annular space enclosed between the two cylinders. Thus, if pressure is maintained at some value in the inner cylinder, the pressure in the annular space will increase and then level off at some value intermediate between the applied pressure and ambient atmospheric pressure. Soil shear strength is determined with a vane-type torsional shear tester which can be mounted concentrically in the center tube.

To determine particle size distrubution, it is necessary to separate a sample into various particle-size ranges by screening and weighing the percentage of the total sample collected with each screen. A rough approximation is achieved by weighing the total sample initially collected and then weighing the amount remaining in the refined sample for diameters less than 300 μ and the amount of the residue after pulverizing and grading. Thus, the percentage of soil sample in three ranges of sizes can be achieved

from the proposed sample pulverizing, grading, and refining system. This gives two points which can be fitted to the summation curve for an assumed normal (Gaussian) distribution, giving a first order approximation of particle size distributions. The three size ranges are particles greater than 5 mm in diameter, particles between 300 μ and 5 mm in diameter (residue), and those particles less than 300 μ in diameter.

5.11.3 RANGE OF PARAMETERS

Soil Type:	Solid rock to loose sand
Raw Sample Size:	500 grams
Weight Range:	0.1 gram < W < 500 grams
Porosity:	0 < p < 50%

5.11.4 PROCEDURE A

(1) Deploy outer bearing sylinder until surface contact is made.

(2) Activate controlled-feed mechanism and measure sinkage and applied forces until limit of travel is reached or a predetermined maximum load is attained. — LOAD DEFLECTION DATA OUT

(3) Survey contact switches for continuity of contact. If sinkage is essentially zero and full contact continuity is not achieved, store data for selection of sample collection method.

(4) Deploy central bearing cylinder until surface contact is made.

(5) Activate controlled-feed mechanism and measure sinkage/force data. — DATA OUT

(6) If sinkage/force data and continuity switches indicate a deformable soil, continue with Steps (7) through (16). If the surface is hard and uneven, continue with Steps (17) through (19).

(7) Insert vane shear probe to predetermined depth in soil.

(8) Apply torque to vane shear tester and determine soil shear strength. DATA OUT

(9) Pressurize inner bearing cylinder to predetermined maximum and monitor decay rate. DATA OUT

(10) If maximum pressure is not achieved, pressurize at predetermined maximum flow rate and record pressure increase in central bearing cylinder and the annular space between the central and outer bearing cylinder. DATA OUT

(11) Rig for sample collection, using aerosol dust sampler.

(12) Collect aerosol dust sample from surface.

(13) Rig for subsurface soil sample collection.

(14) Collect subsurface soil sample batch.

(15) Rig for collection of next subsurface soil sample batch.

(16) Repeat Steps (14) and (15) until all desired sample batches are collected or until soil composition precludes further collection and go to Step (20).

(17) Proceeding from Step (6), if a hard surface is encountered, rig for sample collection, using aerosol dust sampler.

(18) Collect aerosol dust sample.

(19) Redeploy soil mechanics and sample carrier to new site and repeat Steps (1) through (19). Repeat as necessary until a suitable sampling site is located or until sufficient aerosol dust sample is collected to complete an experimental cycle.

(20) Return soil mechanics and sample carrier to laboratory.

(21) Transfer samples to laboratory for further processing.

(22) Recycle soil mechanics and sample collection equipment.

5.11.5 PROCEDURE B

This part of Experiment 11 is performed at the laboratory during the sample grading process and yields an estimate of soil particle size distribution.

(1) Place raw sample batch on weight scale.

(2) Activate scale and uncage.

(3) Servo drive scal torsion bar back to null position and read weight. DATA OUT

(4) Cage scale and remove raw soil sample.

(5) Uncage scale and return to null position.

(6) Recage scale.

(7) Transport raw sample to pulverizer.

(8) Pulverize soil sample.

(9) Transport pulverized soil sample to grader.

(10) Grade sample to separate particle sizes with diameters equal to or less than 300 μ.

(11) Transport graded sample to weight scale.

(12) Repeat Steps (2) through (6). DATA OUT

(13) Transport graded sample to storage.

(14) Transport residue to weight scale.

(15) Repeat Steps (2) through (6). DATA OUT

(16) Transport residue to waste storage.

EXPERIMENT 11- SOIL MECHANICS DETERMINATION

SYMBOLS

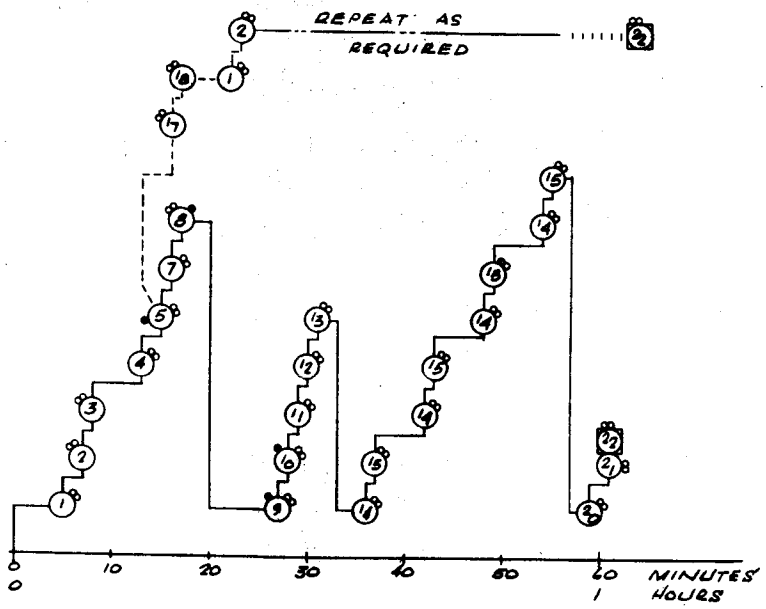
ABL COMMAND

DATA OUTPUT

CYCLE COMPLETION

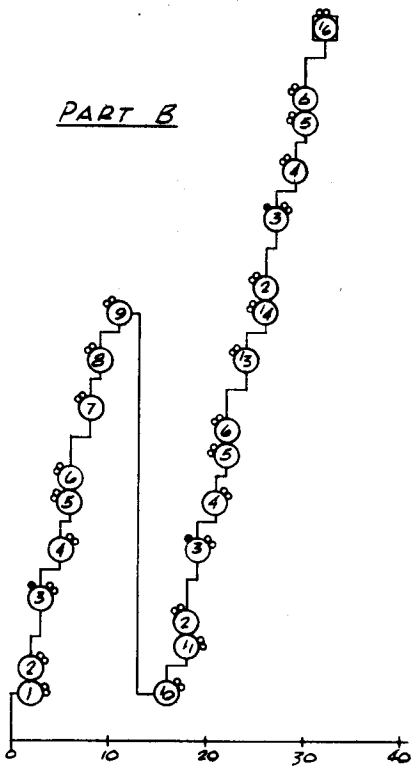
NUMBERED CIRCLES REFER TO EXPERIMENT PROCEDURES

PART A



TIME

PART B



TIME IN MINUTES

5.12 EXPERIMENT NO. 12: SOIL SAMPLE ENCAPSULATION AND PRESERVATION

5.12.1 DESCRIPTION

To encapsulate and preserve for future analysis samples of unprocessed soil. A kilogram of soil (or as much as can be obtained) will be taken at each sample site and will be preserved in a hermetically sealed container and identified by sample site, date of collection, and existing environment (i.e., soil temperature, time of day, atmospheric temperature, atmospheric pressure, atmospheric humidity, and wind velocity).

5.12.2 APPARATUS USED

A plug-type soil sampler will be used to collect a 7-cm-diameter-by-20-cm-long specimen with a minimum of disturbance. A cylinder with end caps will be employed and hermetically sealed.

5.12.3 RANGE OF PARAMETERS

1 kilogram of soil.

5.12.4 PROCEDURE

(1) Deploy sampler.

(2) Force sampler into surface to desired depth, or until maximum load value is reached.

(3) If full depth is reached, do Steps (4) through (11). If maximum load value is reached, go to Step (12).

(4) Close container cover. DATA OUT

(5) Retract sampler.

(6) Extract sample container.

(7) Transport container to sealer unit.

(8) Hermetically seal container.

(9) Transport sealed container to storage.

(10) Introduce tagged sample container into sampler.

(11) Stow sampler.

(12) Orient sample collector over new surface area.

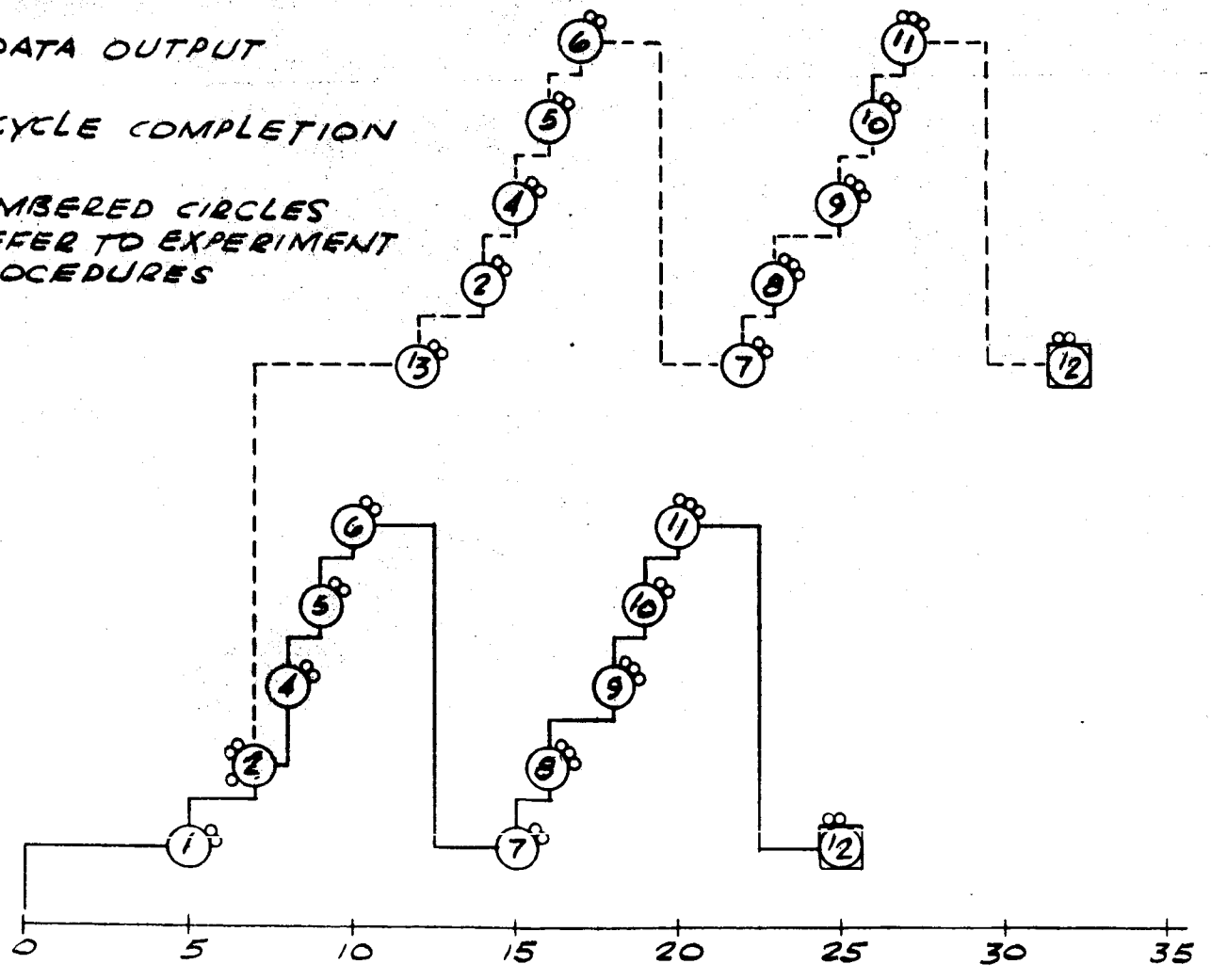
(13) Repeat Steps (2) through (11).

EXPERIMENT 12- SOIL SAMPLE ENCAPSULATION AND PRESERVATION

SYMBOLS

- EARTH COMMAND
- ABL COMMAND
- SELF CONTAINED FUNCTION
- DATA OUTPUT
- CYCLE COMPLETION

NUMBERED CIRCLES REFER TO EXPERIMENT PROCEDURES



5.13 EXPERIMENT NO. 13: ELEMENTAL SOIL ANALYSIS

5.13.1 DESCRIPTION

This experiment determines the elemental composition of the soil as completely as possible without chemical processing. The elements of principal interest are C, N, S, P, Mg, Na, K, Ca, Fe, and Si.

5.13.2 APPARATUS USED

An α-scattering analyzer will be employed using α-source and solid-state semiconductor detectors. This analysis will be conducted "in situ" or in the laboratory, or both.

5.13.3 RANGE OF PARAMETERS

Sample Size: 1g ungraded soil

5.13.4 PROCEDURE

(1) Introduce reference sample into instrument.

(2) Open slit.

(3) Record scattering and compare with calibration data. DATA OUT

(4) Close slit.

(5) Remove reference sample.

(6) Introduce test sample.

(7) Open slit.

(8) Record scattering data. DATA OUT

(9) Close slit.

(10) Secure source.

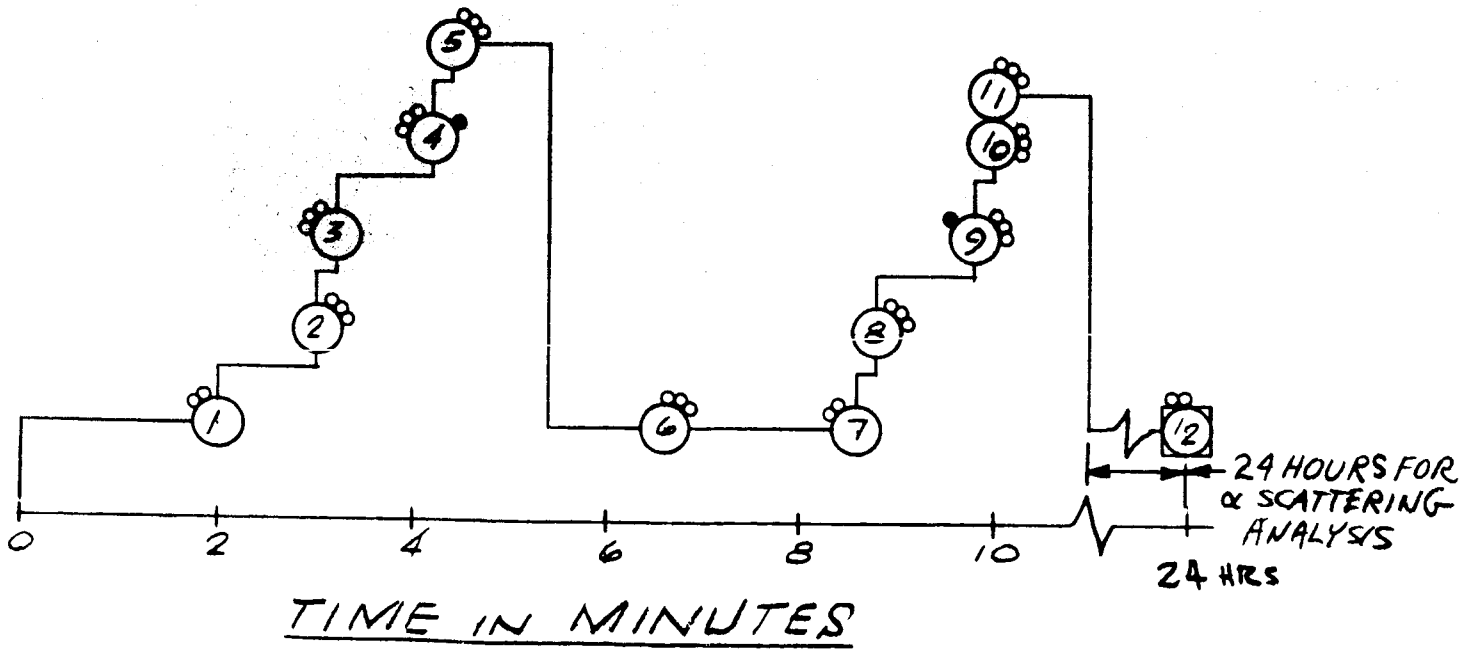
(11) Remove test sample and transport to storage for future analysis.

EXPERIMENT 13 - ELEMENTAL SOIL ANALYSIS

SYMBOLS

- ABL COMMAND
- SELF CONTAINED FUNCTION
- DATA OUTPUT
- CYCLE COMPLETION

NUMBERED CIRCLES REFER TO EXPERIMENT PROCEDURES



5.14 EXPERIMENT NO. 14: SOIL GAS ANALYSIS

5.14.1 DESCRIPTION

This experiment is designed to determine the composition of soil gases extracted as a function of depth by the probe in Experiment 8. Specifically, the gases of interest are CH_4, NH_3, H_20, CO_2, CO, H_2S, O_2, O_3, NO, NO_2, H_2, N_2, and SO_2.

5.14.2 APPARATUS USED

The soil probe in Experiment 8 collects the soil gas sample. The sample will be analyzed with a gas chromatograph employing three columns and using an argon ionization detector followed by a mass spectrometer.

5.14.3 RANGE OF PARAMETERS

Molecular Weights:	Up to 100
Composition:	By percentage

5.14.4 PROCEDURE

(1) Transport soil gas sample from Experiment 8 to metering chamber.

(2) Feed slug of soil gas into gas chromatograph column carrier gas.

(3) Record detector output from gas chromatograph columns at a rate of one data point per second for one minute. DATA OUT

(4) Divert samples of interest from columns into transfer ampules.

(5) Transport samples from Step (4) to mass spectrometer.

(6) Inject samples into mass spectrometer.

(7) Record spectrometer detector output. DATA OUT

(8) Release stored soil gas.

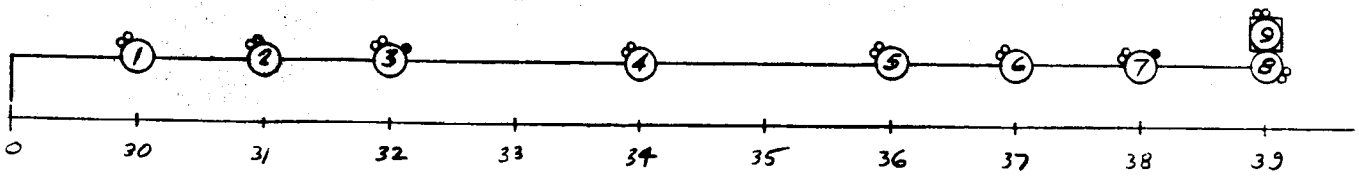
(9) Recycle equipment to ready.

EXPERIMENT 14- SOIL GAS ANALYSIS

SYMBOLS

- ABL COMMAND
- DATA OUTPUT
- CYCLE COMPLETION

NUMBERED CIRCLES REFER TO EXPERIMENT PROCEDURES



TIME IN MINUTES

5.15 EXPERIMENT NO. 15: DETERMINATION OF SOLUBLE INORGANIC IONS AND pH

5.15.1 DESCRIPTION

An aqueous extraction of the soil will be performed to determine pH and presence of inorganic ions. Ions of interest are $CO_3^{=}$, $PO_4^{\equiv}$, H^+, $SO_4^{=}$, NO_3^-, NO_2^-, $S^=$, OH^-, Cl^-, and Na^+. Mg^{++} Fe^{++}, Fe^{+++}, K^+ NH_4^+ Ca^{++}, and (Co^{++}, Cu^{++}, Zn^{++}, Mn^{++}).(1)

5.15.2 APPARATUS USED

A chemical processing chamber utilizing filtration and an aqueous extraction, with extract evaporation on a plate for α-scattering. Analytical measurements are made with a pH meter and an α-scattering analyzer, with solid-state detectors.

5.15.3 RANGE OF PARAMETERS

pH:	1-13
Ion Detection:	By pulse height counter
Range:	Carbon to maximum possible Z with α-scattering technique

5.15.4 PROCEDURE

(1) Check equipment (α-scattering).

(2) Rig reaction chamber to accept soil sample.

(3) Collect a soil sample (one gram of processed sample).

(4) Mechanically process soil sample.

(5) Transfer measured sample (~1 gram) to reaction chamber.

(6) Acquire three volumes of water (3X volume of soil sample).

---

(1) Doubtful by α-scattering. Possibly use X-ray fluorescence spectroscopy for heavy elements.

(7) Add water to soil sample.

(8) Stir for 5 minutes.

(9) Filter.

(10) Retain both filtrate and residue.

(11) Repeat extraction Steps (6) through (10) two more times.

(12) Combine filtrates.

(13) Transfer residue to waste storage.

(14) Evaporate filtrate by dropwise addition onto heated platten.

(15) When evaporation is complete (allow 60 minutes), transfer platten to α-scattering instrumentation.

(16) Determine α-scattering spectrum (allow 24 hours). DATA OUT

(17) Remove platten.

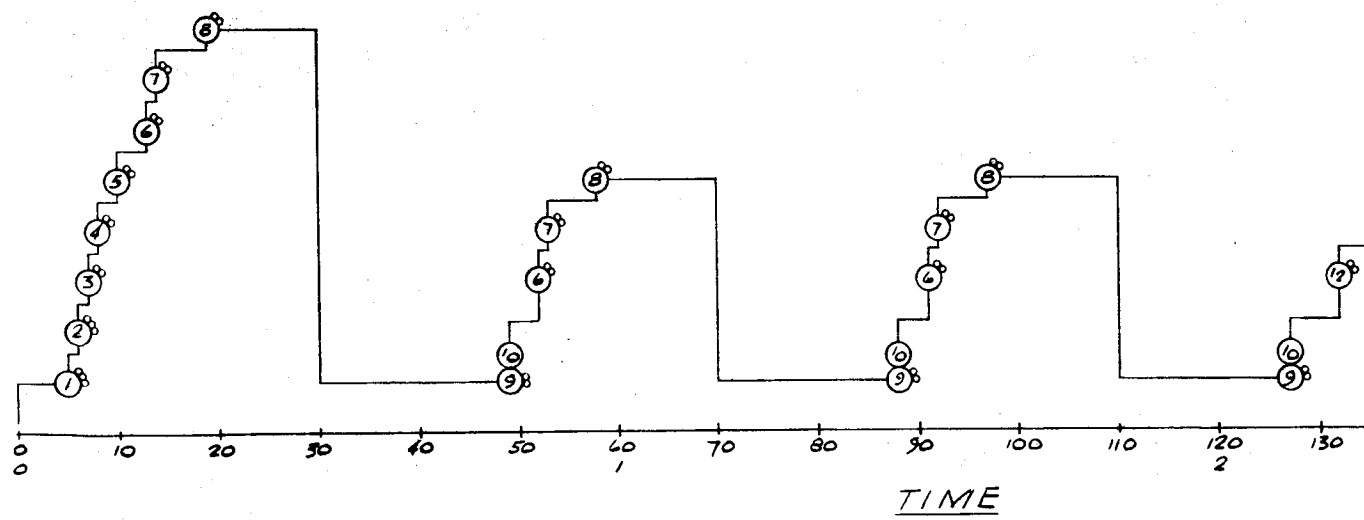
(18) Clean and recycle equipment as necessary.

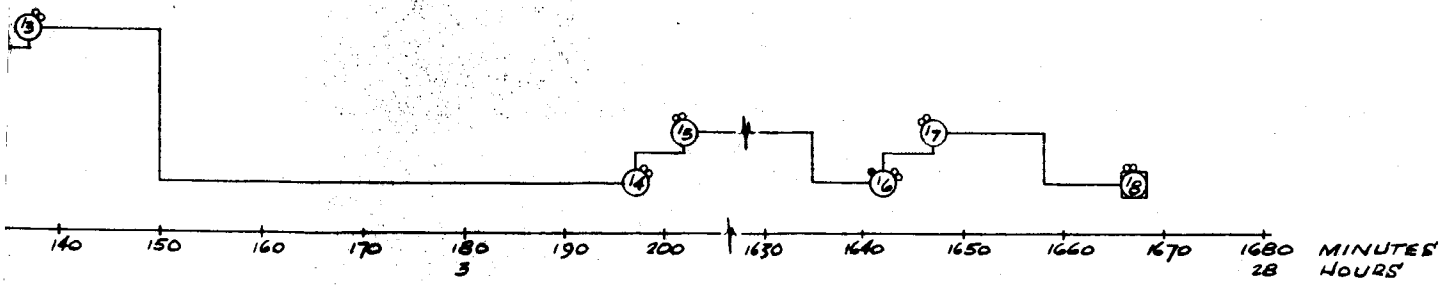
EXPERIMENT 15- DETERMINATION OF SOLUBLE INORGANIC IONS AND pH.

SYMBOLS

- ABL COMMAND
- SELF CONTROLLED FUNCTION
- DATA OUTPUT
- CYCLE COMPLETION

NUMBERED CIRCLES REFER TO EXPERIMENT PROCEDURES

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MINUTES
HOURS

5.16 EXPERIMENT NO. 16: DETECTION OF ORGANIC MATERIAL IN SOIL

5.16.1 DESCRIPTION

This experiment is designed to detect the presence of organic material in a soil sample. The soil sample is subjected to a programmed heating to remove residual H_2O and gases, followed by pyrolysis of solid material. Complex organic materials are detected in the pyrolysis products.

5.16.2 APPARATUS USED

A chemical processing chamber utilizing a programmed heating and pyrolysis with an argon ionization detector and/or fluorometer using an excitation source at 350 mμ is employed.

5.16.3 RANGE OF PARAMETERS

Broad-band fluorescence spectrum from 450 to 700 mμ.

Sample Size: 5g ungraded soil

5.16.4 PROCEDURE

(1) Transfer 5 grams of ungraded soil into plyrolysis cup.

(2) Position pyrolysis cup in pyrolysis oven.

(3) Seal chamber.

(4) Position fluorometer cuvette above argon ionization detector.

(5) Pressurize pyrolysis chamber with argon at 15 psia.

(6) Initiate programmed heat at 3.3°C per minute.

(7) After one minute, draw gases envolved through argon ionization detector. Record temperature with detector output. DATA OUT

(8) Inject gases into cuvette.

(9) Repressurize pyrolysis chamber with argon at 15 psia.

(10) Repeat Steps (7) through (9) until a maximum temperature of 250°C is reached.

(11) Initiate pyrolysis heating until a maximum temperature of 850°C is reached.

(12) Repeat Steps (9) through (11).

(13) Remove pyrolysis residue and transfer to waste storage.

(14) Remove sample cuvette and transfer to fluorometer and allow to stabilize at laboratory temperature of 4°C.

(15) Perform fluorometric analysis. Source excitation at 350 mμ and scan spectrum from 450 mμ to 700 mμ. DATA OUT

(16) Remove sample from fluorometer and transfer to waste storage.

(17) Seal pyrolysis oven.

(18) Pressurize with argon at 15 psia and maintain flow to chamber as required.

(19) Purge the argon ionization detector and pump of residual gases.

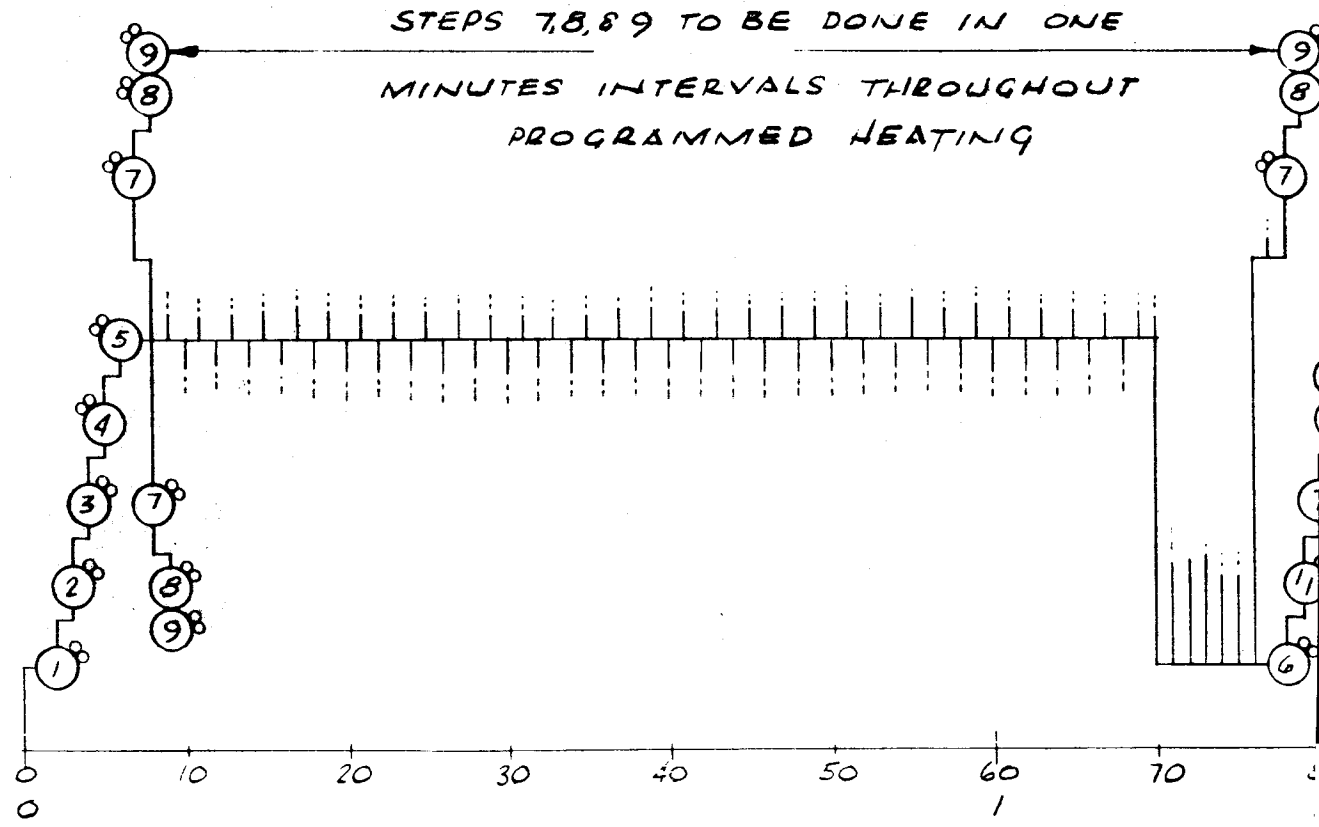
(20) Secure pyrolysis oven.

EXPERIMENT 16- DETECTION OF ORGANIC M

SYMBOLS

- ABL COMMAND
- SELF CONTROLLED FUNCTION
- DATA OUTPUT
- CYCLE COMPLETION
- SPECTRAL ANALYZER SUB-ROUTINE

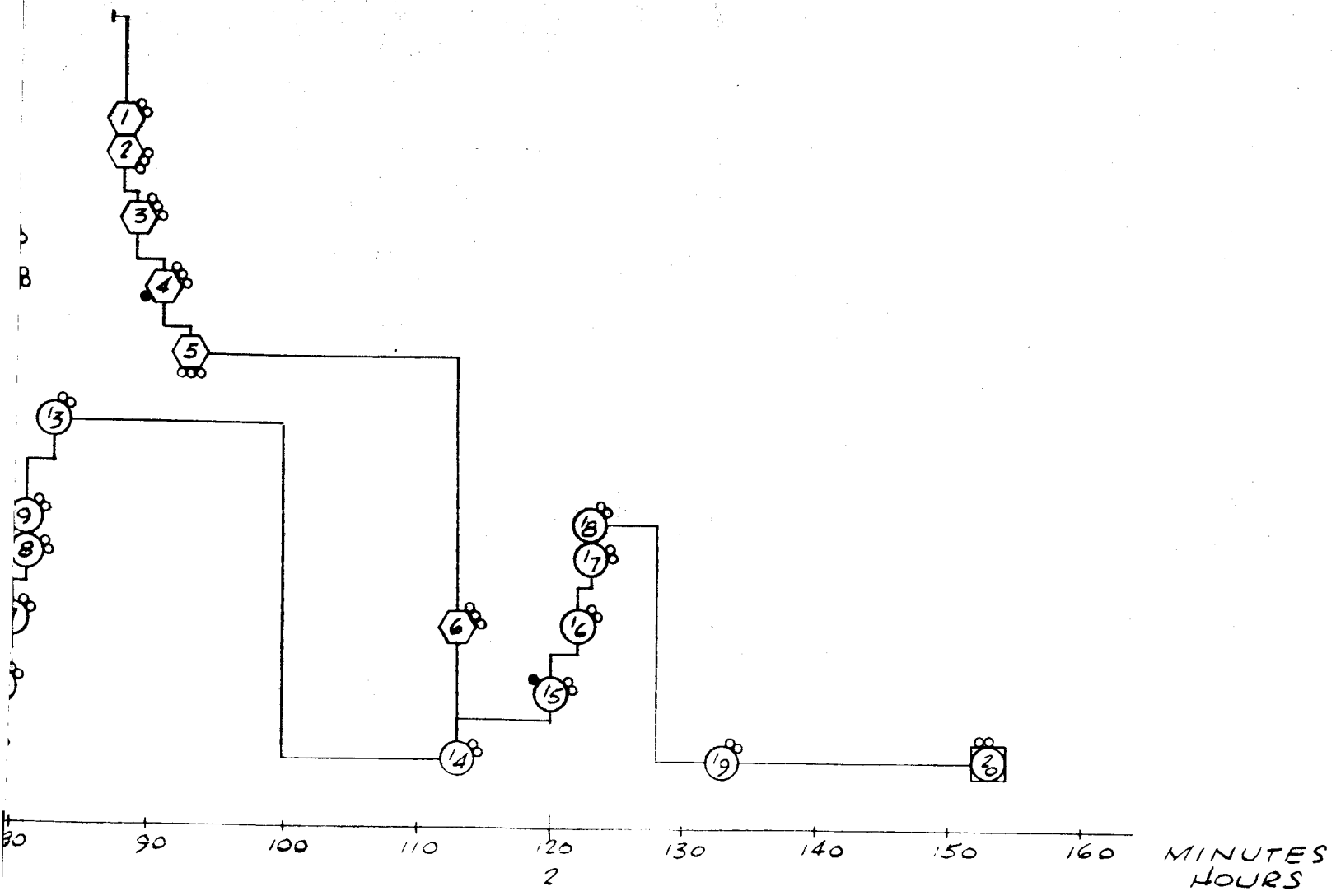
NUMBERED C
PROCEDURES



TIM

ATERIAL IN SOIL

RCLES REFER TO EXPERIMENT



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5.17 EXPERIMENT NO. 17: SOIL GAS EXCHANGE

5.17.1 DESCRIPTION

This experiment evaluates the composition of gases evolved from a soil sample "in situ" for both an undisturbed sample and one to which labeled substrates have been added. Gases of interest are H_2O, CO_2, O_2, CO, CH_4, H_2, NH_3, H_2S, SO_2, NO, NO_2, N_2, and labeled $C^{14}O_2$, $C^{14}O$, $C^{14}H_4$ and $S^{35}O_2$, H_2S^{35} labeled gases.

5.17.2 APPARATUS USED

A gaseous sample collector collects evolved gases, which are then analyzed with a three-column gas chromatograph, followed by a mass spectrometer. Labeled outputs are detected by β radiation counter. The addition of labeled substrates requires the use of a chemical dispenser capable of purging the substrate compound of decomposition products which are radioactively labeled. Argon ionization detector and β counter are employed to detect labeled gases.

5.17.3 PROCEDURE

(1) Deploy three fused silica gas collection chambers on the surface.

(2) Start closed-loop circulation of atmosphere in each chamber.

(3) Withdraw gas sample from each chamber for control analysis.

(4) Inject sample in gas chromatograph and perform analysis . DATA OUT

(5) Repeat Step (4) for sample from each chamber.

(6) Transfer C^{14} labeled substrate from storage to flushing chamber.

(7) Flush substrate with CO_2, followed by N_2, to remove decomposition products.

(8) Transfer C^{14} labeled substrate to injection probe.

(9) Insert probe into soil and inject or infuse a measured quantity of C^{14} substrate in one of the three chambers.

(10) Repeat Step (9) in predetermined pattern within the chamber.

(11) Transfer S^{35} labeled substrate from storage to flushing chamber.

(12) Flush substrate with N_2 to remove decomposition product.

(13) Transfer S^{35} labeled substrate to injection probe.

(14) Insert probe into soil and inject or infuse a measured quantity of S^{35} substrate in another of the three chambers.

(15) Repeat Step (14) in predetermined pattern within the chamber.

(16) At 3-hour intervals, withdraw a gas sample from each of the chambers.

(17) Inject sample in gas chromatograph and perform analysis. DATA OUT

(18) Repeat Step (17) for sample from each chamber.

(19) Collect labeled gas effluent from gas chromatograph.

(20) Transfer to β ionization chamber.

(21) Perform rate count. DATA OUT

(22) Repeat Steps (19) through (21) for each peak recorded on gas chromatograph.

(23) Repeat Steps (16) through (21) for 48 hours

(24) Turn off recirculation pump.

(25) Recycle substrate injectors and stow.

(26) Return chambers to laboratory and secure for future use.

EXPERIMENT 17- SOIL GAS EXCHANGE

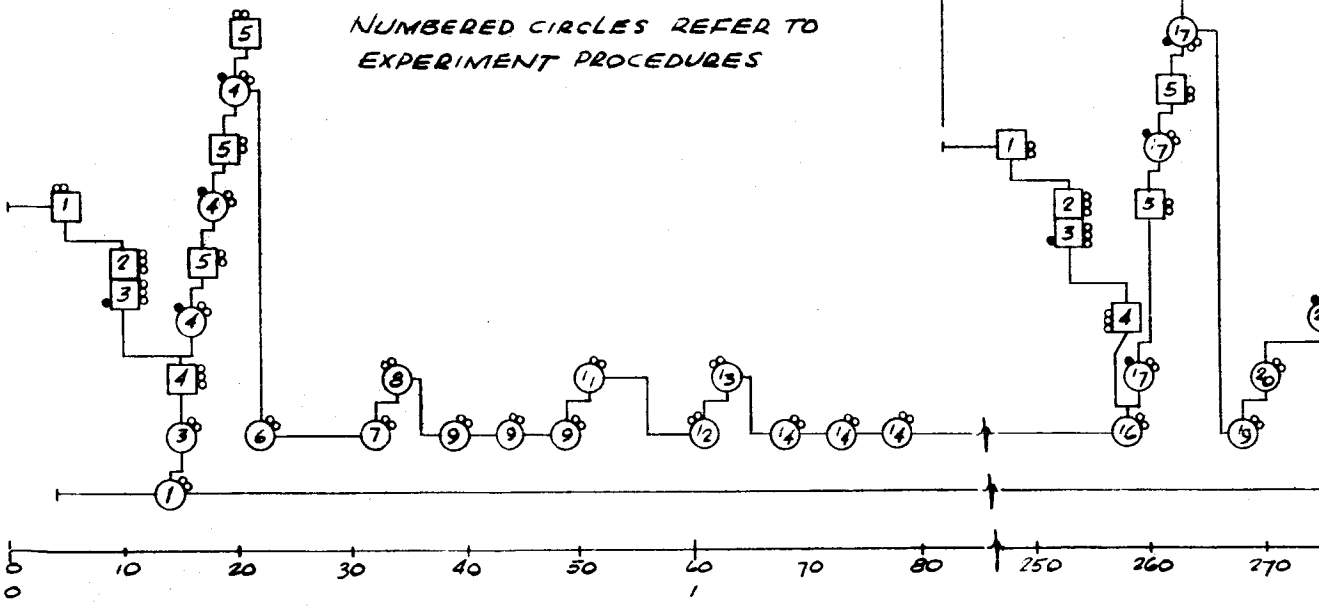
SYMBOLS

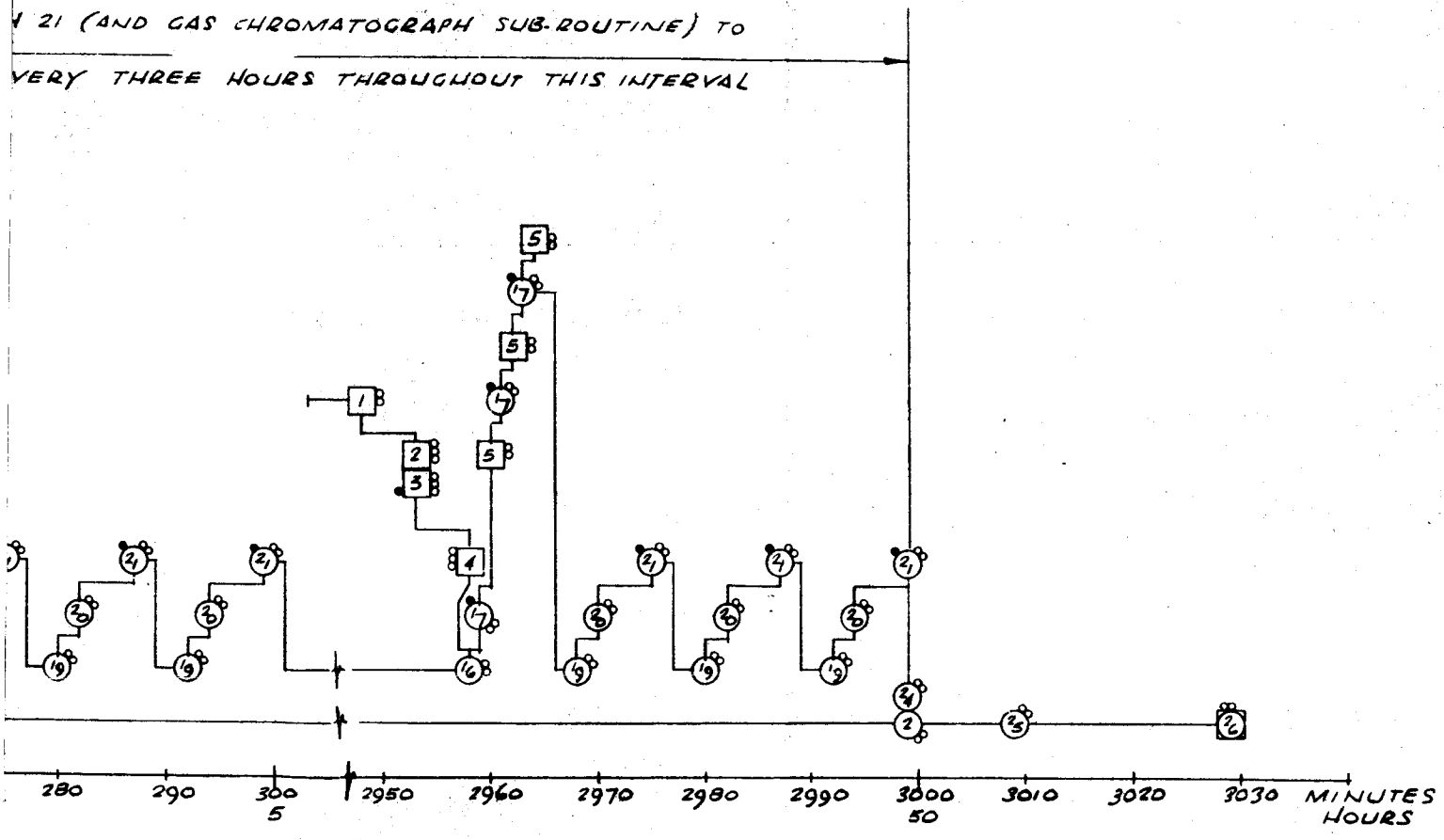
- ABL COMMAND
- SELF CONTROLLED FUNCTION
- DATA OUTPUT
- CYCLE COMPLETION
- GAS CHROMATOGRAPH SUB-ROUTINE

NUMBERED CIRCLES REFER TO EXPERIMENT PROCEDURES

STEPS 16 THROUGH

BE DONE E




21 (AND GAS CHROMATOGRAPH SUB-ROUTINE) TO
VERY THREE HOURS THROUGHOUT THIS INTERVAL
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5.18 EXPERIMENT NO. 18: AMINO ACID ANALYSIS

5.18.1 DESCRIPTION

This experiment is designed to detect amino acids using a programmed heating, followed by pyrolysis of a graded but unprocessed soil sample.

5.18.2 APPARATUS USED

Sample collection equipment.

Sample mechanical processing and transfer equipment.

Gas chromatograph equipped with a pyrolysis attachment. Column: 5-ft-by 1/8-in.-diameter of 15 percent Apiezon L, 4.5-percent Carbowax 20M, and 3-percent phosphoric acid on Chromosorb. Flow rate 50 ml/min at 105° to 107°C. (Surveyor gas chromatograph.)

The pyrolysis oven should be provided for heating to 150°, 325°, and 500°C.

5.18.3 RANGE OF PARAMETERS

Soil Sample: 5g, $d \leqq 300\ \mu$

5.18.4 PROCEDURE

(1) Check equipment and warm up column oven.

(2) Collect sample. A volume of sample ranging from 1 to 3 cc is desired.

(3) Process sample, if required.

(4) Transfer sample to pyrolysis oven.

(5) Select pyrolysis temperature.

(6) Initiate pyrolysis cycle.

(7) Transfer vapors to chromatograph.

(8) Do chromatogram.

(9) Clean column (backflush).

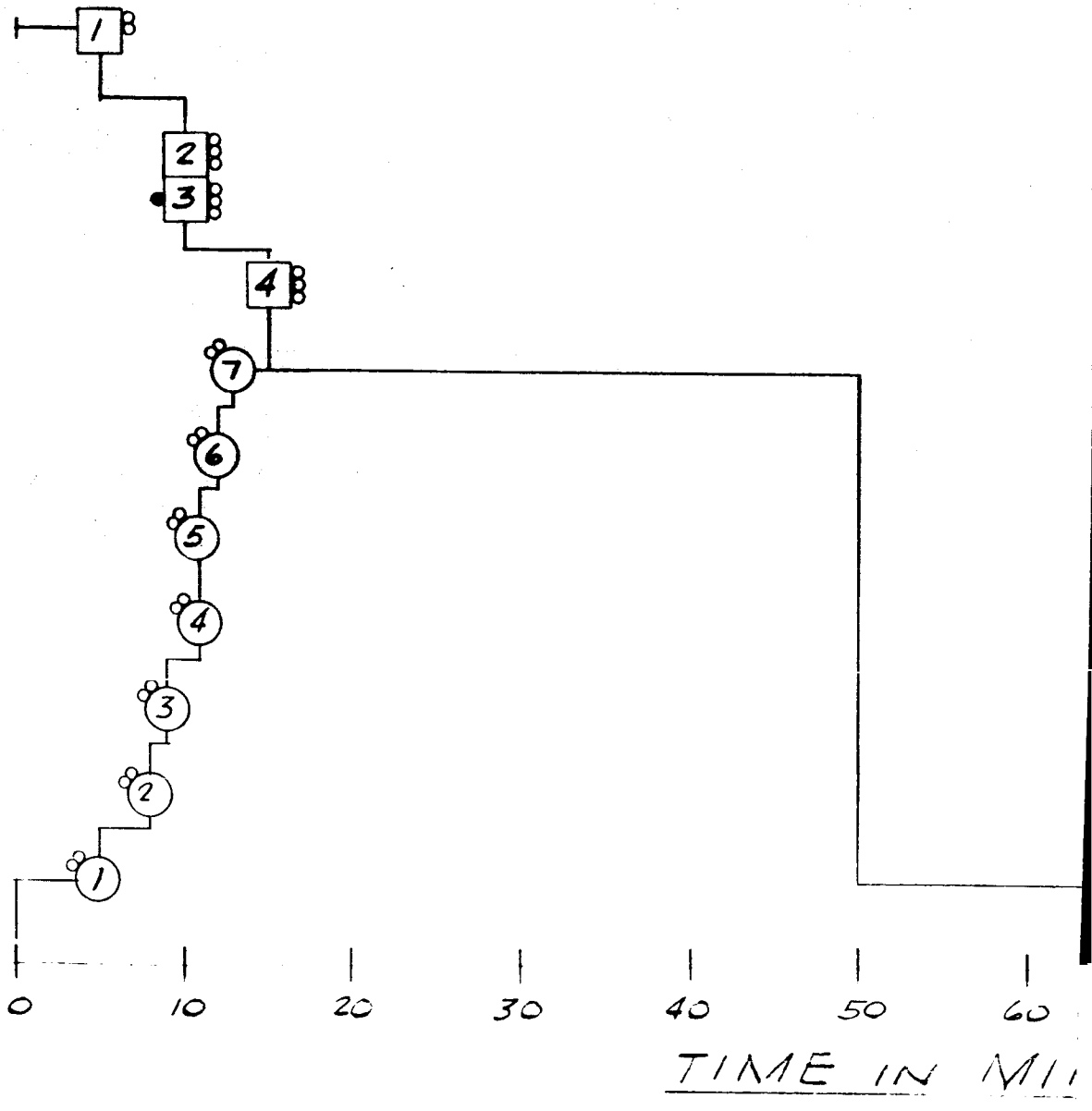
(10) Allow oven and column to cool.

(11) Remove residue from the oven.

(12) Clean oven.

(13) Recycle to ready.

EXPERIMENT 18 - AMINO ACID ANALYSIS

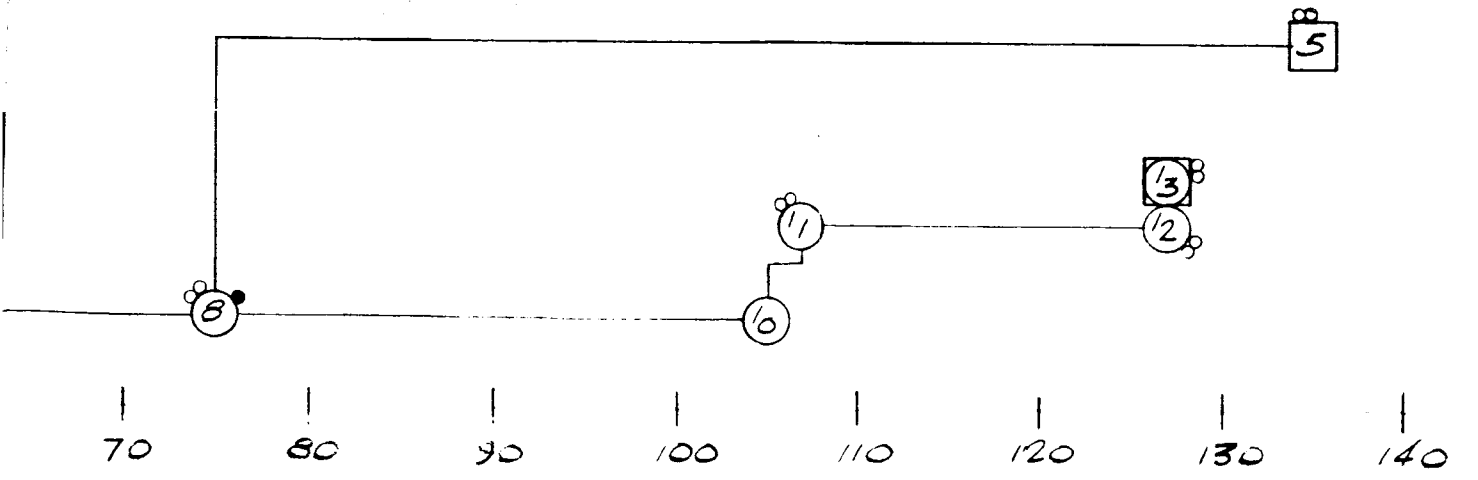


BY PYROLYSIS-GAS CHROMATOGRAPHY

SYMBOLS

- ABL COMMAND
- DATA OUTPUT
- CYCLE COMPLETION
- GAS. CHROMATOGRAPH SUB-ROUTINE

NUMBERED CIRCLES REPRESENT EXPERIMENT STEPS



NOTES

5.19 EXPERIMENT NO. 19: DETECTION OF AMINO ACIDS AND OPTICAL ACTIVITY

5.19.1 DESCRIPTION

This experiment differs from Experiment 18 in that chemical processing is performed involving extraction, filtration, and volatile derivative preparation. The basis of the experiment is that derivatives with two asymmetrical carbons have different steriochemistries and separate in the gas chromatograph columns.

5.19.2 APPARATUS USED

The chemical processor is used to perform extraction, filtration, and derivative preparation. The analysis is performed by a gas chromatograph employing a 1/8-inch-diameter-by-5-foot-long column, followed by an argon ionization detector.

5.19.3 RANGE OF PARAMETERS

Soil Sample: 5g, $d \leq 300\,\mu$

5.19.4 PROCEDURE

(1) Check equipment.

(2) Collect a soil sample (2 to 5 grams).

(3) Mechanically process sample.

(4) Weigh the sample (2 to 5 grams) or measure volume.

(5) Transfer sample to the reaction chamber.

(6) Acquire 10 ml of water.

(7) Add to reaction chamber containing soil sample.

(8) Stir for 5 minutes.

(9) Filter.

(10) Retain both filtrate and residue.

(11) Repeat extraction Steps (6) through (1) two more times.

(12) Combine filtrates.

(13) Transfer residue to waste storage.

(14) Evaporate filtrate to dryness-controlling temperature so that it never rises to point of causing decomposition or polymerization (allow 2 hours).

(15) Treat dry residue with 5 ml d-sec-butyl alcohol (anhydrous).

(16) Saturate with hydrogen chloride gas (allow 5 minutes).

(17) Stir for one hour.

(18) Filter.

(19) Evaporate filtrate to near dryness (~0.5 ml liquid volume should remain). (Allow one hour.)

(20) Transfer an aliquot (few μl) to gas chromatograph.

(21) Do gas chromatograph (allow one hour). DATA OUT

(22) Clean reaction chamber equipment.

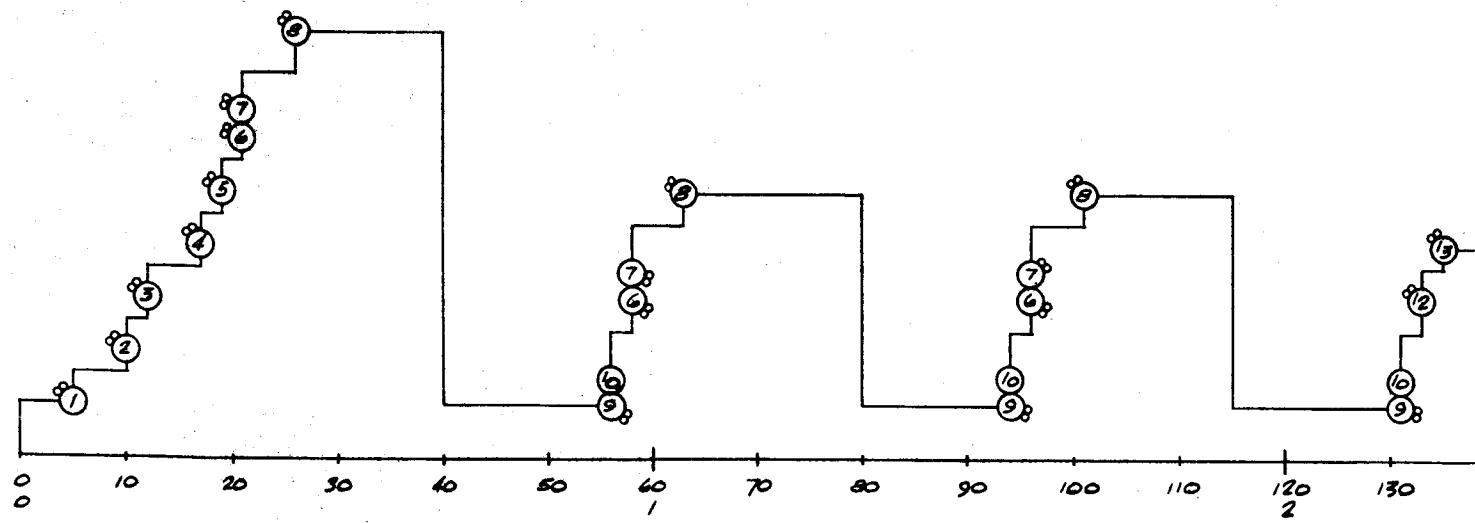
(23) Recycle gas chromatograph and other equipment.

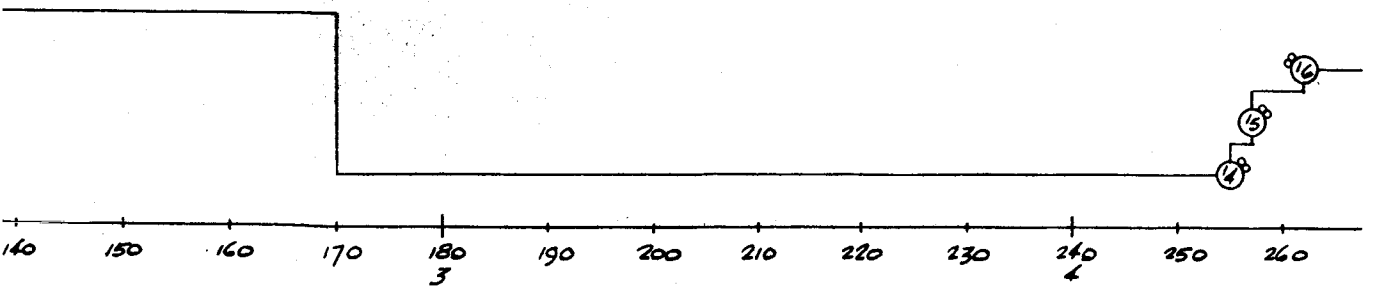
EXPERIMENT 19- DETECTION OF AMINO ACIDS & OPTICAL ACTIVITY

SYMBOLS

- ABL COMMAND
- SELF CONTROLLED FUNCTION
- DATA OUTPUT
- CYCLE COMPLETION
- GAS CHROMATOGRAPH SUBROUTINE

NUMBERED CIRCLES REFER TO EXPERIMENT PROCEDURES

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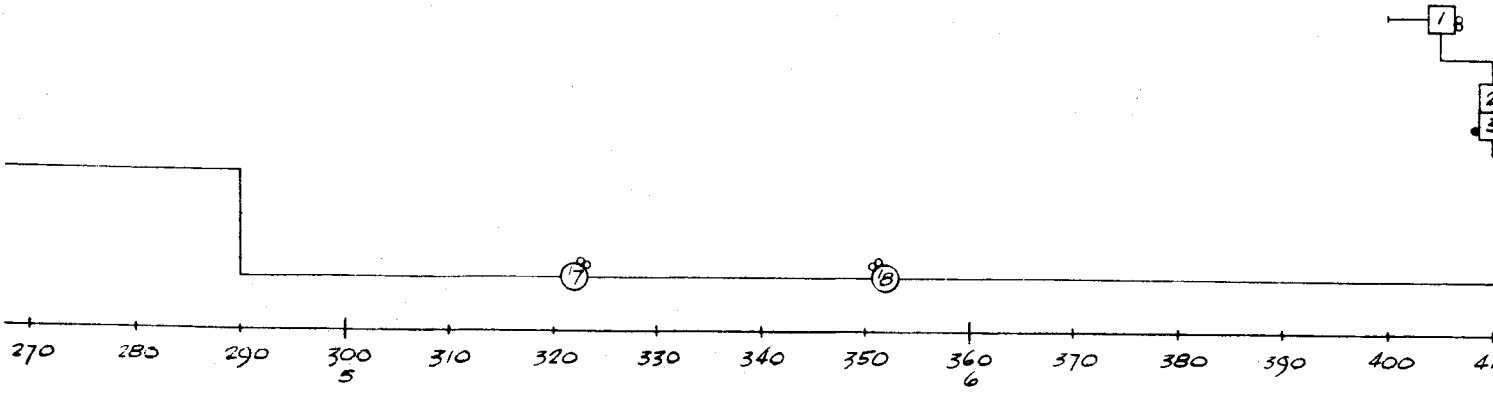
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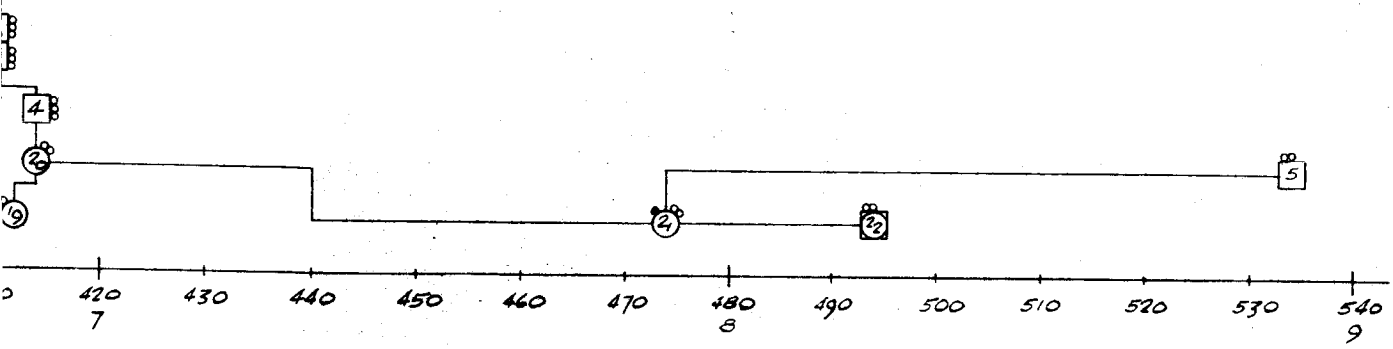
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5.20 EXPERIMENT NO. 20: DETECTION OF PORPHYRINS

5.20.1 DESCRIPTION

To detect porphyrins using chemical extraction and processing followed by a fluorometer analysis of the solution.

5.20.2 APPARATUS USED

A chemical processor is used to perform a soil extraction, homogenization, and filtration. A fluorometer with an exciting source at 405 mμ Hg line and capability of scanning from 600 to 700 mμ is used.

5.20.3 RANGE OF PARAMETERS

Fluorescent Intensity:	4 decades of variation
Wavelength Scan:	To 1 mμ resolution
Sample Size:	10g, $d \leq 300\mu$

5.20.4 PROCEDURE

(1) Check equipment.

(2) Collect sample (surface soil sample preferred). Present estimate of sample size is 10 grams.

(3) Grade sample by sieving through a coarse screen.(2)

(4) Weigh sample. Sample should weigh about 10 grams.(3)

DATA OUT

---

(1) Equipment check should consist of checking out fluorometer and sample processing equipment.

(2) The sample grading may be bypassed if the sample is collected in a manner which pulverizes large chunks or excludes such chunks as rocks. However, to assure that the homogenizer is not damaged, removal of large, hard pieces is recommended. Thus, the primary purpose of grading is to prevent homogenizer damage.

(3) A rough weighing to about ± 0.2 gram is probably adequate. Volume measuring technique might also suffice.

(5) Acquire 30 ml of 4:1 ethyl acetate-acetic acid.

(6) Transfer weighed sample to the homogenizer.

(7) Add solvent to homogenizer containing the sample.

(8) Homogenize for a period of 10 minutes.

(9) Filter the homogenate.

(10) Retain filtrate[4]

(11) Remove residue. RESIDUE

(12) Transfer filtrate to the reaction chamber.

(13) Acquire 10 ml of EDTA solution.[5]

(14) Add EDTA to filtrate.

(15) Mix.

(16) Bubble oxygen gas through the solution for a period of 5 minutes.[6]

(17) Fill fluorometer cell.

(18) Transfer cell to fluorometer.

---

[4] The filtrate will be an ethyl acetate-acetic acid solution containing porphyrins with their metal ions and porphyrinogens.

[5] The EDTA is suggested to be a 0.01-M solution in 4:1 ethyl acetate-acetic acid. The EDTA serves the function of chelating metal ions, both those extracted from soil by the solvent and those associated with the porphyrins or porphyrinogens. These metal ions, unless chelated, may interfere in the fluorescence spectrum. A laboratory check of this procedure is required to define such problems and to define the details of the procedure.

[6] The act of bubbling oxygen through the solution may effect the desired mixing, so that Step (15) might be eliminated. The oxygen serves to oxidize the porphyrinogens to porphyrins.

(19) Excite fluorescence with source illumination having a wavelength of 400 mμ.[7]

(20) Record fluorescent spectrum between 600 and 750 mμ. DATA OUT

(21) If the fluorescence intensity is so high that the detector is saturated, go to Step (22). If the fluorescence is detectable but does not saturate the detector over the wavelength region from 600 to 750 mμ, go to Step (30).

(22) Reduce intensity of excitation source by one-half and go to Step (20).

(23) Remove cell from fluorometer.

(24) Bubble through gaseous HCl for a period of 5 minutes to make 2M in HCl.

(25) Return cell to fluorometer.

(26) Scan spectral region from 600 to 750 mμ as before. DATA OUT

(27) Remove cell.

(28) Shut off fluorometer source.

(29) Recycle fluorometer.

(30) Remove sample from cell. SAMPLE

(31) Clean cell and processing equipment.

(32) Recycle all equipment to ready position.

---

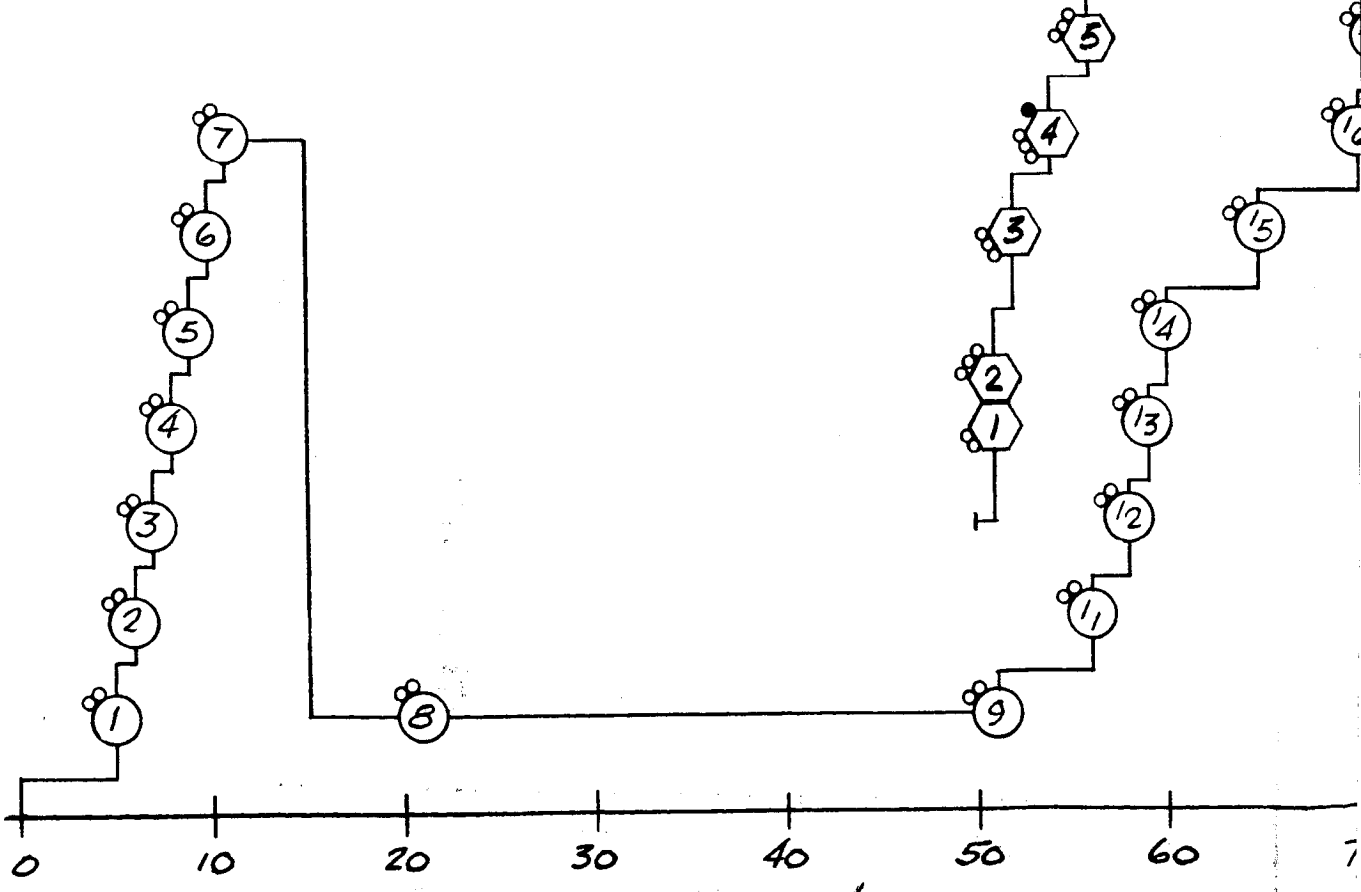
[7] The Soret absorption band of the porphyrins at 400 mμ is used to absorb excitation energy. An isolated 405 mμ Hg line could also be used for this purpose. The porphyrin fluorescence is shifted considerably to the red region of the visible spectrum.

EXPERIMENT 20- DETECTION OF PORPHY
EXTRACTION AND FLUO

SYMBOLS

- EARTH COMMAND
- ABL COMMAND
- SELF-CONTROLLED FUNCTION
- DATA OUTPUT
- CYCLE COMPLETION
- SPECTRAL ANALYZER SUB-ROUTINE

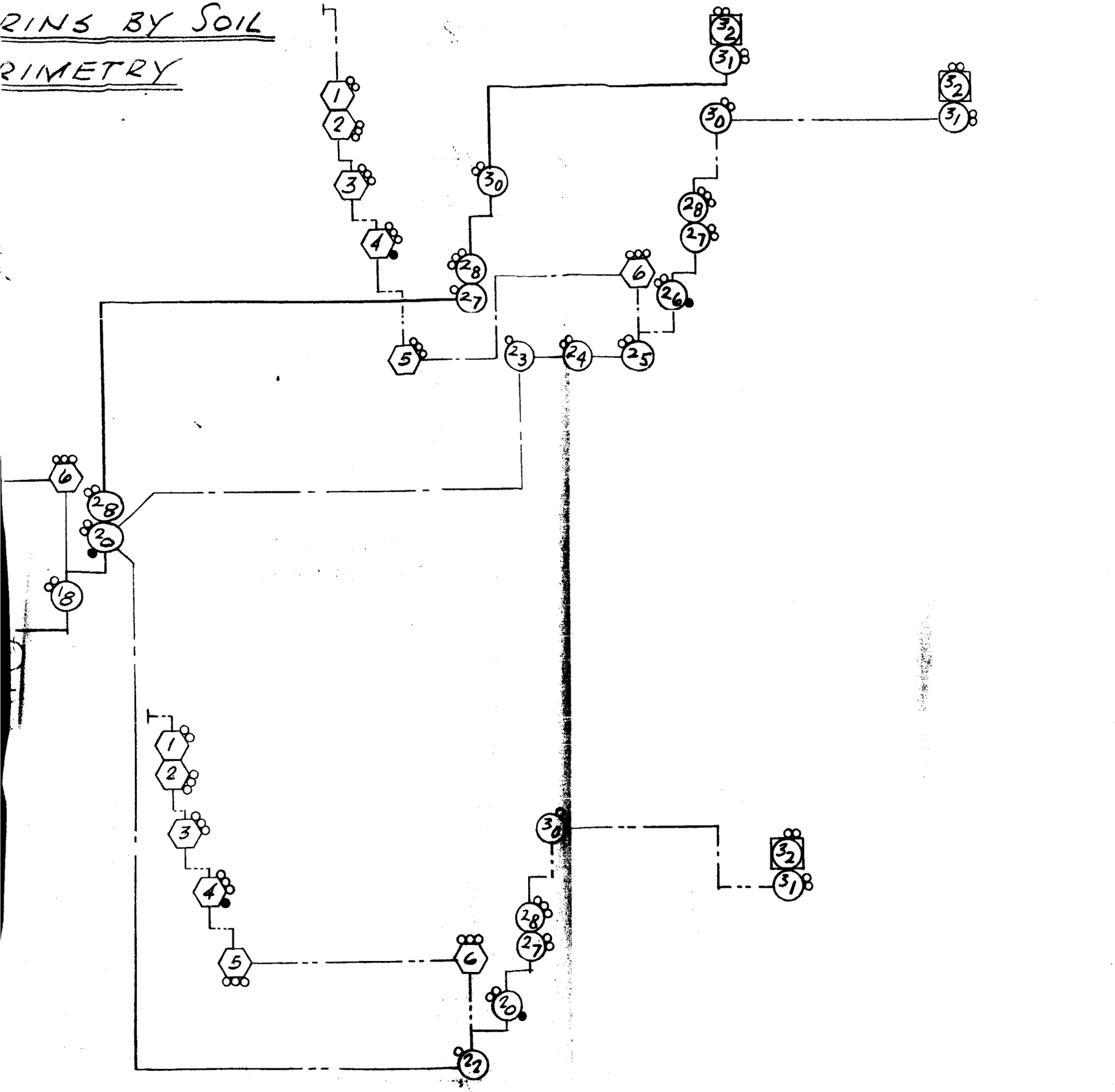
NUMBERS IN CIRCLES REFER TO EXPERIMENT STEPS



5-63-1

RINS BY SOIL

RIMETRY



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TIME IN MINUTES

5-63-2

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5.21 EXPERIMENT NO. 21: DETECTION OF FLAVINS

5.21.1 DESCRIPTION

This experiment is designed to detect flavins by a spectrofluorometric analysis.

5.21.2 APPARATUS USED

A chemical processor is required capable of performing a solvent extraction, homogenization, programmed heating, and a liquid/liquid phase separation. The analysis is performed with a fluorometer with the excitation source at 445 mμ and capability of scanning from 500 to 600 mμ.

5.21.3 RANGE OF PARAMETERS

Fluorescent Intensity:	4 decades of variation
Wavelength Scan:	To 1 mμ resolution
Sample Size:	5g, d $\leq$ 300μ

5.21.4 PROCEDURE

In the following procedure, flavins are extracted from a sample with water and sulfuric acid. Since flavins are practically insoluble in chloroform, the acidic extract is treated with chloroform to remove fluorescent materials which would later interfere in the detection of lumiflavin fluorescence. The extract is next made basic and photolyzed, converting the flavins to lumiflavins. Since lumiflavins are chloroform-soluble, and since all other fluorescent, chloroform-soluble compounds were previously removed, a chloroform extract of the photolysis mixture will contain lumiflavin free of interfering substances. Fluorescence of the chloroform extract indicates the presence of lumiflavins and, therefore, the presence of flavins in the original sample.

(1) Check equipment.[1]

(2) Collect a sample of soil or suspected biological material. An excess of over 5 grams is desired if the sample is soil; over 1 gram if biological material.

---

[1] A check for functioning of the processing equipment, the fluorometer, and the photolysis light source is required.

(3) Mechanically process the sample for subsequent homogenization.[2]

(4) Weigh the sample (1 to 5 grams).[3] DATA OUT

(5) Transfer the sample to the homogenizer.

(6) Acquire 10 ml of water.

(7) Add to homogenizer.

(8) Homogenize for a period of 10 minutes.

(9) Heat to 80°C for 15 minutes while continuing to homogenize.

(10) Acquire 3 ml of 1 N H_2SO_4.

(11) Add to homogenate.

(12) Heat at 80°C for 15 minutes while continuing to homogenize in sealed container.

(13) Cool to ambient (~25°C).

(14) Centrifuge or filter the homogenate.

(15) Retain supernatant or filtrate (~13 ml).

(16) Remove residue.[4] RESIDUE

(17) Acquire 1 ml of chloroform.

---

[2] If the sample is soil, rough-crushing and collection of particles less than a critical size (~50μ) is sufficient. If the sample is biological material, it should be cut into small pieces or should be ground.

[3] A knowledge of the approximate sample weight (± 0.2 gram) is adequate. A knowledge of the gross sample density and its volume would also suffice.

[4] The extraction of the flavins from the sample (if any) is now complete. The next sequence of steps is intended to remove interfering, chloroform-soluble materials.

(18) Add to the filtrate from Steps (15) and (22).

(19) Mix for a period of 15 minutes.

(20) Allow a period of 30 minutes for layers to separate.

(21) Separate layers.

(22) Retain H_2SO_4 layer.

(23) Fill fluorometer cell with chloroform (bottom) layer.

(24) Transfer cell to fluorometer.

(25) Activate fluorometer source (445 mμ).

(26) Detect fluorescence (500 to 600 mμ).

(27) Remove cell.

(28) Remove chloroform from cell and excess chloroform solution.

(29) If fluorescence is detected in Step (26), go to Step (17). If no significant fluorescence is detected in the chloroform extract, go to Step (30).[5]

(30) Acquire 3 ml of 1 N NaOH.

(31) Add to sample solution.

(32) Mix.

(33) Evaporate water under reduced pressure to a total solution volume of 2 ml.

(34) Acquire 2 ml of 1 N NaOH.

(35) Add to sample.

(36) Mix

---

[5] Extraction of interfering substances is now complete. The next sequence of steps is the alkaline photolysis of flavins to lumiflavins.

(37) Filter or centrifuge.

(38) Retain filtrate or supernatant.

(39) Remove residue.

(40) Transfer sample solution (filtrate or supernatant) to photolysis apparatus.

(41) Activate photolysis light source.

(42) Illuminate the sample for a period of 30 minutes.[6]

(43) Remove the sample from photolysis apparatus.

(44) Acquire 4 ml of chloroform.

(45) Add to sample.

(46) Mix.

(47) Allow layers to separate.

(48) Separate layers.

(49) Remove aqueous (top) layer.

(50) Retain chloroform (bottom) layer.

(51) Fill fluorometer cell with chloroform layer.

(52) Transfer cell to fluorometer.

(53) Excite fluorescence with source illumination having a wavelength of 445 mμ.

(54) Record fluorescent spectrum between 500 and 600 mμ. DATA OUT

[6] The period of photolysis is determined by the light source intensity and the maximum expected concentration of flavins. Thirty minutes is probably more than enough time to achieve the necessary photolysis.

(55) If the fluorescence intensity is so high that the detector is saturated, go to Step (56). If the fluorescence is detectable but does not saturate the detector over the wavelength region from 500 to 650 mμ, go to Step (58).

(56) Reduce intensity of excitation source by one-half.

(57) Go to Step (54).

(58) Remove cell.

(59) Shut off fluorometer source.

(60) Recycle fluorometer.

(61) Remove sample from cell. SAMPLE

(62) Clean cell and processing equipment.

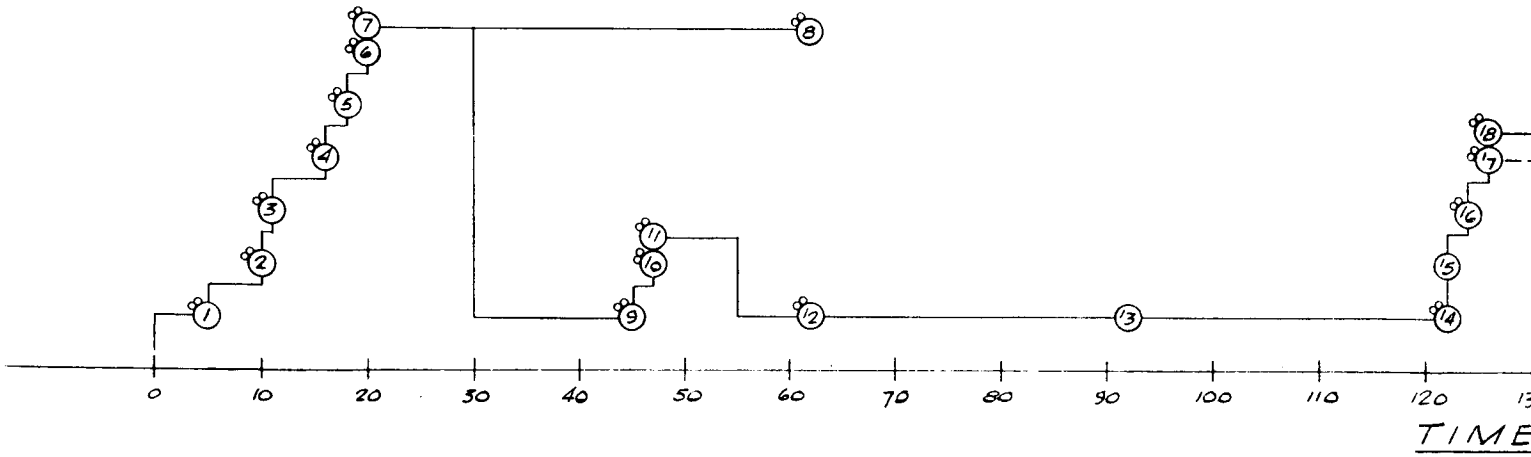
(63) Recycle all equipment to ready position.

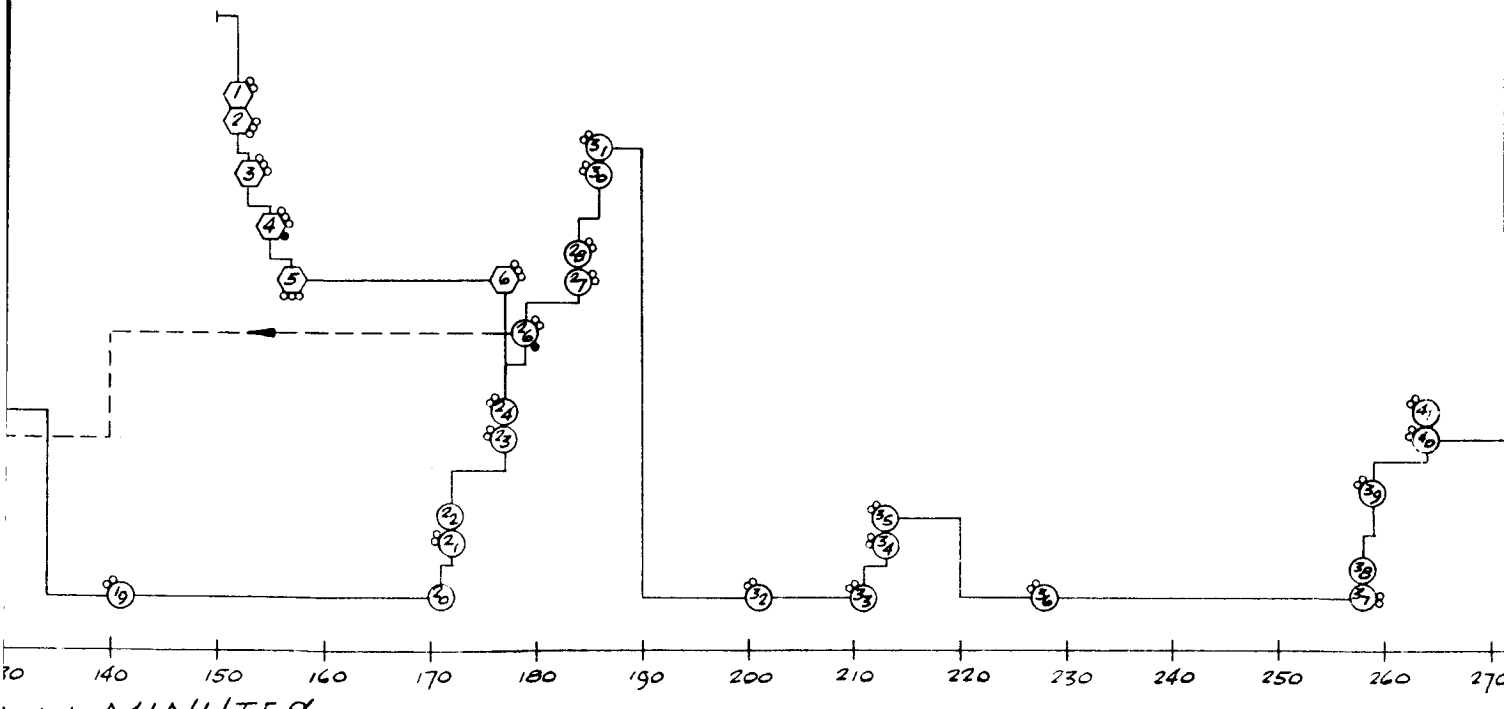
EXPERIMENT 21 - DETECTION OF FLAVINS

SYMBOLS

- ABL COMMAND
- DATA OUTPUT
- CYCLE COMPLETION
- SPECTRAL ANALYZER SUB-ROUTINE

NUMBERED CIRCLES REPRESENT EXPERIMENT STEPS

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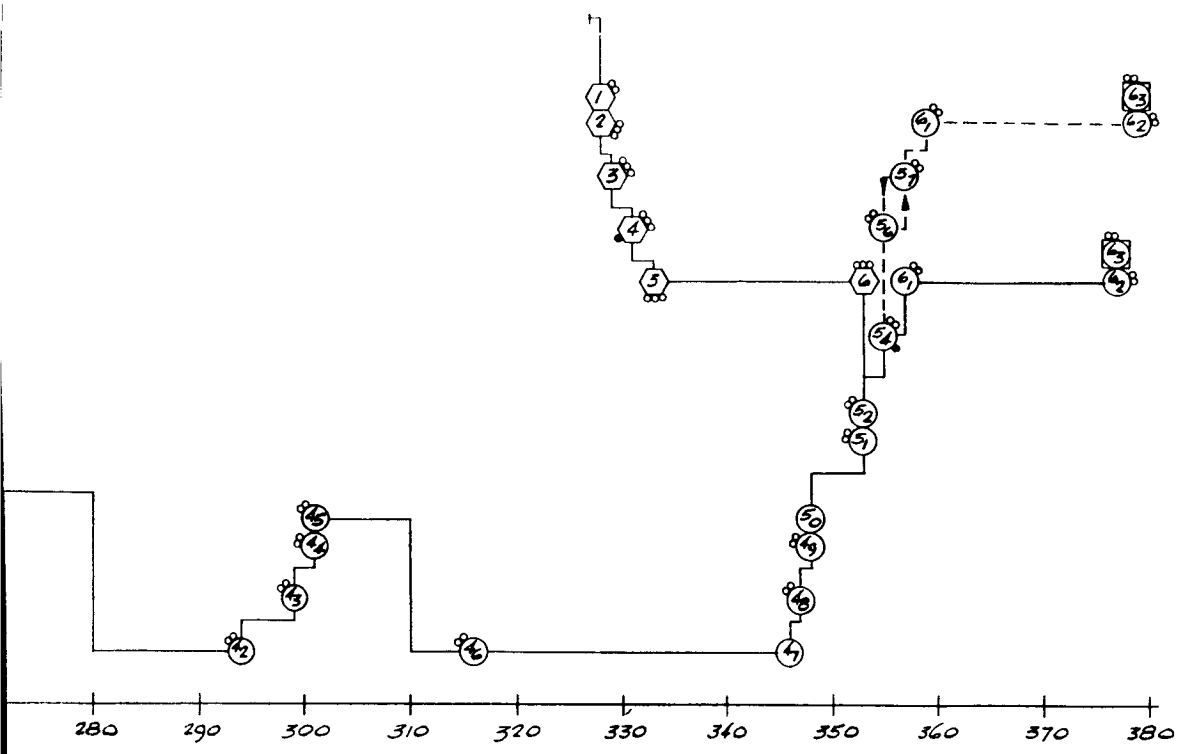
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5.22 EXPERIMENT NO. 22: DETECTION OF NONSAPONIFIABLE LIPIDS

5.22.1 DESCRIPTION

This experiment is designed to detect nonsaponifiable lipids by means of a programmed volatilization of an organic soil extract and gas chromatograph analysis.

5.22.2 APPARATUS USED

A chemical processor capable of performing a solvent extraction filtration, homogenization, programmed heating and evaporation, and liquid/liquid phase separation. A gas chromatograph utilizing a column 1/8 inch in diameter by 60 cm long followed by a mass spectrometer detector is used to perform the analysis.

5.22.3 RANGE OF PARAMETERS

Sample Size:	10 g, $d \leq 300\mu$ (same sample used in Experiment 23)

5.22.4 PROCEDURE

The following procedure is intended to separate steroids and nonsaponifiable lipids from other materials in soil, concentrate the steroid-containing extract, and chromatograph it. Steps (1) to (26) are common to both Experiment 22 and Experiment 23.

(1) Check equipment.[1]

(2) Warm up chromatograph to operating temperature.

(3) Collect a sample.[2] In excess of 10 grams are desired.

(4) Grade and mechanical-process the sample for subsequent homogenization.

---

[1] Chemical processing equipment (see equipment list above) and the volatilization-gas chromatograph should be checked out.

[2] Any reasonable means of collecting in excess of 10 grams of sample is acceptable. If 10 grams is not available, proceed with whatever sample is available down to a minimum sample size (determined by the sensitivity of the method and the expected concentration of steroids in the sample) of probably one gram.

(5) Weigh the processed sample (should weigh about 10 grams).[3]

(6) Transfer sample to homogenizer.

(7) Acquire 50 ml of 2:1 ether-acetone.

(8) Add to homogenizer.

(9) Homogenize for a period of 10 minutes.

(10) Centrifuge or filter through a diatomaceous earth-sintered glass filter.[4]

(11) Remove residue.

(12) Retain supernatant or filtrate.

(13) Evaporate solvent.[5]

(14) Retain residue.

(15) Acquire 1 ml of 1 N NaOH.

(16) Add to residue.

(17) Mix. Continue mixing through Step (22).

(18) Heat to 100°C at one atmosphere of pressure for a period of 30 minutes.

(19) Cool to 25°C.

(20) Acquire 2 ml of chloroform.

(21) Add to sample.

---

[3] Either a balance or a sample volume measuring device can be used.

[4] Suggested filtration method only. Others may be better.

[5] Use of a stream of nitrogen gas directed on the liquid surface is one possibility. Distillation at reduced pressure (ambient pressure?) is another.

(22) Continue mixing for a period of 10 minutes, then cease mixing.

(23) Allow a settling time of 30 minutes.

(24) Separate layers.

(25) Transfer the bottom layer (chloroform) to temporary storage.

(26) Retain the top aqueous layer and repeat Steps (20) through (24).

(27) Combine chloroform layers.

(28) Dry chloroform.[6]

(29) Evaporate chloroform.[5]

(30) Retain residue.

(31) Acquire 0.1 ml of dimethyl formamide.

(32) Add to residue.

(33) Mix (homogenize at low speed).

(34) Allow a period of 30 minutes for settling or centrifuge or filter.

(35) Transfer 1 to 10 $\mu\ell$ (microliter) of the clear dimethyl formamide layer to gas chromatograph.[7]

(36) Do volatilization gas chromatography routine. DATA OUT

(37) Clean, rinse, and dry contaminated equipment.

(38) Recycle to ready.

---

[6] The chloroform solution is treated with a drying agent to remove traces of water.

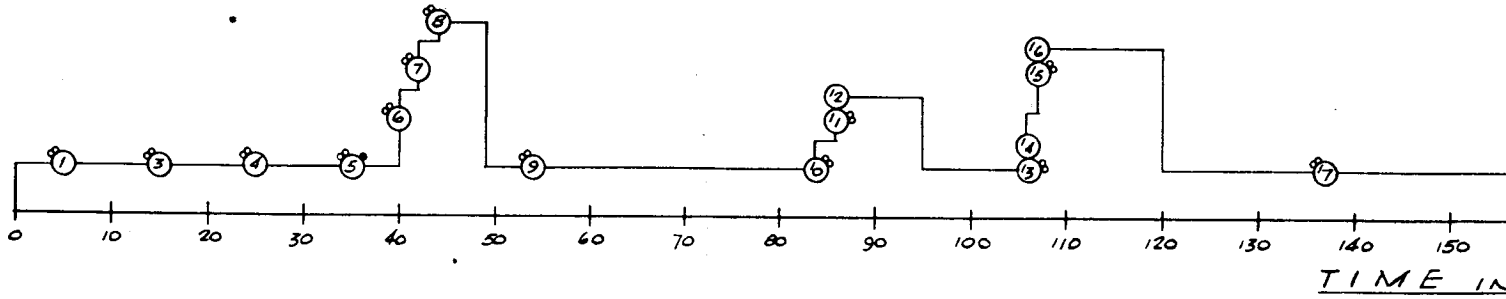
[7] Start with one $\mu\ell$ and, if no peaks detected, run another aliquot of, say, two $\mu\ell$, and then, if necessary, do 10 $\mu\ell$.

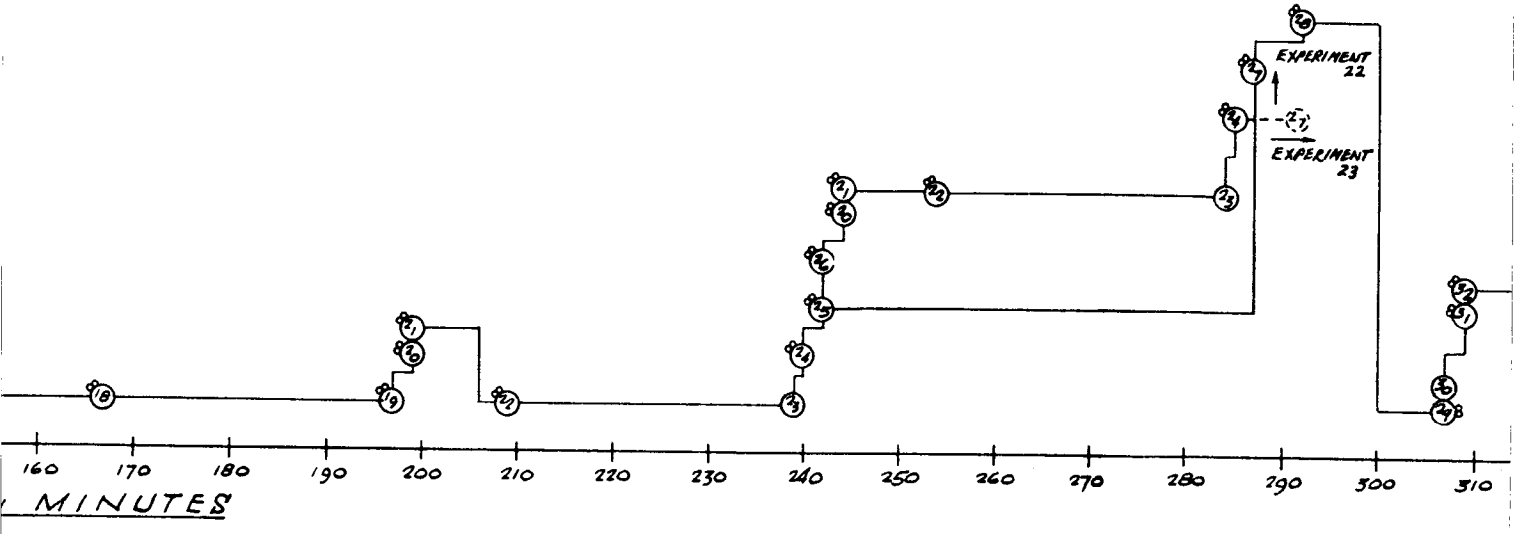
EXPERIMENT 22 - DETECTION OF NON-SAPONIFIABLE LIPIDS

SYMBOLS

- EARTH COMMAND
- ABL COMMAND
- SELF-CONTROLLED FUNCTION
- DATA OUTPUT
- CYCLE COMPLETION
- GAS CHROMATOGRAPH SUB-ROUTINE

NUMBERS IN CIRCLES REFER TO EXPERIMENT STEPS




EXPERIMENT 22
EXPERIMENT 23
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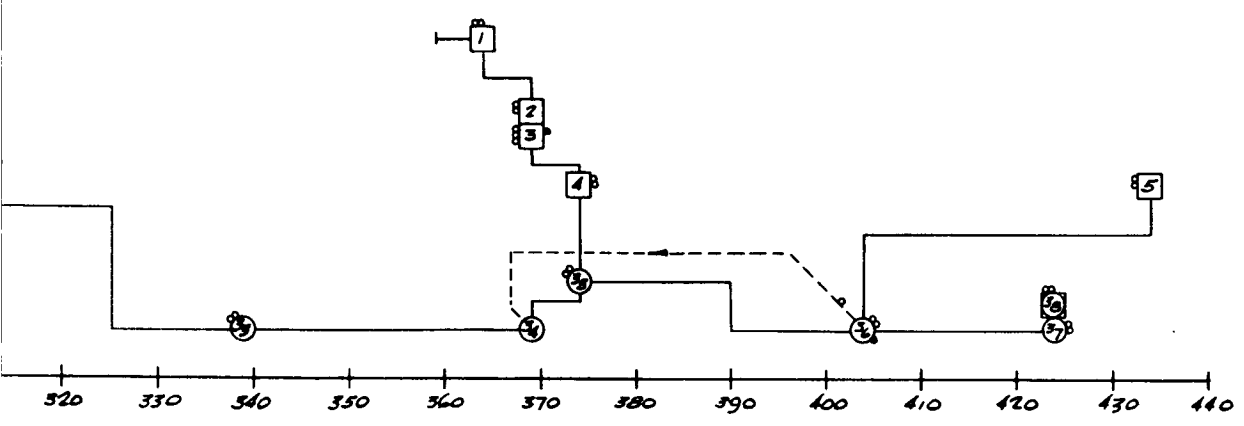
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5.23 EXPERIMENT NO. 23: DETECTION OF SAPONIFIABLE LIPIDS

5.23.1 DESCRIPTION

This experiment is an extension of Experiment 22 to detect the saponifiable lipids also by means of a programmed volatilization of the organic solution obtained in the liquid/liquid phase separation of Experiment 22 and a gas chromatograph analysis.

5.23.2 APPARATUS USED

A chemical processor is employed capable of performing a solvent extraction, filtration, homogenization, liquid/liquid phase separation. Analysis is performed on substances extracted by organic solvents from the neutralized aqueous solution. A single-column gas chromatograph with a special packing is used to perform the analysis.

5.23.3 RANGE OF PARAMETERS

Sample: From Experiment 22

5.23.4 PROCEDURE

The first 26 steps of this procedure are common with Experiment 22. The aqueous basic layer from Step (26) is retained and used for the saponifiable lipid analysis. To maintain continuity, Experiment 23 begins with Step (27).

(27) Retain top basic, aqueous layer.

(28) Transfer aqueous layer to reaction chamber.

(29) Acquire 1.2 ml of 1 N HCl.

(30) Add to aqueous layer (to make it acidic and precipitate fatty acids).

(31) Acquire 2 ml of petroleum ether.

(32) Add petroleum ether to aqueous phase.

(33) Mix for a period of 30 minutes.

(34) Allow a period of 10 minutes for layers to separate.

(35) Separate layers.

(36) Transfer top layer (petroleum ether) to temporary storage.

(37) Retain aqueous layer and do Steps (31) through (35).

(38) Combine petroleum ether extracts.

(39) Remove aqueous layer. LAYER

(40) Dry petroleum ether solution (by treating with a drying agent to remove water).

(41) Filter.

(42) Retain the filtrate.

(43) Remove any residue (drying agent).

(44) Evaporate the petroleum ether solution to a total volume of about 0.1 ml.(1)

(45) Rig chromatograph to accept sample.

(46) Inject one $\mu\ell$ of the petroleum ether solution into the chromatograph.

(47) Do chromatography. DATA OUT

(48) Backflush column.

(49) Recycle column to initial ready position to accept new sample.

(50) If the chromatogram shows peaks (besides the solvent peak), go to Step (54). If no peaks are detected, go to Step (51).

(51) Inject 10 $\mu\ell$ of the petroleum ether solution into the chromatograph.

(1) Or evaporate to dryness and redissolve the residue in 0.1 ml of petroleum ether.

(52) Do chromatography. DATA OUT

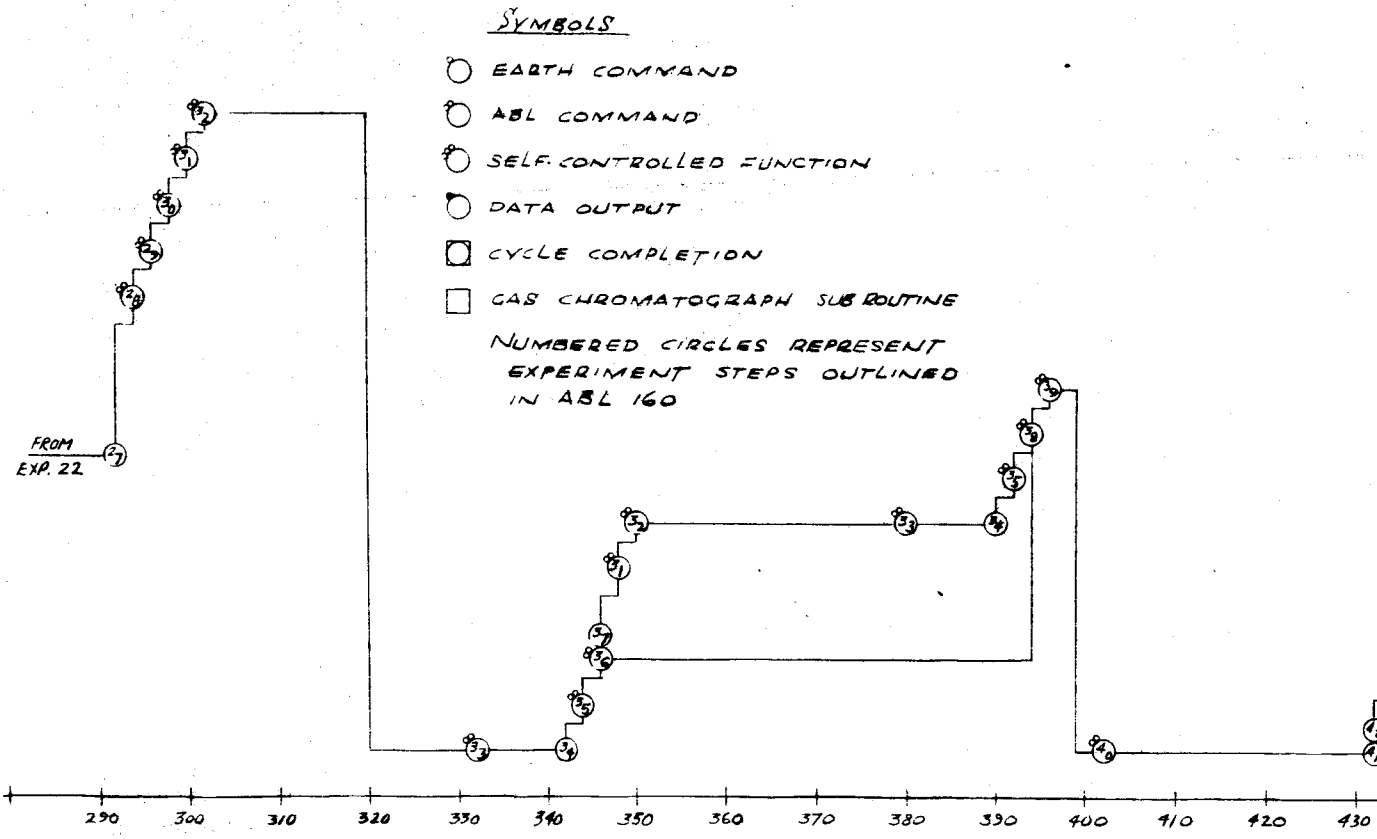
(53) Backflush column.

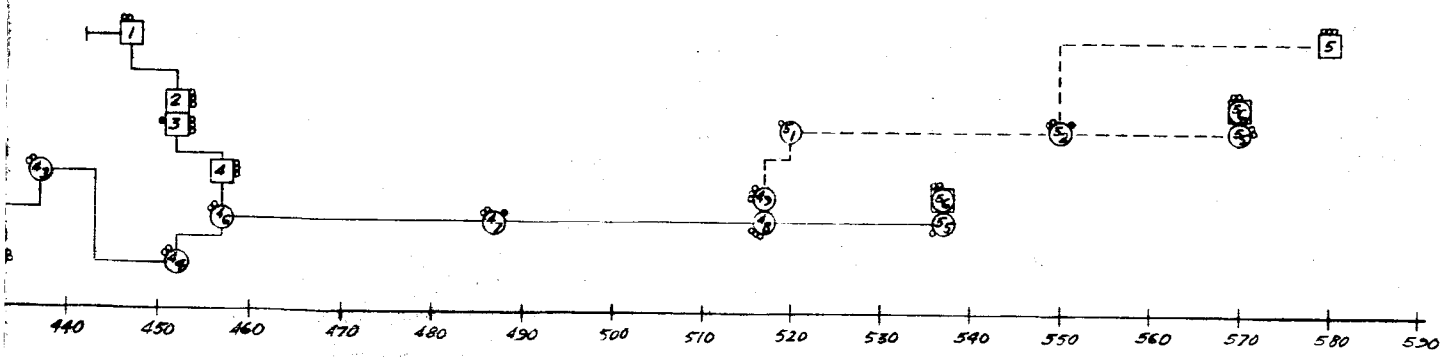
(54) Allow column and ovens to cool to ambient temperature.

(55) Clean processing equipment.

(56) Recycle.

EXPERIMENT 23 - DETECTION OF SAPONIFIABLE LIPIDS

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5.24 EXPERIMENT NO. 24: DETECTION OF MACROMOLECULES BY ABSORPTION IN THE VISIBLE SPECTRUM

5.24.1 DESCRIPTION

This experiment is designed to detect macromolecules such as proteins and nucleic acid by measuring changes in abosrbance of a dye/extract solution or by chemical reactions with the extract and employing a spectrophotometer in the visible spectrum.

5.24.2 APPARATUS USED

A chemical processor is required capable of performing a solvent extraction of a soil sample, a filtration, a dialysis, and preparation of a dye/extract solution or performing chemical reactions. The analysis is performed using an optical-null spectrophotometer operating in the visible spectrum 400 to 700 mμ. Either a tungsten lamp or xenon lamp source can be used.

5.24.3 RANGE OF PARAMETERS

Absorbance:	4 decades of variation
Wavelength Scan:	400 to 700 mμ with one mμ resolution
Sample Size:	10 g, d ≤ 300 μ (sample also used for Experiments 25 and 26)

5.24.4 PROCEDURE

(1) Check equipment.[1]

(2) Collect sample of about one gram weight.

(3) Grade sample by crushing and sieving[2] if dry soil; break up and crush if hard, brittle biological material; go directly to homogenizer if soft, flexible biological material.

---

[1] Check out the sample processing equipment and the spectrophotometer.

[2] Sample should be reduced to powder size (possibly fairly coarse) for efficient homogenization and mixing.

(4) Weigh sample. Sample should weigh about one gram.[3]

DATA OUT

(5) Transfer sample to homogenizer.

(6) If the sample is soil, go to Step (7). If the sample is biological material, go to Step (10).

(7) Acquire 3 ml of 0.5 N NaOH.

(8) Add to homogenizer containing the sample.

(9) Go to Step (12).

(10) Acquire 3 ml of water.

(11) Add to homogenizer containing the sample.

(12) Homogenize for a period of 10 minutes.

(13) Filter homogenate.[4]

(14) Retain filtrate.

(15) Remove residue.

RESIDUE

(16) Transfer the filtrate to ultrafiltration apparatus.

(17) Do ultrafiltration (with carbowax).

(18) Reconstitute retentate by dissolving in one ml of 0.002 M neutral buffer.[5]

---

[3] A rough weighing is sufficient. If sample weighs less than one gram and if additional sample cannot be collected expediently, proceed with the available amount of sample.

[4] Or centrifuge.

[5] Volumes mentioned are tentative. A certain exposure time will be required in order to effect complete reconstitute. The actual mechanics of this process has to be worked out.

(19) Prepare dye solution.[6]

(20) Add one ml of dye solution to one ml of reconstituted retentate.

(21) Mix.

(22) Fill spectrophotometer reference cell with neutral buffer of 0.001 M concentration.

(23) Fill spectrophotometer sample cell with dye-retentate solution from Step (21).

(24) Activate spectrophotometer (i.e., cycle through source warmup).

(25) Place cells in appropriate positions in spectrophotometer.

(26) Measure absorption spectrum from 450 to 700 mμ.[7] DATA OUT

(27) Remove cells.

(28) Remove liquids from cells. LIQUIDS

(29) Clean cells and apparatus.

(30) Recycle all equipment to ready position.

---

[6] The dibenzothiocarbocyanine dye will probably be stored in the dry state (to survive sterilization). The dye preparation procedure involves dissolving the solid dye in water. Some sort of mixing chamber is implied. The concentration of the dye solution must be known, perhaps by dissolving a known weight of dye in a known volume of water. Alternatively, the concentration of the dye solution can be ascertained by a measurement on the dye-only solution using the spectrophotometer. Desired concentration is about 10^{-5} M. The dye in the solid state may be as a film deposited on a nonreactive surface.

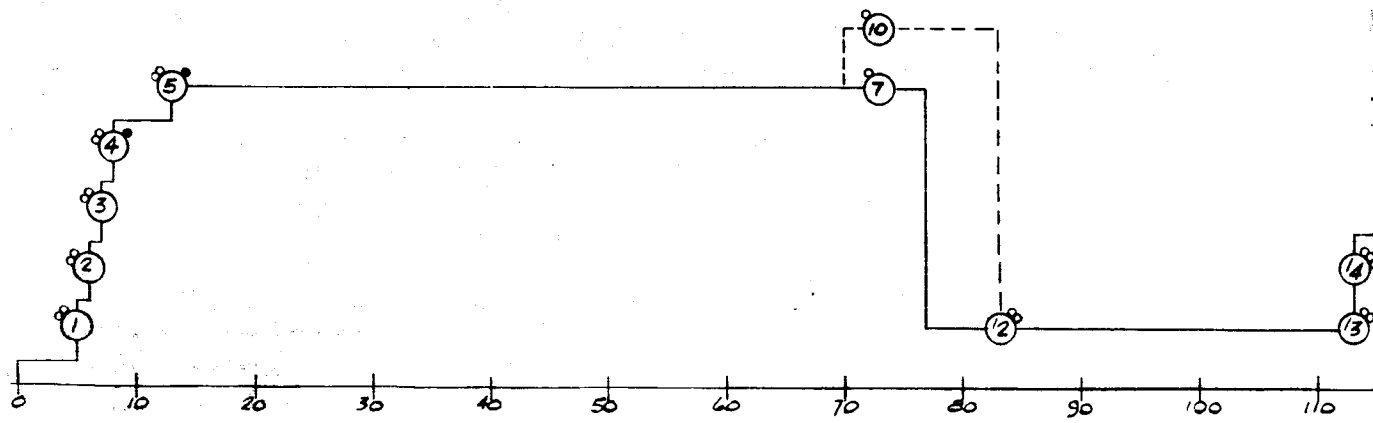
[7] If discrete wavelength settings are used, measure absorption at 640, 570, 550, 535, and 510 mμ.

EXPERIMENT 24- DETECTION OF MACROMOLECULES BY ABSORPTION IN

SYMBOLS

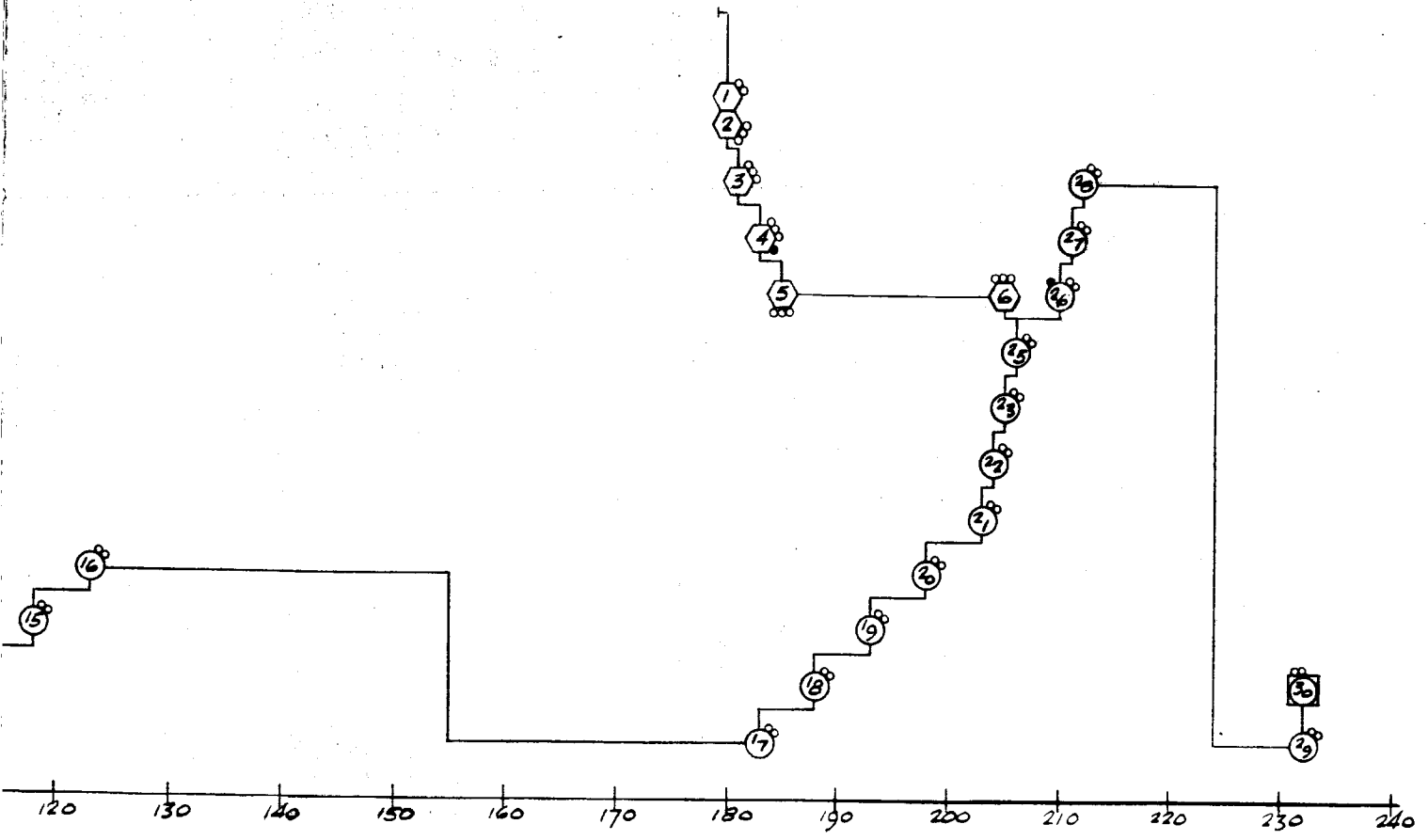
- EARTH COMMAND
- ASL COMMAND
- SELF-CONTROLLED FUNCTION
- DATA OUTPUT
- CYCLE COMPLETION
- SPECTRAL-ANALYZER SUB-ROUTINE

NUMBERS IN CIRCLES REFER TO EXPERIMENT STEPS



TIME IN MINUTES

THE VISIBLE SPECTRUM



5.25 EXPERIMENT NO. 25: DETECTION OF MACROMOLECULES BY ABOSRPTION IN THE ULTRAVIOLET SPECTRUM

5.25.1 DESCRIPTION

This experiment is designed to detect macromolecules such as proteins and nucleic acid by means of abosrbance of the extract solution using an ultraviolet spectrophotometer.

5.25.2 APPARATUS USED

A chemical processor is required capable of performing a solvent extraction, a filtration, and a dialysis of the solution. The analysis is performed using an optical null spectrophotometer operating in the ultraviolet spectrum from 240 to 350 mμ. A xenon or hydrogen vapor lamp source may be employed.

5.25.3 RANGE OF PARAMETERS

Absorbance:	4 decades of variation
Wavelength Scan:	240 to 350 mμ with 1 mμ resolution
Sample:	Same as for Experiment 24

5.25.4 PROCEDURE

(1) Check equipment.

(2) Collect a sample in excess of one gram. (One gram of mechanically processed sample is required.)

(3) Mechanically process the soil sample.

(4) Weigh sample. (Approximately one gram.)

(5) Transfer sample to homogenizer.

(6) If the sample is soil, go to Step (7). If the sample is biological material, go to Step (10).

(7) Acquire 3 ml of 0.5 N NaOH.

(8) Add to homogenizer containing the sample.

(9) Go to Step (12).

(10) Acquire 3 ml of water.

(11) Add to homogenizer containing the sample.

(12) Homogenize for a period of 30 minutes.

(13) Filter homogenate.

(14) Retain filtrate.

(15) Transfer residue to waste storage.

(16) Transfer the filtrate to ultrafiltration apparatus.

(17) Do ultrafiltration (with carbowax) for 60 minutes.

(18) Reconstitute retentate by dissolving in one ml of 0.002 M neutral buffer.

(19) Fill spectrophotometer reference cell with neutral buffer of 0.001 M concentration.

(20) Fill spectrophotometer sample cell with retentate solution.

(21) Activate spectrophotometer (i.e., cycle through source warmup).

(22) Place cells in appropriate positions in spectrophotometer.

(23) Measure abosrption spectrum from 240 to 350 mμ. DATA OUT

(24) Remove cells.

(25) Remove liquids from cells.

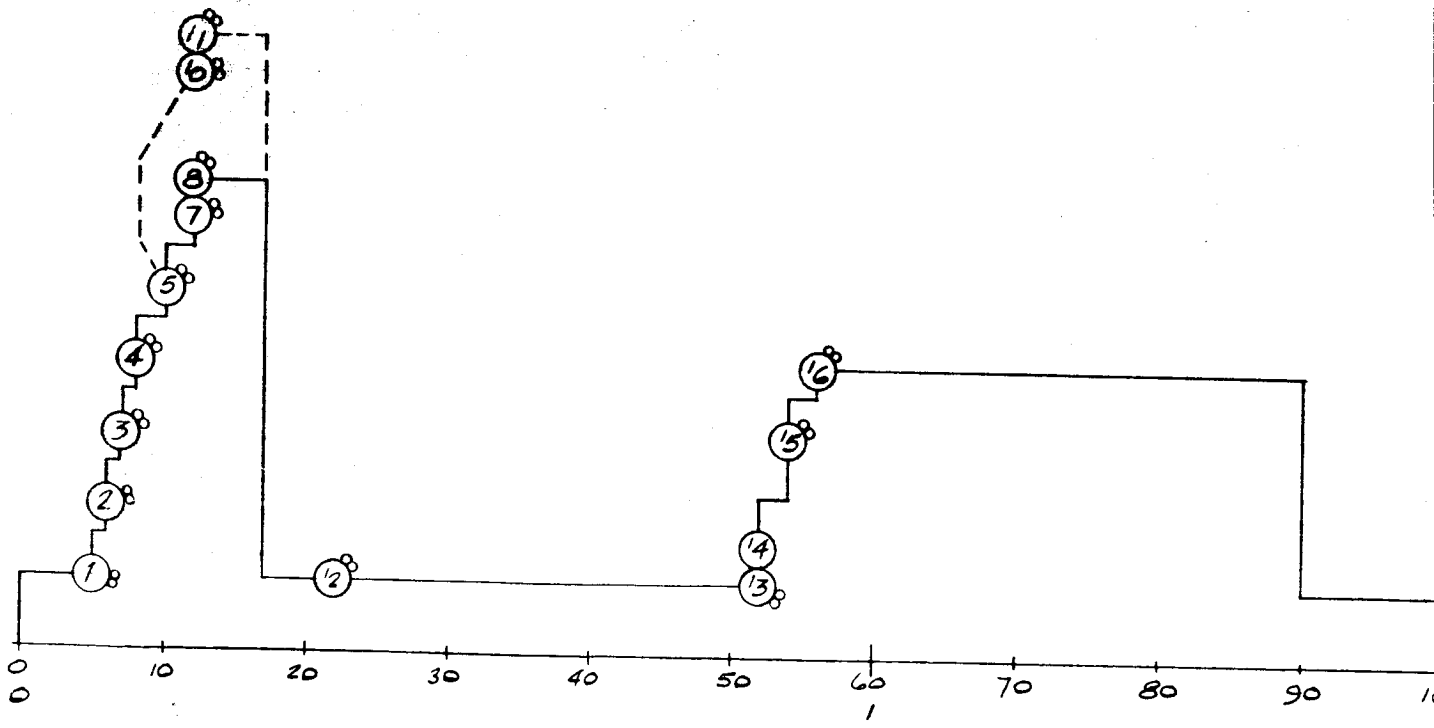
(26) Clean cells and apparatus.

(27) Recycle all equipment to ready position.

EXPERIMENT 25- DETECTION OF MACROMOLECULES BY

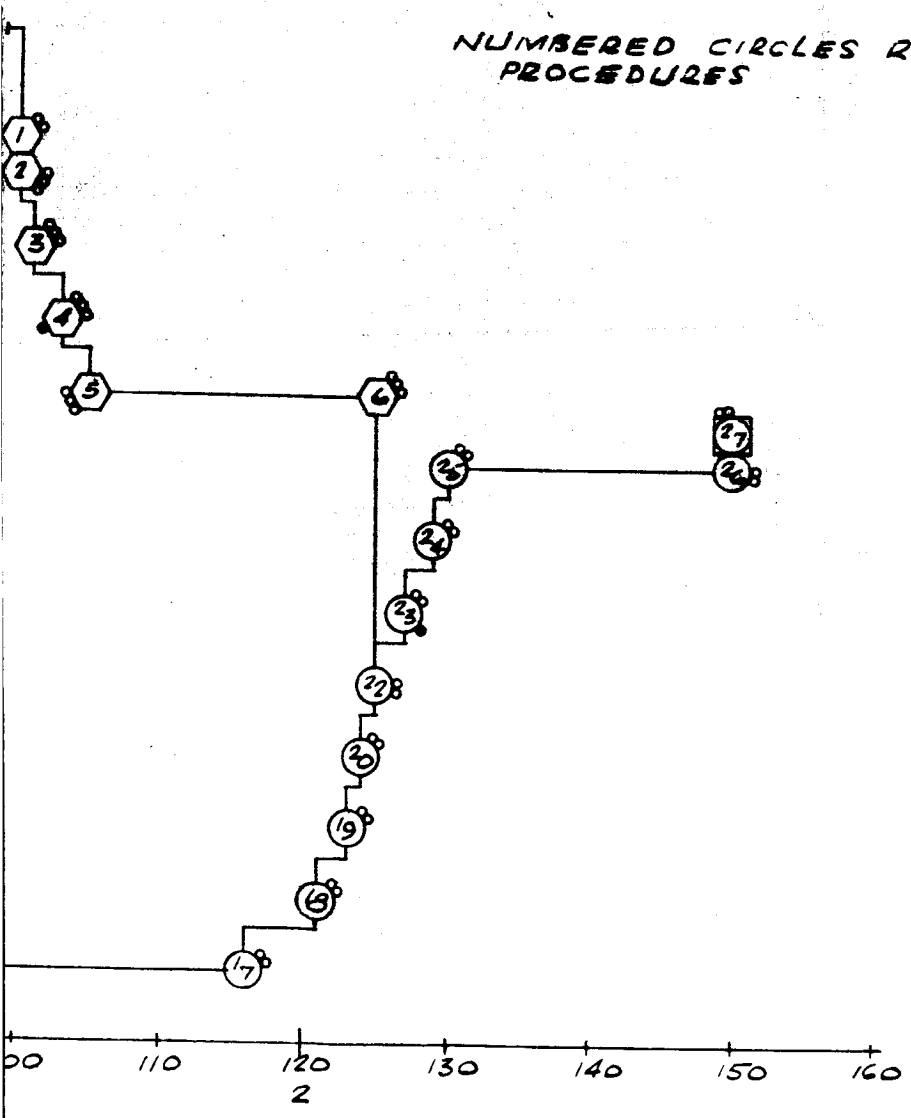
SYMBOLS

- ABL COMMAND
- SELF CONTROLLED FUNCTION
- DATA OUTPUT
- CYCLE COMPLETION
- SPECTRAL ANALYZER SUB-ROUTINE



ABSORPTION IN THE ULTRAVIOLET SPECTRUM

NUMBERED CIRCLES REFER TO EXPERIMENT PROCEDURES



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5.26 EXPERIMENT NO. 26: OPTICAL ACTIVITY OF WATER-SOLUBLE MACROMOLECULES

5.26.1 DESCRIPTION

This experiment is designed to detect macromolecules soluble in water by means of optical rotation in the 270 and 290 mμ range with a backup mode-determining rotation using the Na-D line. Optical rotation will be measured before and after dialysis of the solution.

5.26.2 APPARATUS USED

A chemical processor is required capable of performing a solvent extraction, filtration, and dialysis of the solution. The analysis is performed by means of a polarimeter operating in the UV spectrum at 270 to 290 mμ. A xenon or hydrogen vapor lamp source can be used. A sodium vapor lamp is used in the backup mode.

5.26.3 RANGE OF PARAMETERS

Rotation:	**To the nearest 10^{-2} degree in either direction**
Variation in Beam Intensity:	**To four decades**
Sample:	**Same sample as in Experiment 24**

5.26.4 PROCEDURE

(1) Check equipment.

(2) Collect sample (about one gram).

(3) Mechanically process the soil sample.

(4) Weigh sample. (Approximately one gram.) A rough weighing is sufficient. If sample weighs less than one gram and if additional sample cannot be collected expediently, proceed with the available amount of sample. DATA OUT

(5) Transfer sample to homogenizer.

(6) Acquire 3 ml of one-percent NaCl solution.

(7) Add to homogenizer containing the sample.

(8) Homogenize for 15 minutes.

(9) Filter homogenate.

(10) Retain filtrate.

(11) Retain residue.

(12) Repeat Steps (6) through (10).

(13) Combine filtrates.

(14) Transfer residue to waste storage.

(15) Fill polarimeter cell with filtrate.

(16) Transfer cell to polarimeter.

(17) Measure optical rotation in 270 to 290 mμ region. DATA OUT

(18) Remove cell.

(19) Transfer the filtrate to ultrafiltration or dialysis apparatus.

(20) Clean polarimeter cell.

(21) Do ultrafiltration or dialysis.

(22) Reconstitute retentate by dissolving in one ml of 0.002 M neutral buffer.

(23) Fill polarimeter cell.

(24) Transfer cell to polarimeter.

(25) Measure optical rotation. DATA OUT

(26) Remove sample cell.

(27) Remove solution from cell.

(28) Clean, rinse, and dry cell and contaminated apparatus.

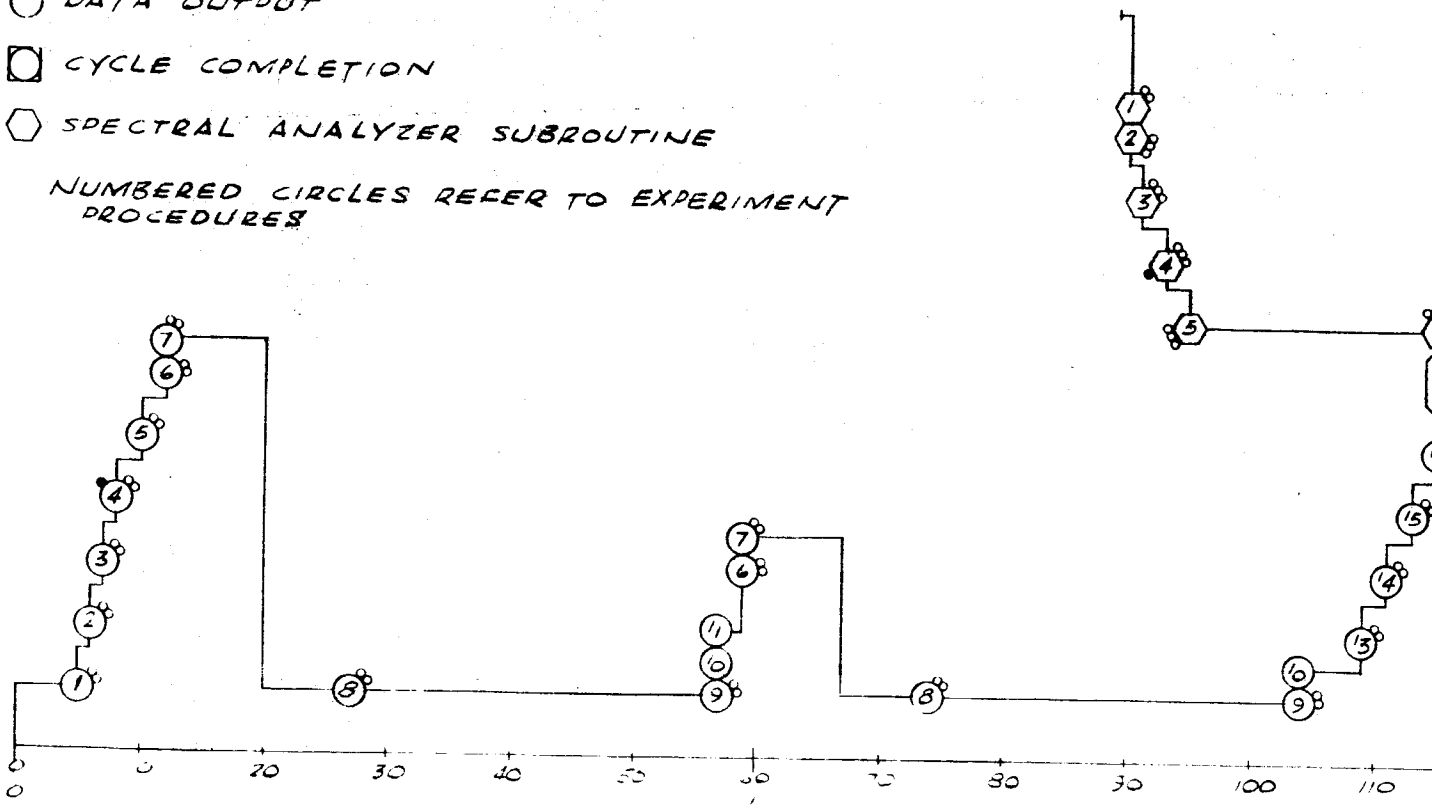
(29) Recycle all equipment.

EXPERIMENT 26- OPTICAL ACTIVITY OF WATER SOLUBLE MACROMO

SYMBOLS

- ABL COMMAND
- SELF CONTROLLED FUNCTION
- DATA OUTPUT
- CYCLE COMPLETION
- SPECTRAL ANALYZER SUBROUTINE

NUMBERED CIRCLES REFER TO EXPERIMENT PROCEDURES



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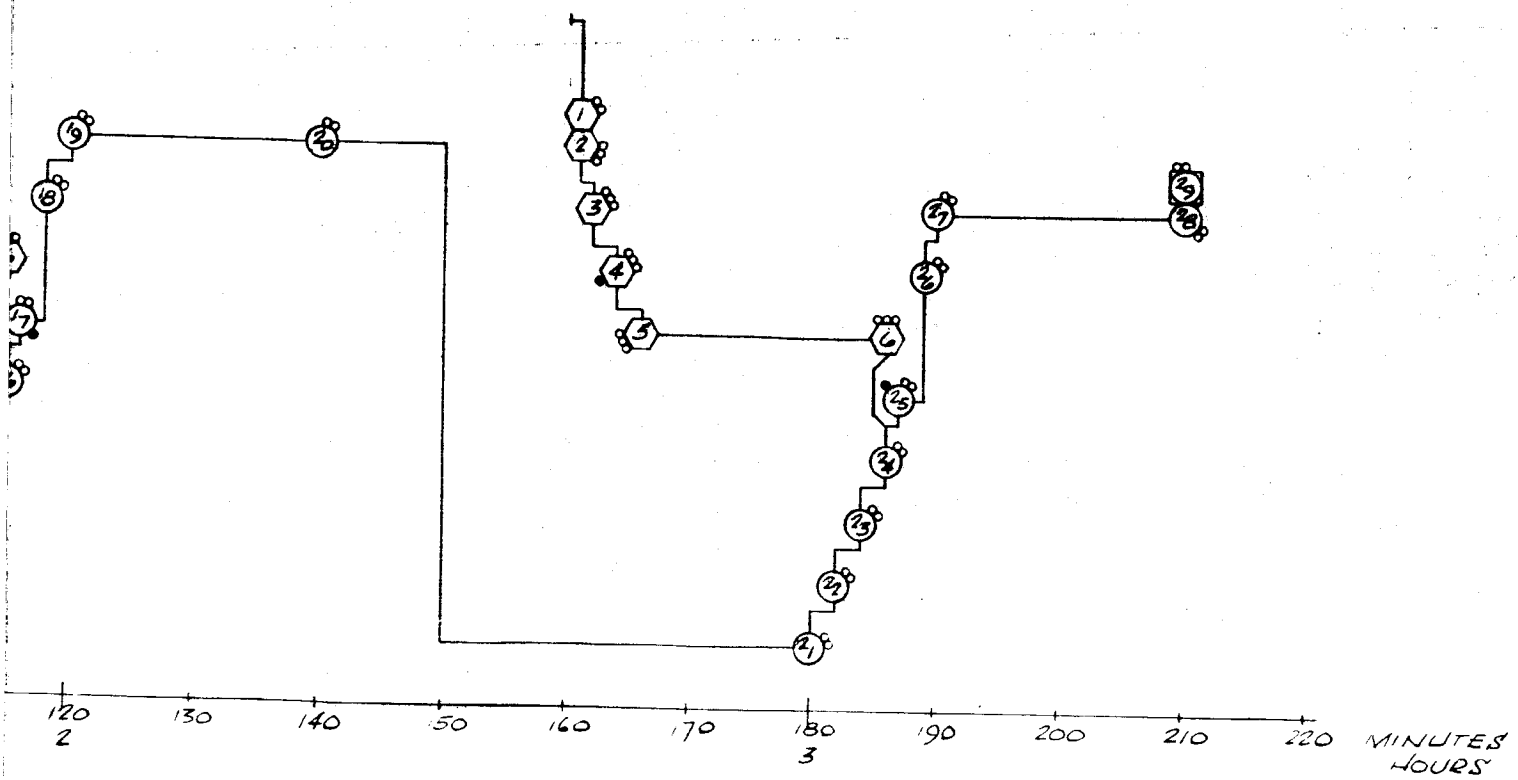

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5.27 EXPERIMENT NO. 27: DETECTION OF WATER-SOLUBLE MACROMOLECULES BY PYROLYSIS GAS CHROMATOGRAPHY

5.27.1 DESCRIPTION

This experiment is designed to detect water-soluble macromolecules using a gas chromatograph analysis.

5.27.2 APPARATUS USED

A chemical processor is required capable of performing a solvent extraction, filtration, and dialysis and a programmed heating with pyrolysis. The analysis is performed with a gas chromatograph employing a column 1/8 inch in diameter by 5 feet long and using a mass spectrometer as a detector.

5.27.3 RANGE OF PARAMETERS

Sample:	Uses solution from Experiment 26
Molecular Weight:	Up to 500

5.27.4 PROCEDURE

(1) Check equipment and warm up pyrolysis oven.

(2) Transport aqueous extract (Experiment 26) from storage to pyrolysis oven.

(3) Evaporate extract to dryness under reduced pressure and controlled temperature.

(4) Select pryolysis temperature. DATA OUT

(5) Pyrolyze sample.

(6) Collect evolved gases.

(7) Transfer evolved gases to gas chromatograph.

(8) Determine gas chromatograph of pyrolysis products. DATA OUT

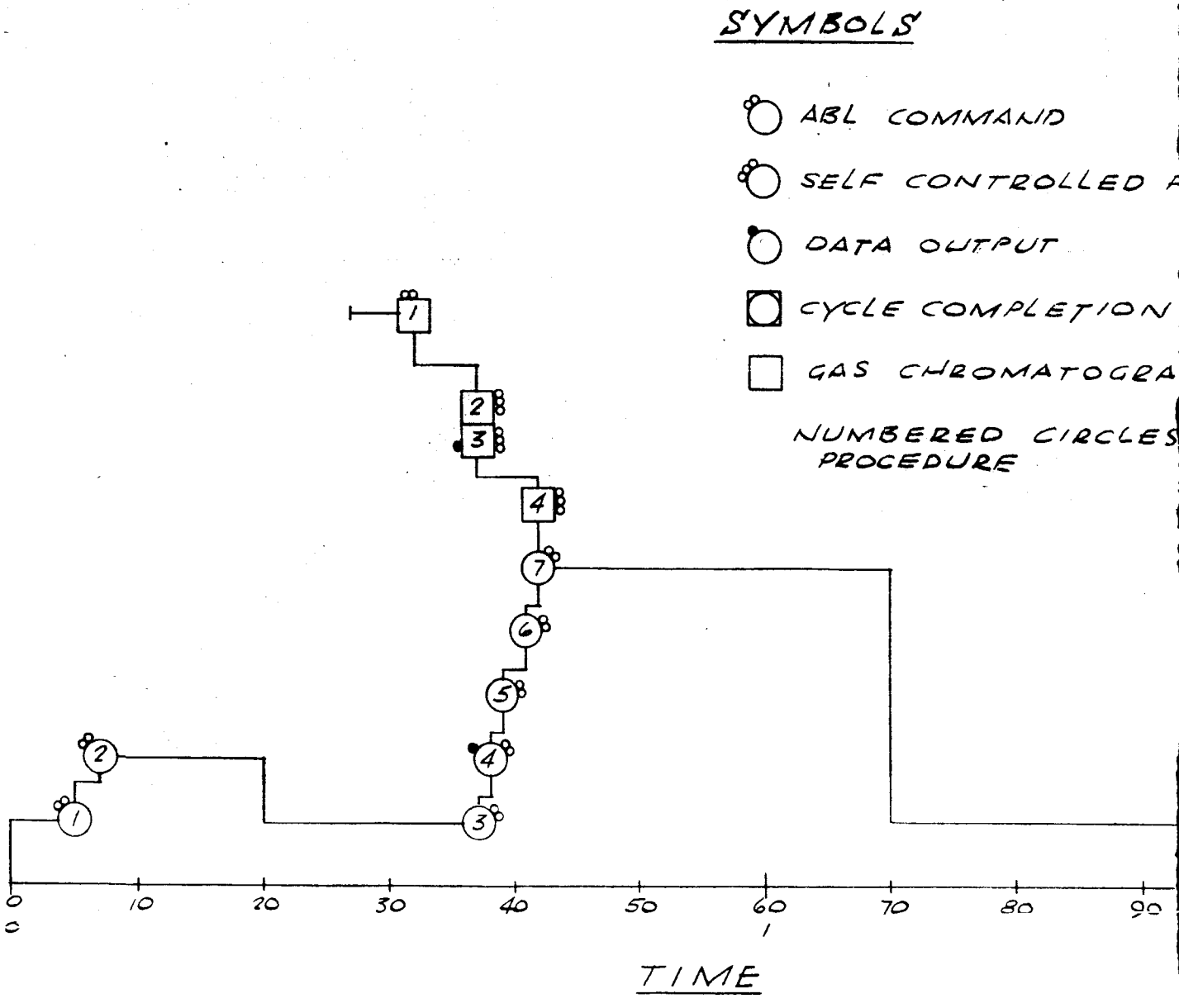
(9) Allow pyrolysis oven to cool.

(10) Remove residue.

(11) Clean oven and equipment.

(12) Recycle to ready.

EXPERIMENT 27- DETECTION OF WATER SOLUBLE MACRO
PYROLYSIS GAS CHROMATOGRAPHY



MOLECULES BY

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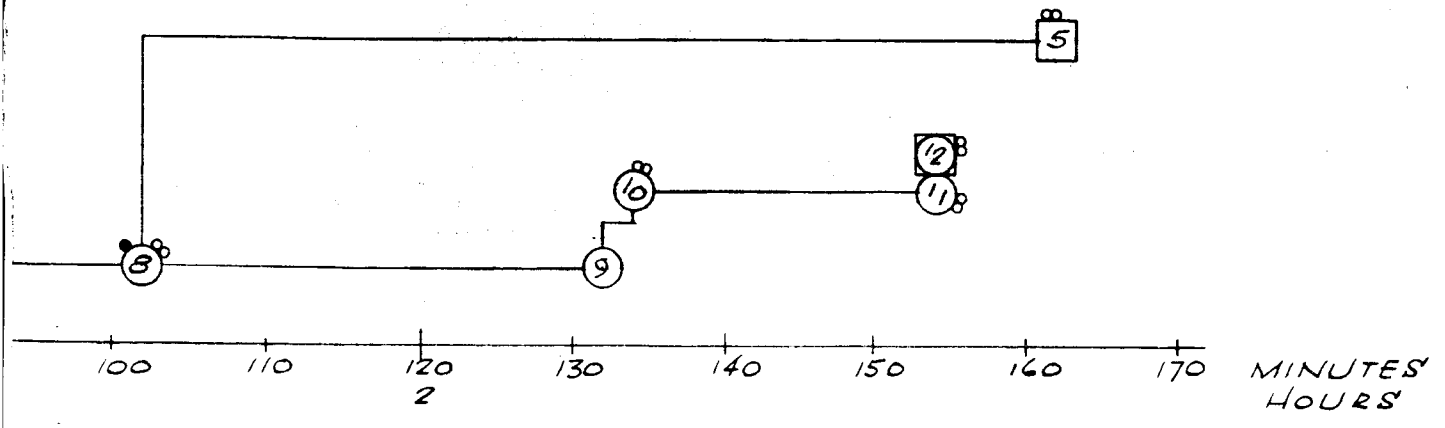


5.28 EXPERIMENT NO. 28: FUNCTIONAL GROUP ANALYSIS

5.28.1 DESCRIPTION

This experiment is designed to identify and categorize the functional groups such as CH, C = C, NH_2, OH, C = O, HC = O, and COOH using infrared spectrophotometry.

5.28.2 APPARATUS USED

A chemical processor is required capable of performing extraction and filtration. The analysis is performed with an infrared spectrometer operating from 2 to 14μ with a resolution of 0.1μ. Either a photodiode or bolometer detector will be used.

5.28.3 RANGE OF PARAMETERS

Absorbance Range:	4 decades
Wavelength Scan:	2 to 14μ
Sample Size:	10 g, d $\leq$ 300μ

5.28.4 PROCEDURE

The following procedure is to separate carbon tetrachloride soluble materials from a soil sample, concentrate the CCl_4 extract, and obtain its infrared absorption spectrum.

Check the chemical processing equipment and warm up the infrared spectrophotometer.

Run a calibration check on the spectrophotometer for wavelength position.

(1) Collect a soil sample. An excess of 10 grams is desired. If 10 grams is not available, proceed with whatever sample is available down to a minimum sample size (determined by the sensitivity of the method and the expected concentration of steroids in the sample) of probably one gram.

(2) Mechanically process the soil sample.

(3) Transfer the sample to the extraction apparatus (reaction chamber).

(4) Acquire 20 ml (approximate) of carbon tetrachloride (CCl_4).

(5) Add to reaction chamber.

(6) Stir for 10 minutes.

(7) Filter.

(8) Retain filtrate and sample residue.

(9) Repeat Steps (6) through (10) twice.

(10) Combine the filtrates. Volume will be about 60 ml.

(11) Transfer residue to waste storage.

(12) Evaporate the CCl_4 extract to about 0.5 ml.

(13) Fill spectrophotometer sample cell with the extract.

(14) Fill reference cell with CCl_4.

(15) Transfer the cells to the spectrophotometer.

(16) Determine the infrared absorption from 2 to 8μ (allow 15 minutes). DATA OUT

(17) Remove cells.

(18) Remove liquids.

(19) Rinse and dry cells.

(20) Clean processing equipment.

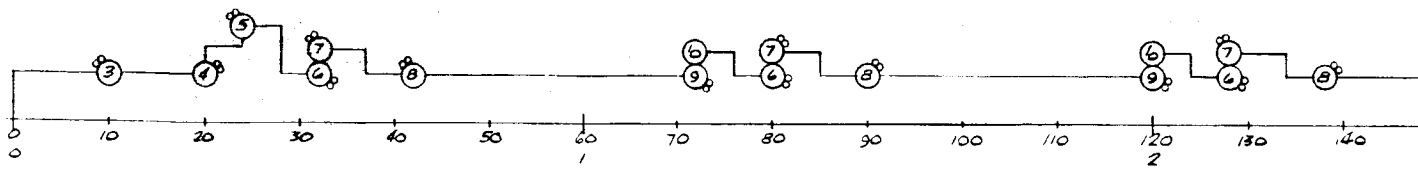
(21) Recycle all equipment as required.

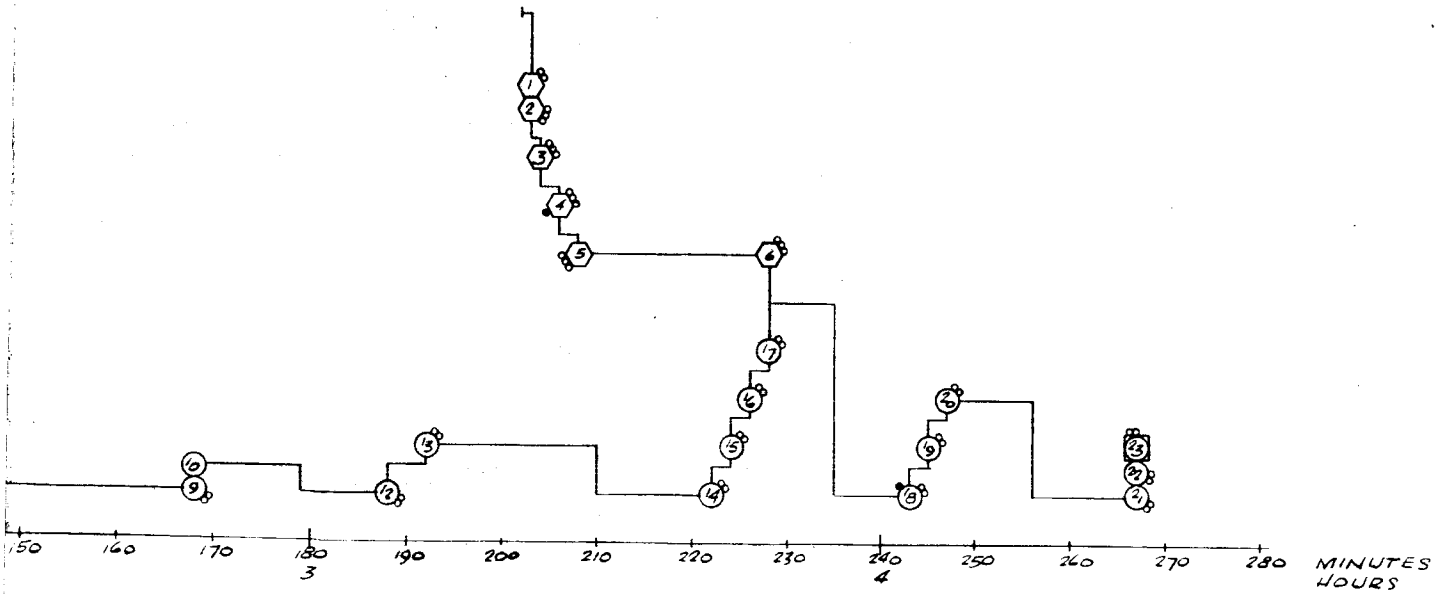
EXPERIMENT 28- FUNCTIONAL GROUP ANALYSIS

SYMBOLS

- ABL COMMAND
- SELF CONTROLLED FUNCTION
- DATA OUTPUT
- CYCLE COMPLETION
- SPECTRAL ANALYZER SUB-ROUTINE

NUMBERED CIRCLES REFER TO EXPERIMENT PROCEDURES

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260
270
280
3
4
MINUTES
HOURS

5.29 EXPERIMENT NO. 29: LIGHT-STIMULATED $C^{14}O_2$ FIXATION AND DARK $C^{14}O_2$ FIXATION AS A FUNCTION OF TEMPERATURE

5.29.1 DESCRIPTION

This experiment is designed to determine the amount of CO_2 fixed in an incubated soil sample. The samples are incubated at two different temperatures, in light and dark, with a tagged ($C^{14}O_2$) carbon dioxide in the chamber atmosphere. After incubation, the soil is pyrolized to determine the amount of $C^{14}O_2$ fixed by counting the tagged carbon released.

5.29.2 APPARATUS USED

Six chemical processing chambers are to be used as culture chambers and combustion chambers. Two are lighted with white light from a tungsten lamp source. Four are dark. Two chambers are sterilized with heat to provide a sterile control. The tagged carbon is counted with β ionization detector.

5.29.3 RANGE OF PARAMETERS

Incubation Temperature:	$0\text{-}1^{\circ}$ and $25^{\circ}C$
Incubation Atmosphere:	N_2 and $C^{14}O_2$ (8 mb)
Chamber Pressure:	Martian ambient
Incubation Time:	24 and 48 hours
Count Rate:	Up to 10^6 counts per minute
Sample Size:	2 g per chamber, 12 g total, $d \leq 300\mu$

5.29.4 PROCEDURE

(1) Collect sample.[1] An excess of 6 grams is required.

---

[1] Preferred sample is a surface scraping of Martian soil. In order of preference, sample may be surface soil, subsurface soil, material (dust, etc.) filtered from air. Depth of surface scraping is not specified but presumed to be on the order of one centimeter. A vacuum cleaner would be used in case the surface is like desert pavement. The first step may be preceded by an equipment check procedure if deemed desirable.

(2) Grade sample to isolate material of mesh finer than 20 to 40.(2)

(3) Measure processed sample into approximately one-gram portions.(3)

(4) Weigh each portion accurately and identify. DATA OUT

(5) Distribute unit portions of the processed sample to each of four dark incubation chambers (D_1, D_2, D_3, and D_4).(4)

(6) Distribute unit portions of the processed sample to each of two illumination chambers (L_1 and L_2).(4)

(7) Admit sterilizing gas (mixture of ethylene oxide and carbon monoxide) to chambers D_1 and D_2 and continue exposure to gas for 4 hours.(5)

(8) While D_1 and D_2 are being sterilized, the other chambers, D_3, D_4, L_1, and L_2, may be exposed to the ambient Martian atmosphere.(6)

---

(2) Method of sample grading for a soil sample suggested is dry-sieving. Reason for grading is to obtain sample uniformity (eliminate coarse material).

(3) This measurement can be made on a volume basis, using either estimated or measured sample density information. If an excess of 6 grams of sample is not available, divide the available sample into six approximately equal portions and proceed with samples of reduced weight.

(4) At this point it is not critical whether the chambers are dark or illuminated (perhaps by being opened to admit the sample). Note also that the sample weight may be less than one gram (see Note (3)).

(5) Complete sterilization may require much longer times but is not essential. It is sufficient to achieve significant reduction in biological activity in D_1 and D_2 by reducing the organism population through a 4-hour exposure to the gas.

(6) Flushing gas of nitrogen-argon mixture may be used instead of Martian atmosphere. If Martian atmosphere is used, it should be filtered to remove dust. The flow rate must be very small to prevent sample loss.

(9) Flush all six incubation chambers with a moderate flow (rate?) of carbon dioxide free gas mixture of nitrogen, argon, and oxygen (low partial pressure of oxygen). Continue this flow through Step (13).

(10) Determine desired temperatures T_1 and T_2.[7]

(11) Equilibrate chambers D_1 (sterile control), D_3, and L_1 to temperature T_1.

(12) Equilibrate chambers D_2 (sterile control), D_4, and L_2 to temperature T_2.

(13) Monitor temperatures of chambers and adjust accordingly.

DATA OUT

(14) Discontinue purge gas flow.

(15) Turn on illumination for L_1 and L_2.

(16) Determine partial pressure of CO_2 in Martian atmosphere.[8]

(17) Pressurize each chamber to partial pressure of $C^{14}O_2$ as determined in Step (16).[9]

(18) Determine length of incubation, t_1.[10]

---

[7] For the first time through the procedure,

$T_1 = 0^{\circ}$ to $1^{\circ}C$

$T_2 = 25^{\circ}C$

[8] This data comes from the memory of ABL or from environmental experiment on atmospheric pressure and composition.

[9] Probably 8 mb. Can use this figure for design parameter until better definition of CO_2 partial pressure is obtained.

[10] For initial run through the procedure, the time t_1 is 2 hours. If results of experiment fail to show $C^{14}O_2$ fixation, the procedure will be repeated with an incubation time between 24 and 48 hours.

(19) Maintain incubation temperatures (T_1 and T_2) for t_1.[11]

(20) Add 1 ml of 10 N HCl to each of the six incubation chambers.

(21) Open exhaust port.

(22) Begin flush with nitrogen/argon flush gas and continue flow (rate?) through Step (30). Exhaust gas to outside.

(23) Heat each chamber to boiling at the ambient pressure to evaporate to dryness.[12]

(24) Monitor temperatures of chambers and adjust accordingly.[13]

(25) Maintain temperature below critical value.

(26) Monitor drying of each of the six chambers.[14]

(27) If drying is complete, go to Step (31). If drying is incomplete, go to Step (28).

(28) Wait time t_2.

(29) Continue heating.

(30) Go to Step (24).

---

[11] It is desirable to incubate all six chambers simultaneously. If the power drain is too high, it is, however, permissible to incubate chambers serially.

[12] Gases exhausted to the outside are $C^{14}O_2$, flush gas, water vapor, and hydrogen chloride. It may be possible (may or may not be desirable) to recover water and HCl.

[13] Temperature should not become so high that pyrolysis organic material sets in.

[14] A sensor is implied sensitive to water vapor in the exhaust gas.

(31) Discontinue heating.

(32) Wait until temperature drops to T_4.[15]

(33) Discontinue flush gas.

(34) Close exhaust port.

(35) Pressurize dried sample and sample chambers with oxygen.

(36) Monitor temperature of each chamber.

(37) Heat each chamber to 850°C[16] for a time t_3[17] to burn organic carbon and release fixed C^{14} as $C^{14}O_2$.

(38) Open valve between incubation chamber and β-counting chamber.

(39) Transfer gas to β-counting chamber.[18]

(40) Count radioactivity of gas in counting chamber. DATA OUT

(41) Purge counting chamber with N_2/A flush gas or with filtered atmosphere.

(42) Remove solid residue from incubation chambers. RESIDUE

(43) Wash.

---

(15) T_4 is probably close to 25°C. Forced cooling may be needed to achieve cooling in a reasonable time.

(16) This may be accomplished serially.

(17) Time t_3 is on the order of minutes.

(18) It may not be necessary to effect complete transfer by, say, pumping. That is, the total gas chamber volume may include the incubation chamber and the β-counting chamber.

(44) Rinse.

(45) Dry.

(46) If there is a detectable difference in

(a) illuminated and sterile control,

(b) dark and sterile control,

(c) illuminated samples from the two different temperatures, T_1 and T_2, or

(d) dark samples from two different temperatures, T_1 and T_2,

recycle to ready. If there is no detectable difference between (a), (b), (c), or (d), repeat Steps (1) through (45) with t_1 = 24 to 48 hours and then go to Step (47).

(47) If still no difference (as defined in Step (46)), do Steps (1) through (6) and then go to Step (48).

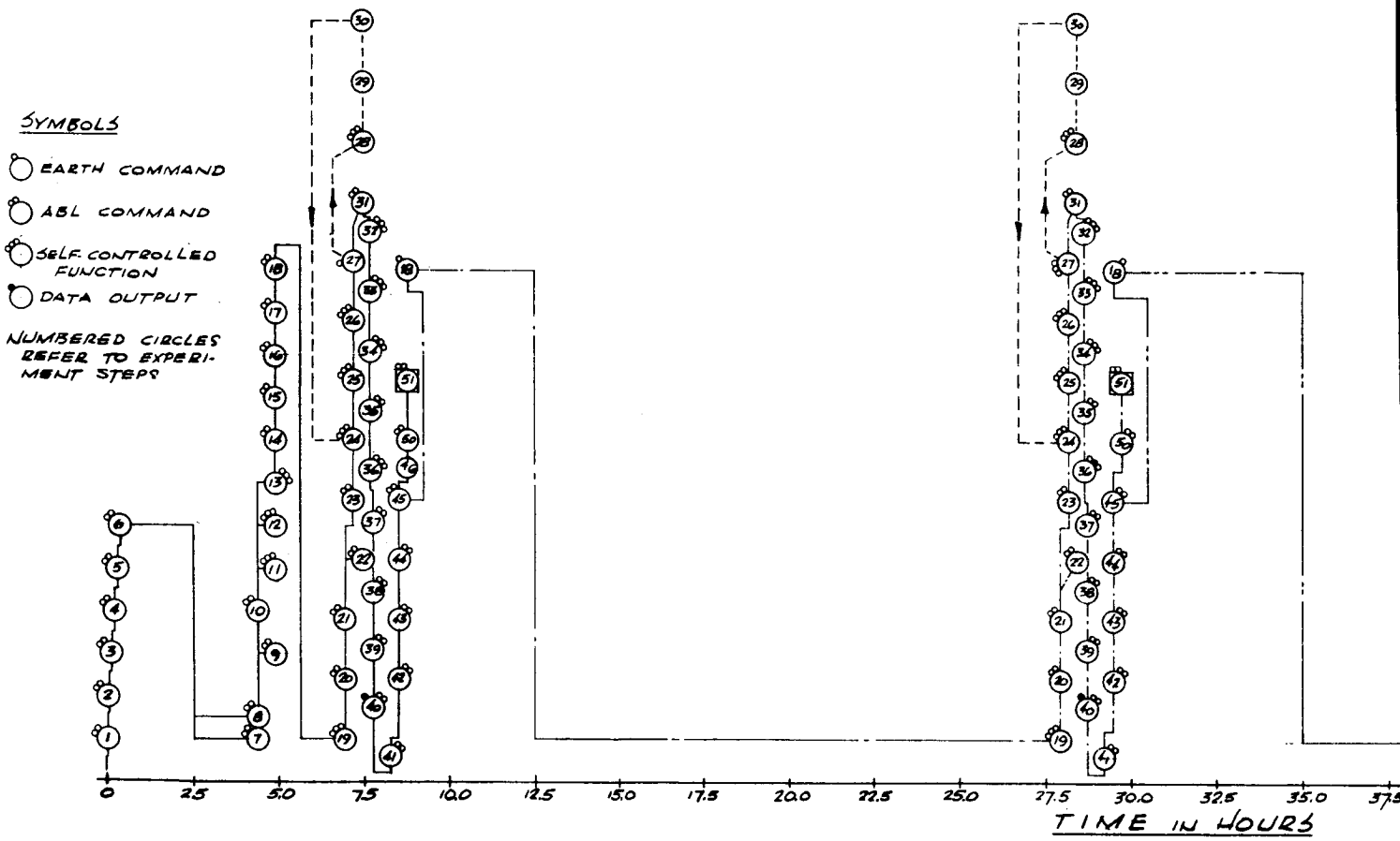
(48) Add 1 ml of aqueous culture media to each incubation chamber.

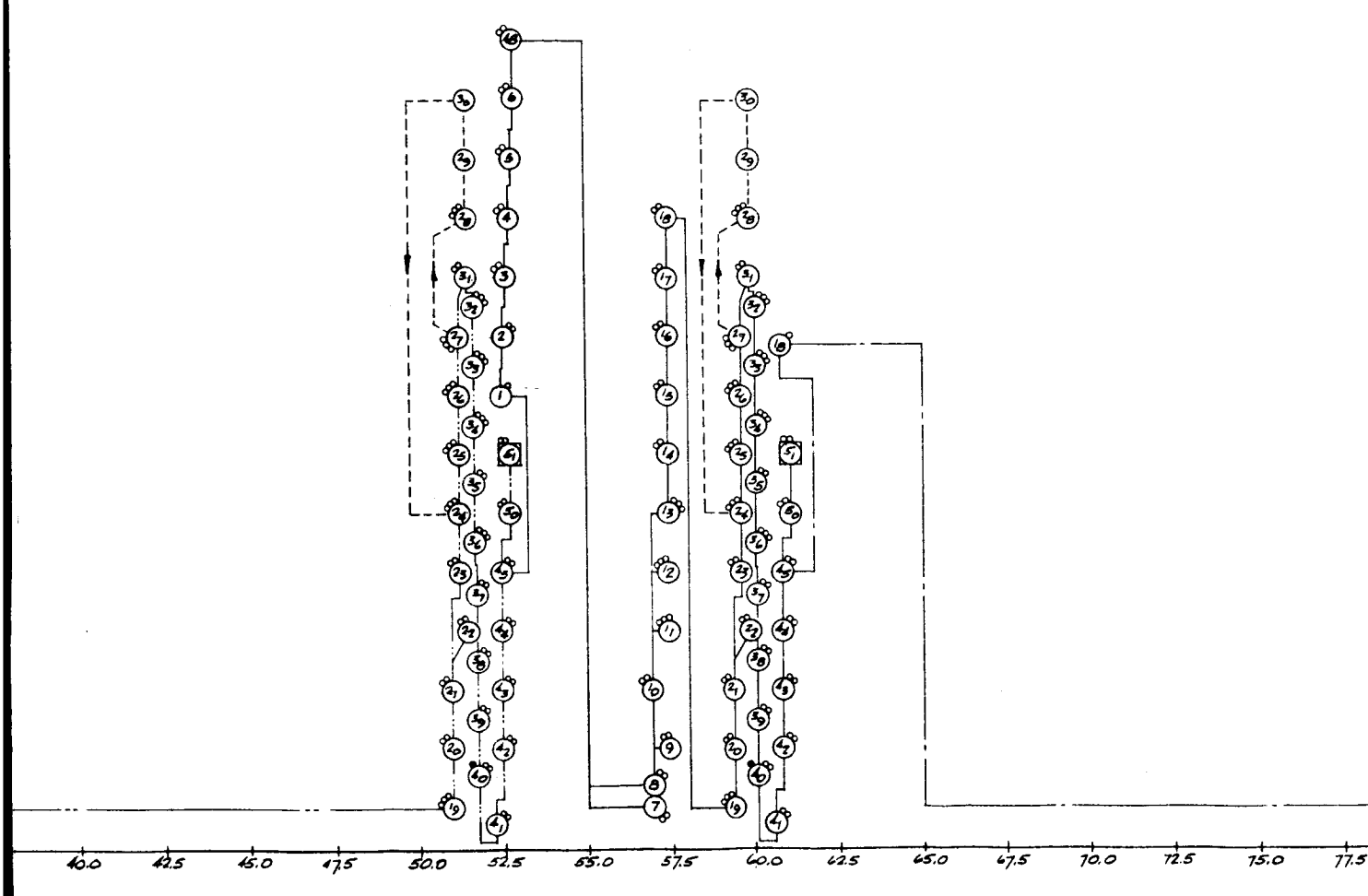
(49) Do Steps (7) through (45) and then go to Step (50).

(50) Check equipment.

(51) Recycle to ready.

EXPERIMENT 29 - LIGHT SIMULATED $C^{14}O_2$ FIXATION & DARK $C^{14}O_2$ FIXATION AS A FUNCTION OF TEMPERATURE

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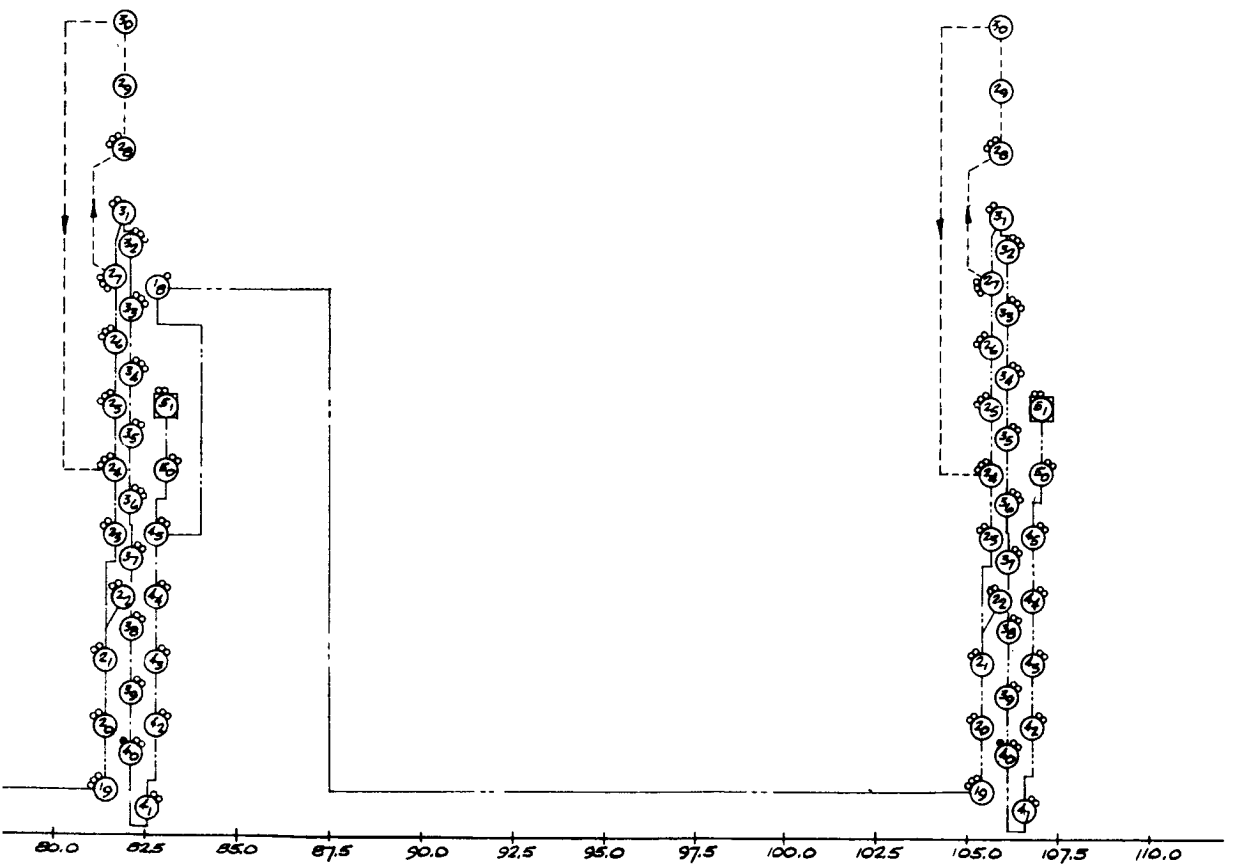
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5.30 EXPERIMENT NO. 30: EVOLUTION OF CO_2 BY NORMAL METABOLISM

5.30.1 DESCRIPTION

This experiment is designed to detect the evolution of CO_2 during normal metabolism and will be performed "in situ" and in culture chambers. Using culture chambers, the chamber atmosphere is purged of CO_2 and maintained as a N_2 atmosphere with a low O_2 content. The soil is incubated at two temperatures for a given time, and the increase in CO_2 content of the chamber atmosphere is determined by a CO_2 detector such as $Ba(OH)_2$ solution. The increase in CO_2 is compared with that obtained in a sterile control specimen for each incubation temperature.

5.30.2 APPARATUS USED

Four chemical processing chambers are used to provide controlled heating for incubation, heat sterilization of the two sterile control chambers, and a control CO_2-free initial atmosphere. The conductance of a $Ba(OH)_2$ solution is used as the CO_2 detector.

5.30.3 RANGE OF PARAMETERS

Incubation Temperatures:	0-1°C and 30-35°C
Incubation Atmosphere:	N_2 and low O_2
Chamber Pressure:	Martian ambient
Incubation Time:	24 and 48 hours
Carbon Dioxide Content:	Change in conductivity $Ba(OH)_2$ solution
Soil Sample Size:	2 grams/chamber (with particle diameter $d \leq 300\ \mu$), 8 grams total

5.30.4 PROCEDURE

(1) Check equipment.[1]

---

[1] The equipment check probably should involve injection of test signals into electronics and checking mechanical operations, transducers, motors, etc.

(2) Collect sample.[2] Four grams are desired.

(3) Grade sample to isolate material of mesh finer than 40.[3]

(4) Measure sample into approximately one-gram portions (four similar samples desired).[4]

(5) Transfer samples to each of four incubation chambers (D_1, D_2, D_3, and D_4).

(6) Admit sterilizing gas to chambers D_1 and D_2 and continue exposure to the gas for four hours.[5]

(7) During the sterilization of D_1 and D_2, D_3 and D_4 may be exposed to the ambient Martian atmosphere (filtered) or to flushing gas ($N_2|Ar|O_2$) mixture.

(8) Flush all chambers with flushing gas.

(9) Equilibrate D_1 (sterile control) and D_3 while flushing to 0-1°C.

(10) Equilibrate D_2 (sterile control) and D_4 while flushing to 30-35°C.

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(18) Add med

(19) Do

(20) Ren pre

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(22) Rin

(23) Dry

(24) Ste

(25) Rec

[2] The sample may be surface or subsurface material. If the sample is collected in a manner so that particle sizes are finer than 40 mesh, Step (3) can be eliminated from the procedure.

[3] Sieving is assumed, but other methods of grading sample on size basis are acceptable. The reason for sample sizing is to obtain samples having similar contents of biological material.

[4] This measurement may be made with a balance or with a sample volume measuring technique, and the data from the soil density measurement experiment (Environmental Experiment 2.5, Soil Density) may be used to determine volume of a one-gram soil sample. If the total available sample is less than 4 grams, the sample should be divided into four equal portions by volume. Proceed with samples of reduced volume.

[5] Four hours is adequate to achieve at least partial reduction in microorganism populations.

[6] Carbon dioxide significantly sterile contro (0-1°C) sample

p flow of flushing gas.

l incubation chambers.

itor change in carbon dioxide partial
ssures in each chamber over a
hour period.(6)

DATA OUT

difference(6) in CO_2 evolution is
ected, go to Step (20). If no
ference(6) detected, go to Step (15).

tinue to monitor CO_2 for an additional
hours.

DATA OUT

difference detected, go to Step (20).
no difference is detected, go to
p (17).

Steps (1) through (5).

l one ml of aqueous, sterile culture
lia to each chamber.

Steps (6) through (15).

ıove samples (and culture media if
ɛsent) from each chamber.

SAMPLES
(MEDIA)

ɜh each chamber.

ıse.

'.

ɛrilize.

ycle to ready.

—

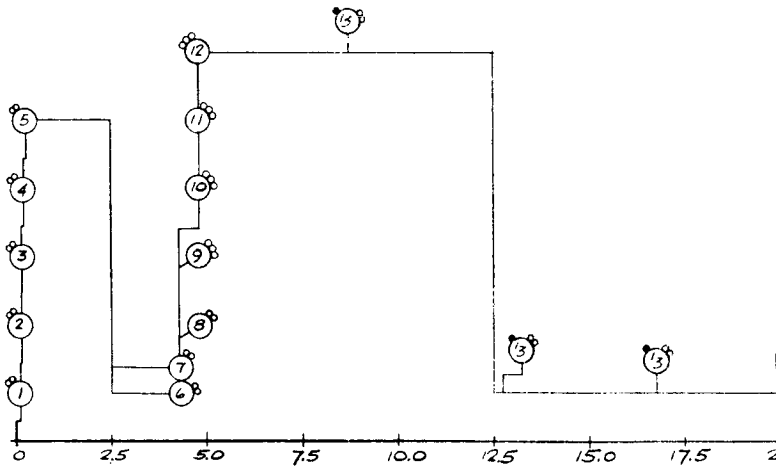
evolution because of normal metabolism is indicated by
ıigher CO_2 partial pressures in samples compared to the
. and in elevated temperature sample compared to the cold

EXPERIMENT 30 - EVOLUTION OF CARBON DIO

SYMBOLS

- EARTH COMMAND
- ABL COMMAND
- SELF-CONTROLLED FUNCTION
- DATA TO STORAGE
- CYCLE COMPLETION

NUMBERED CIRCLES REFER TO EXPERIMENT STEPS



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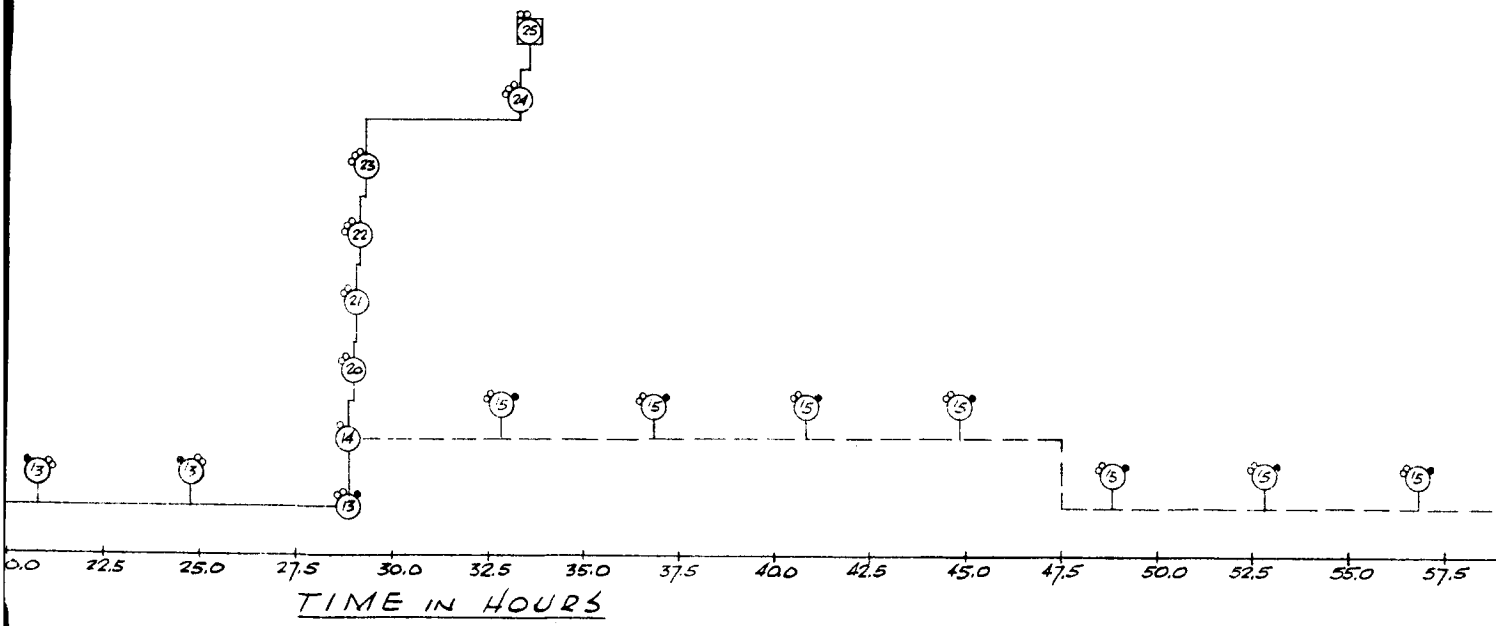
IDE DURING NORMAL METABOLISM



TIME IN HOURS

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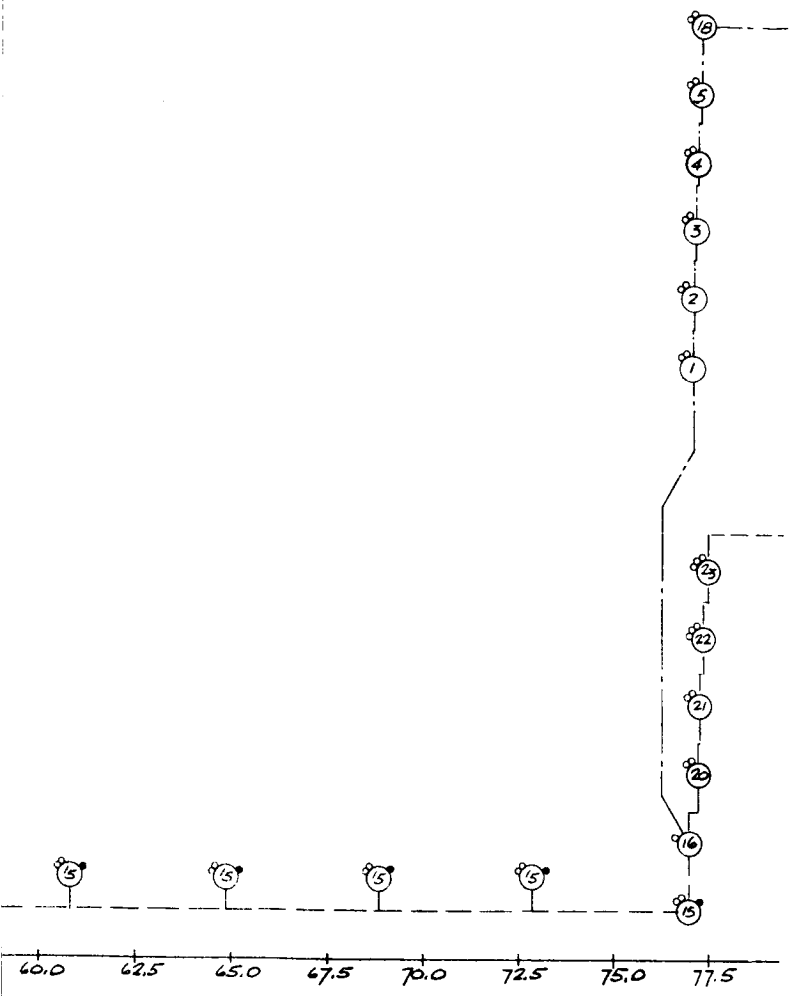
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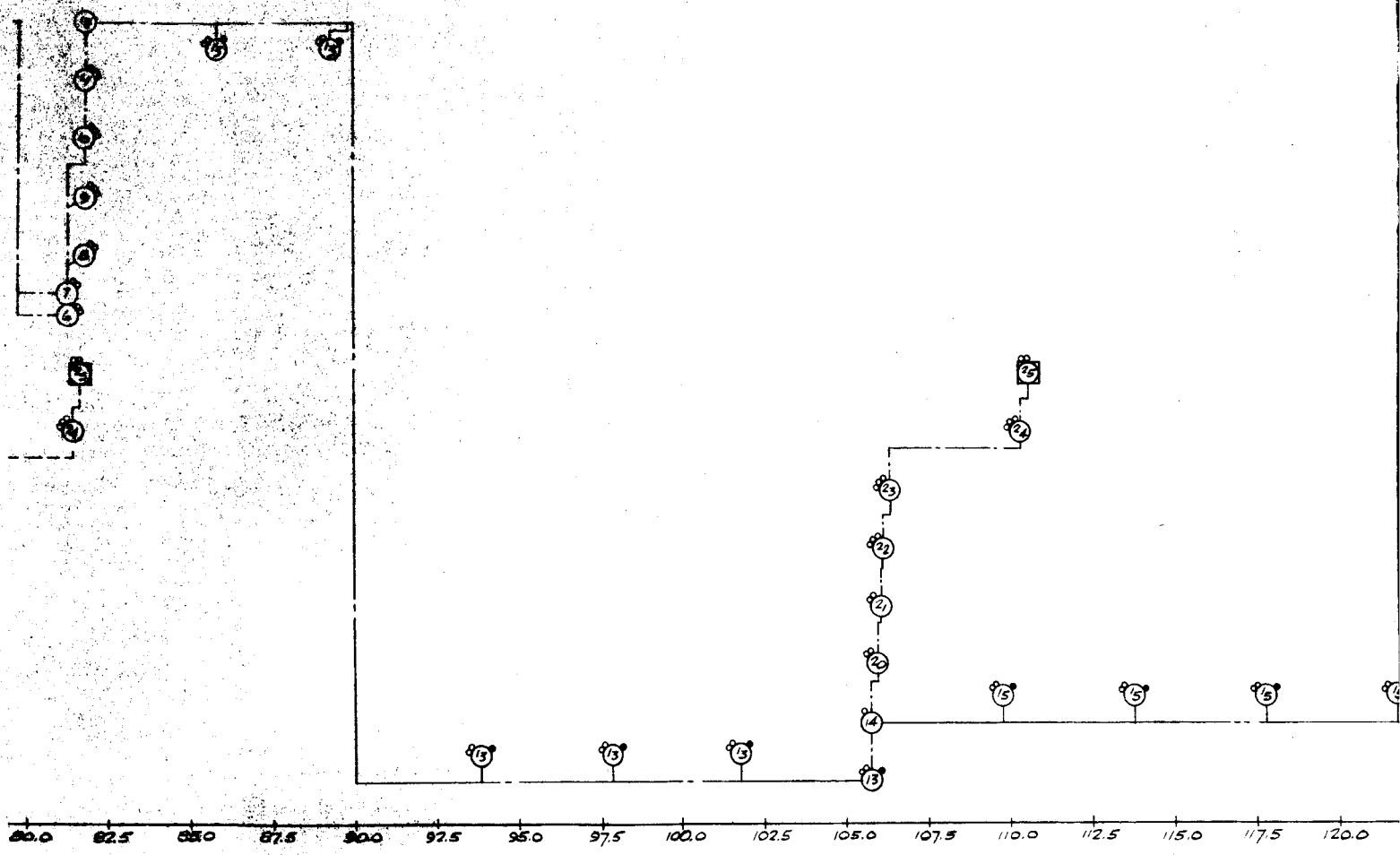
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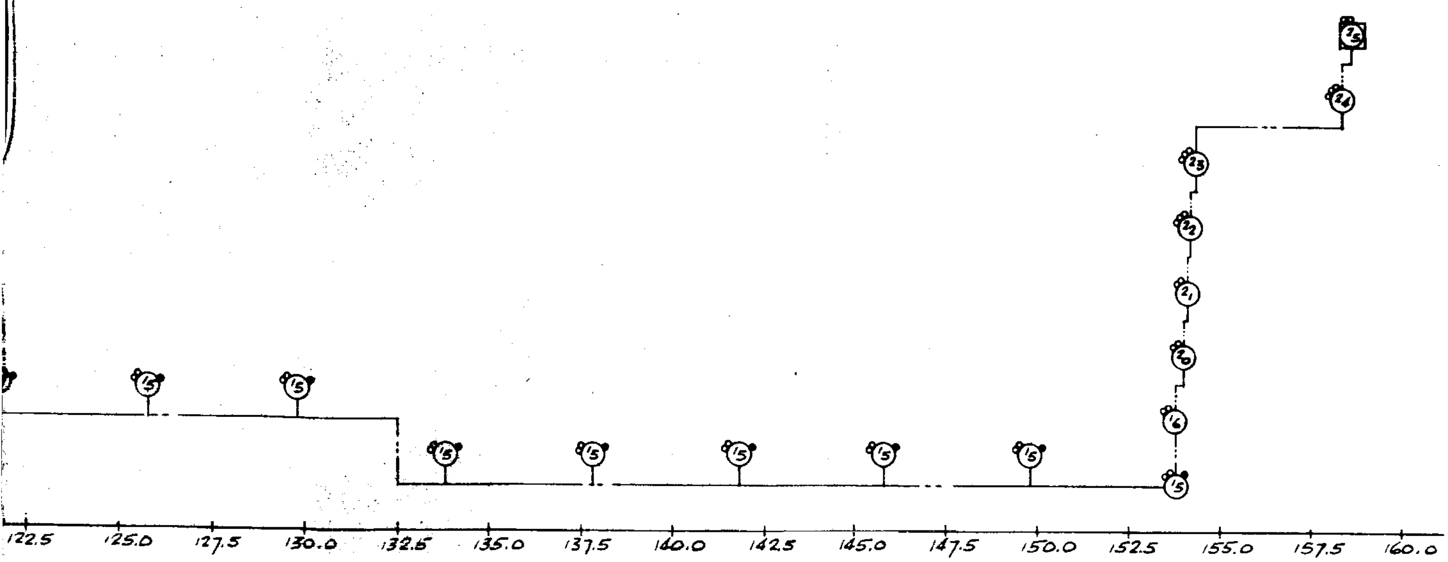
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5.31 EXPERIMENT NO. 31: $C^{14}O_2$ EVOLUTION FROM LABELED SUBSTRATE

5.31.1 DESCRIPTION

This experiment measures the amount of $C^{14}O_2$ evolved from an incubated soil sample to which a labeled substrate has been added. The labeled substrates are C^{14} formate and glucose-U-C^{14}. Two samples are heated to 120°C for 15 minutes, after which one sterile and one control sample are equilibrated at 0-1°C and the other two at 30°C. The labeled substrate purged of $C^{14}O_2$ is added after incubation temperatures have reached equilibrium. Incubation proceeds for a given time, and the rates of evolution of $C^{14}O_2$ are compared by measuring β activity.

5.31.2 APPARATUS USED

Four chemical processing chambers are required, capable of providing a programmed heating up to 150°C, heat sterilization of the two sterile control chambers, and an initial atmosphere of N_2 or of Martian atmospheric gases. The chambers will also be capable of adding a controlled amount of labeled substrate. A β-ionization detector is used to compare rates of evolution of $C^{14}O_2$ by counting β activity.

5.31.3 RANGE OF PARAMETERS

Programmed Heating:	0-120°C for 15 minutes
Incubation Temperatures:	0-1°C and 30°C
Incubation Time:	24 and 48 hours
Counting Rate:	10^6 counts per minute
Soil Sampling Size:	2 grams/chamber (having particle diameter d $\leq$ 300 μ), 8 grams total

5.31.4 PROCEDURE

(1) Check equipment.[1]

(2) Collect sample.[2] About one gram is desired.

---

[1] The equipment check probably should involve injection of test signals into electronics and checking mechanical operations, transducers, motors, etc.

[2] The sample may be surface or subsurface material. If the sample is collected in a manner so that particle sizes are finer than 40 mesh, Step (3) can be eliminated from the procedure.

(3) Grade sample to isolate material of mesh finer than 40.[3]

(4) Acquire formaldehyde - C^{14} substrate.

(5) Remove accumulated $C^{14}O_2$ by flushing with carbon dioxide for a period of 60 minutes.[4]

(6) Acquire glucose-U-C^{14} substrate.

(7) Do Step (5).

(8) Measure samples (volume technique) into four approximately 0.2 g portions.[5]

(9) Transfer samples to each of four incubation chambers (D_1, D_2, D_3, and D_4).

(10) Sterilize D_1 and D_2 by heating at 120° for 15 minutes (provides two sterile control samples).[6]

(11) Equilibrate D_1 (sterile control) and D_3 at 0-1°C.

---

[3] Sieving is assumed, but other methods of grading sample on a size basis are acceptable. The reason for sample sizing is to obtain samples having similar contents of biological material.

[4] The substrates undergo self-decomposition during storage, liberating $C^{14}O_2$, which must be removed before the substrates are used in the subsequence steps. Otherwise, this $C^{14}O_2$ would interfere in the detection of $C^{14}O_2$ evolved by metabolism of the substrate.

[5] This measurement may be made with a balance or with a sample volume-measuring technique. The data from the soil density measurement experiment (Environmental Experiment 2.5, Soil Density) could be used to determine volume of a one-gram soil sample. If the total available sample is less than one gram, the sample should be divided into four equal portions by volume. Proceed with samples of reduced volume.

[6] Alternatively, sterilization may be achieved by exposure to "carboxide" gas for an extended period of time (in excess of 4 hours), followed by flushing with nitrogen/argon or nitrogen gas.

(12) Equilibrate D_2 and D_4 at 30 to 35°C.

(13) Monitor temperatures of all chambers. DATA OUT

(14) Inject 0.2 ml of CO_2-flushed formaldehyde-C^{14} substrate solution into each chamber.

(15) Inject 0.2 ml of CO_2-flushed glucose-U-C^{14} substrate solution into each chamber.

(16) Mix.[7]

(17) At intervals of Δt, record β-activity of evolved gases from the separate chambers. DATA OUT

(18) After a period t_1 = 24 hours, exhaust chambers.

(19) Remove samples.

(20) Wash and clean chambers.

(21) Rinse chambers.

(22) Dry.

(23) Flush with filtered ambient atmosphere.

(24) If in the 24-hour period a detectable difference was found in

(a) sample and steril control at 0-1°C,

(b) sample and sterile control at 30-35°C, or

(c) samples at the two different temperatures,

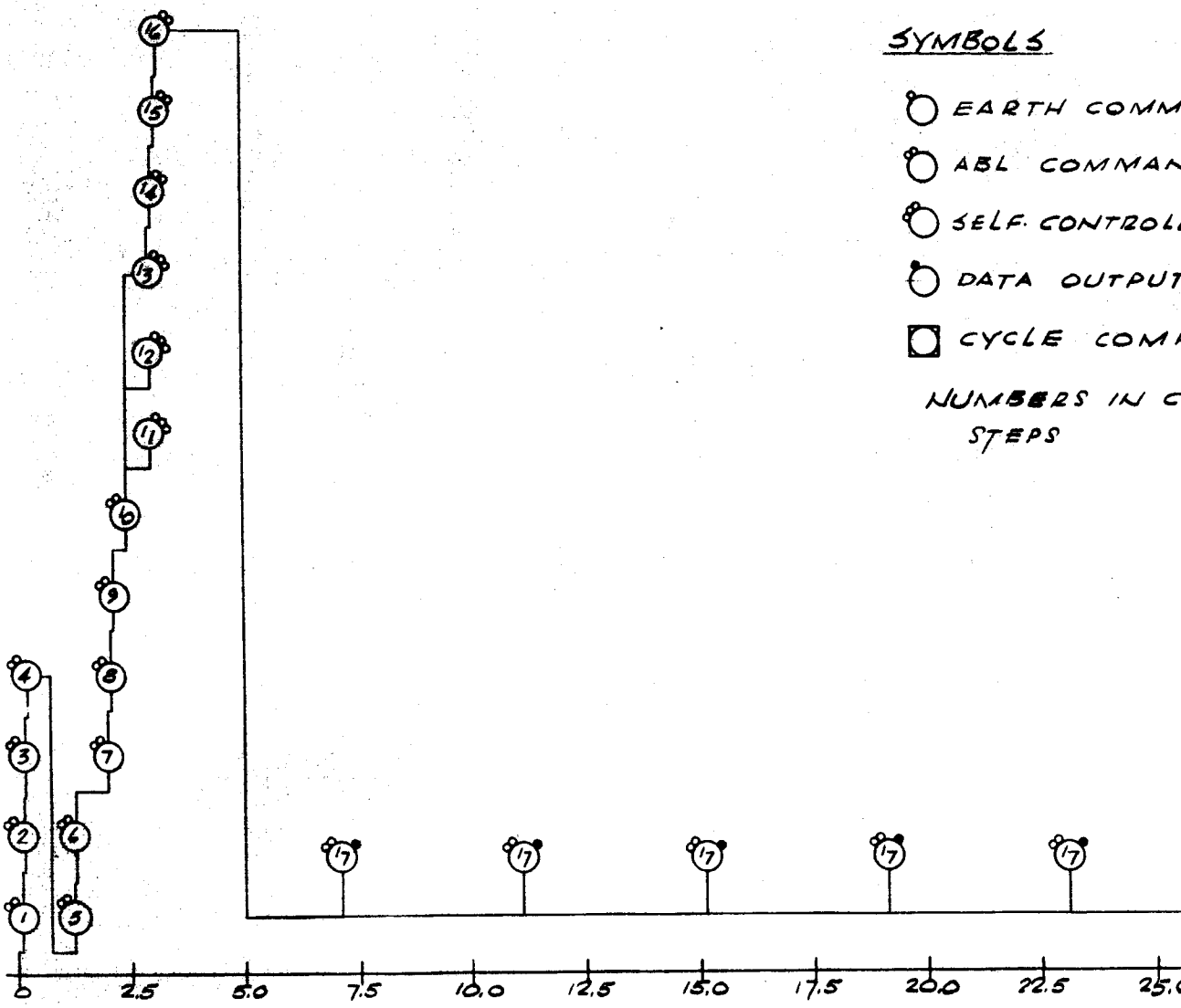
go to Step (25). If no difference was detected, do Steps (1) through (23) with a larger sample of four grams (one gram for each chamber) and let t_1 = 48 hours.

(25) Recycle to ready.

---

[7] The injection process may produce sufficient mixing by itself so that this step might be unnecessary.

EXPERIMENT 31 - $C^{14}O_2$ EVOLUTION FROM LABELED SUBST



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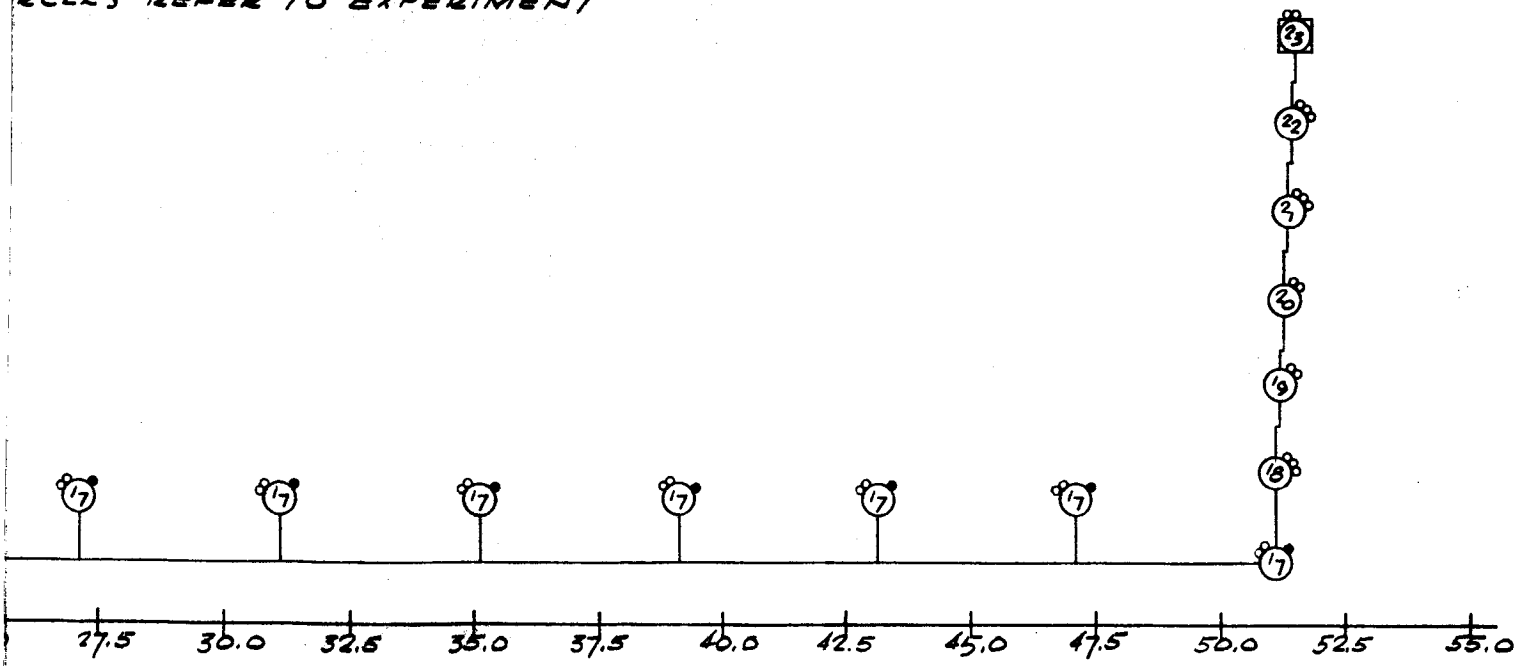
ED FUNCTION

LETION

RCLES REFER TO EXPERIMENT



5

5.32 EXPERIMENT NO. 32: $C^{14}O_2$ UPTAKE IN LIGHT AND DARK AND SUBSEQUENT EVOLUTION BY METABOLISM

5.32.1 DESCRIPTION

This experiment is designed to compare the amount of $C^{14}O_2$ fixed in light and dark with the amount of $C^{14}O_2$ re-evolved by metabolism. The samples consist of soil samples incubated in light and dark and a sterile control sample. The samples are incubated at 25°C for a given time in an atmosphere containing $C^{14}O_2$ (8 mb partial pressure). After exposure to $C^{14}O_2$ atmosphere, the chambers are purged of $C^{14}O_2$, and one set each of light, dark, and sterile samples are monitored to determine rate of re-evolution of $C^{14}O_2$. The other set of samples is burned with oxygen, and the amount of $C^{14}O_2$ fixed is determined.

5.32.2 APPARATUS USED

Six chemical processing chambers are required, capable of programmed heating. Two chambers must be lighted, and two must be sterilized with heat. Three chambers must be capable of burning the soil samples in an oxygen atmosphere. β-ionization counters are used to determine rate of $C^{14}O_2$ re-evolution and the amount of $C^{14}O_2$ released by combustion.

5.32.3 RANGE OF PARAMETERS

Incubation Temperature:	25°C
Incubation Times:	24 and 48 hours
$C^{14}O_2$ Re-evolution Count Rate:	10^6 counts per minute
$C^{14}O_2$ Fixation Count Rate:	10^6 counts per minute
Soil Sample Size:	2 grams/chamber (having particle diameters $\leq 300\mu$), 12 grams total

5.32.4 PROCEDURE

(1) Check equipment.[1]

---

[1] The equipment check probably should involve injection of test signals into electronics and checking mechanical operations, transducers, motors, etc.

(2) Collect sample.[2] Six grams of graded sample are ultimately desired.

(3) Grade sample to isolate material of mesh finer than 40.[3]

(4) Measure sample into approximately one-gram portions (six similar samples desired).[4]

(5) Transfer one-gram portions to each of the chambers L_1, D_1, D_3, L_2, D_2, and D_4.

(6) Admit sterilizing gas to chambers D_1 and D_2 and continue exposure to gas for 4 hours.[5] Gas pressure should be maintained.

(7) During the sterilization of D_1 and D_2, the other samples may be exposed to the ambient Martian atmosphere (filtered) or to flushing gas ($N_2|Ar|O_2$) mixture.

---

[2] Preferred sample is a surface scraping of Martian soil. In order of preference, sample may be surface soil, subsurface soil, material (dust, etc.) filtered from air. Depth of surface scraping is not specified but presumed to be on the order of one centimeter. If the sample is collected in a manner so that particle sizes are finer than 40 mesh, Step (3) can be eliminated from the procedure.

[3] Sieving is assumed, but other methods of grading sample on size basis are acceptable. The reason for sample sizing is to obtain samples having similar contents of biological material.

[4] This measurement may be made with a balance or with a sample volume measuring technique, and the data from the soil density measurement experiment (Environmental Experiment 2.5, Soil Density) may be used to determine volume of a one-gram soil sample. If the total available sample is less than the stated 6 grams, the sample should be divided into six equal portions by volume. Proceed with samples of reduced volume.

[5] Complete inactivation is not essential. Only a significant reduction of biological activity is required. Four hours is adequate to achieve at least partial reduction in micro-organism populations.

(8) Equilibrate all chambers to 25°C.

(9) Monitor temperature of thermostated chambers.

(10) Open gas exhaust ports.

(11) Flush out all chambers with $N_2|Ar|O_2$ or filtered Martian air for a period of 30 minutes.

(12) Initiate illumination of L_1 and L_2.

(13) Close exhaust ports.

(14) Pressurize all chambers to the ambient CO_2 partial pressure (estimated as 8 mb) with $C^{14}O_2$.[6]

(15) Incubate all six chambers at 25°C for a time t_1 = 2 hours.[7]

(16) Open exhaust ports and cease illumination.

(17) Flush $C^{14}O_2$ from all chambers with $C^{12}O_2$ for a period of 30 minutes.

(18) Flush all chambers with $N_2|Ar|O_2$ mixture or ambient, filtered atmosphere for a period of 30 minutes.

(19) At the end of the flush period, cease flow through chambers L_2, D_2 and D_4.

---

[6] The information of the ambient CO_2 partial pressure is derived from an atmospheric composition experiment (Environmental Experiment 5.1)

[7] It is desirable to incubate all six chambers simultaneously. However, if the power drain is too high, it is permissible to incubate chambers serially.

(20) Close ports of L_2, D_2, and D_4.

(21) Follow rate of evolution of $C^{14}O_2$ from L_2, D_2, and D_4 for a period t_2 = 24 hours. Simultaneously, continue with Steps (23) through (48).[8] DATA OUT

(22) At the end of the period t_2, go to Step (49). Steps (23) through (48) should have been completed.

(23) Add one ml of 10 N HCl to each of incubation chambers L_1, D_1, and D_3.[9]

(24) Continue to flush chambers L_1, D_1, and D_3 and exhaust gas to outside.

(25) Heat each chamber to boiling at the ambient pressure to evaporate to dryness.[10]

(26) Monitor temperatures of chambers.[11]

(27) Maintain temperature below critical value.

(28) Monitor drying of each of the three chambers.[12]

---

[8] Determine differences in ratios for L_2/D_4 and D_4/D_2 as a function of time. Positive results are indicated by a greater continued rate of $C^{14}O_2$ evolution from the light compared to the dark (L_2/D_4) and the dark compared to the sterile control (D_4/D_2). The other series of samples in chambers L_1, D_1, and D_3 should be processed concurrently.

[9] Steps (23) through (48) apply only to incubation chambers L_1, D_1, and D_3.

[10] Gases exhausted to the outside are $C^{14}O_2$, flush gas, water vapor, and hydrogen chloride. It may be possible (may or may not be desirable) to recover water and HCl.

[11] Temperature should not become so high that pyrolysis of organic material sets in.

[12] A sensor is implied sensitive to water vapor in the exhaust gas, for example.

(29) If drying is complete, go to Step (33). If drying is incomplete, go to Step (30).

(30) Wait time t_3.

(31) Continue heating.

(32) Go to Step (26)

(33) Discontinue heating.

(34) Wait until temperature drops to T_4.[13]

(35) Discontinue flush gas.

(36) Close exhaust port.

(37) Pressurize dried sample and sample chambers with oxygen.

(38) Monitor temperature of each chamber.

(39) Heat each chamber to 1000°C[14] for a time t_4[15] to burn organic carbon and release fixed C^{14} as $C^{14}O_2$.

(40) Open valve between incubation chamber and β-counting chamber.

(41) Transfer gas to β-counting chamber.[16]

---

[13] T_4 is probably close to 25°C. Forced cooling may be needed to achieve cooling in a reasonable time.

[14] This may be accomplished serially.

[15] Times t_3 and t_4 are on the order of minutes.

[16] It may not be necessary to effect complete transfer by, say, pumping. That is, the total gas chamber volume may include the incubation chamber and the β-counting chamber.

(42) Count radioactivity of gas in counting chamber.[17] DATA OUT

(43) Purge counting chamber with flush gas.

(44) Remove solid residue from incubation chambers, L_1, D_1, and D_3. RESIDUE

(45) Wash L_1, D_1, and D_3.

(46) Rinse.

(47) Dry.[18]

(48) If results from Step (42) are positive (i.e., differences as defined in Note (17) are found), and if results from Step (21) are positive (i.e., differences as defined in Note (8) are found), go to Step (49) If results from Step (42) are positive and the results from Step (21) are negative, go to Step (49). If results from Step (42) are negative and the results from Step (21) are negative, go to Step (61) (repeat experiment with longer incubation time). If results from Step (42) are negative and the results from Step (21) are positive, go to Step (65) (equipment check on combustion) and terminate.

(49) Illuminate L_2.

(50) Pressurize L_2, D_2, and D_4 to the ambient CO_2 partial pressure (estimated as 8 mb) with $C^{14}O_2$.[19]

---

[17] Fixation of $C^{14}O_2$ is indicated by a difference in light and dark samples, light and sterile samples, or dark and sterile samples.

[18] The dry cycle will probably resemble Steps (24) through (35).

[19] Subsequent steps apply only to chambers L_2, D_2, and D_4. Chambers L_1, D_1, and D_3 have been already cleaned and recycled.

(51) Incubate at 25°C for a time t_5 = 24 hours.[20]

(52) Do Steps (16) through (20).

(53) Follow rate of evolution of $C^{14}O_2$ for a period t_2 = 24 hours. DATA OUT

(54) After the period t_2, exhaust chambers L_2, D_2, and D_4.

(55) Remove samples from L_2, D_2, and D_4.

(56) Wash and clean chambers L_2, D_2, and D_4.

(57) Rinse chambers L_2, D_2, and D_4.

(58) Dry chambers L_2, D_2, and D_4.[18]

(59) Flush chambers L_2, D_2, and D_4 with filtered ambient atmoshpere or flush gas.

(60) Recycle to ready and stop.

(61) Do Steps (55) through (59).

(62) Do Steps (1) through (47) with a new t_1 = 24 hours.[21]

(63) If results of Steps (42) and (21) are both still negative (no $C^{14}O_2$ fixation occurs), or if both are now positive, or if results of Step (42) are positive and the results from Step (21) are negative (means that $C^{14}O_2$ is fixed but not re-evolved), go to Step (64). If results from Step (42) are negative but results from Step (21) are positive (implies inconsistency, $C^{14}O_2$ evolved but not fixed), go to Step (65).

---

[20] Actually t_5 may range from 2 to 24 hours as desired.

[21] The incubation time t_1 may actually range from 4 to 24 hours as desired.

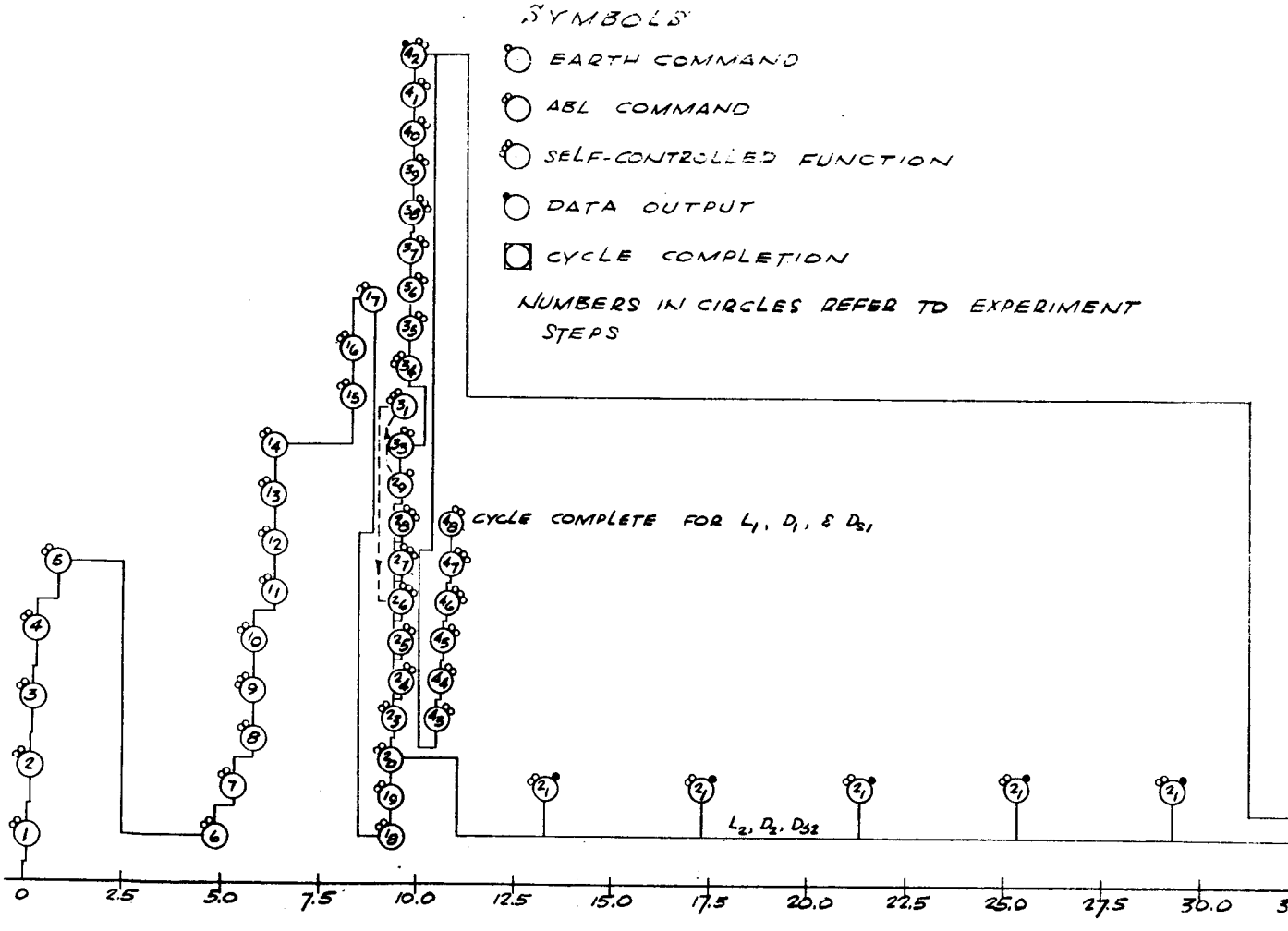
(64) Do Steps (55) through (60).

(65) Check combustion equipment for possible malfunction.

(66) Check $C^{14}O_2$ evolution equipment for false positive result.

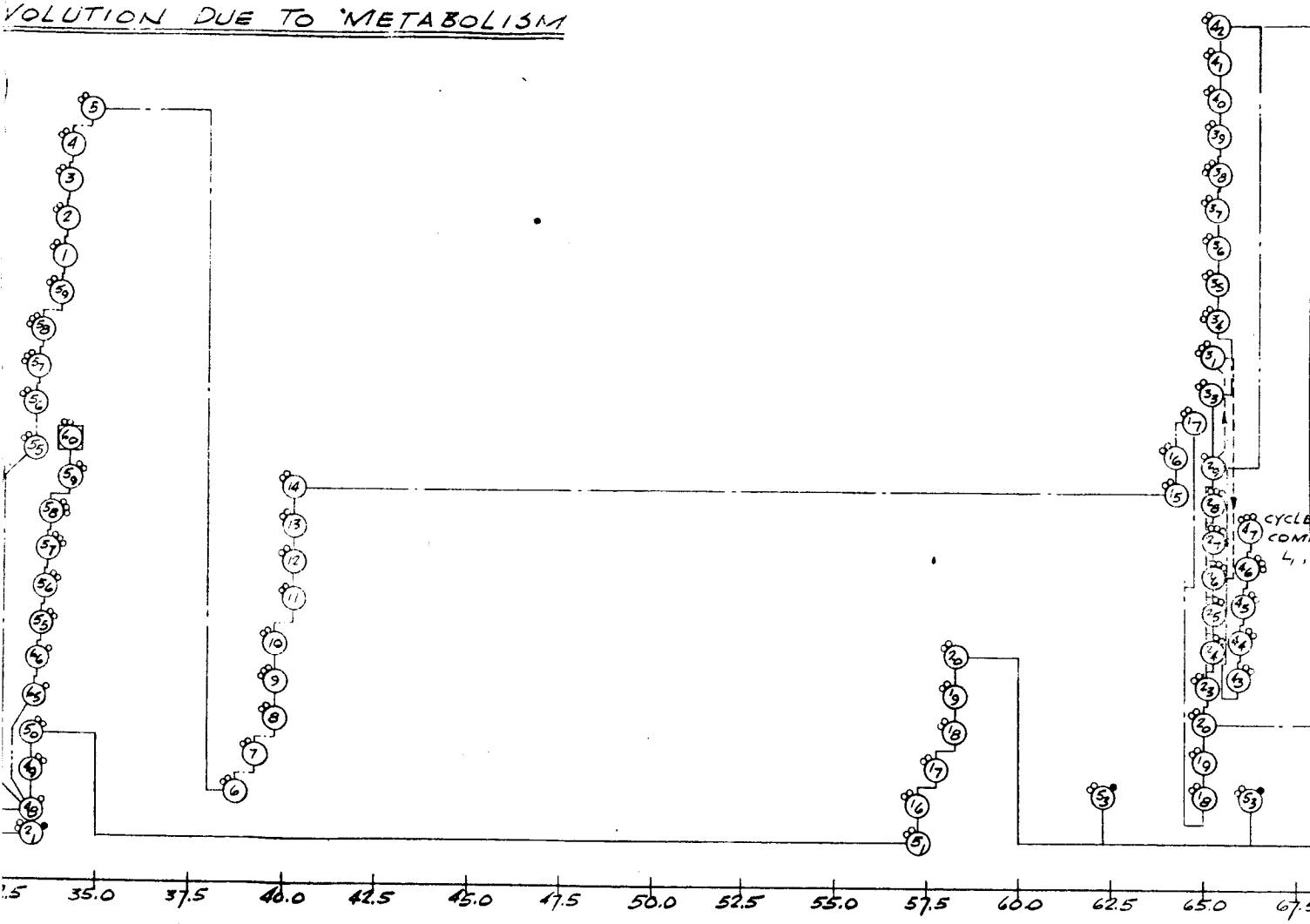
(67) Do Steps (55) through (60).

EXPERIMENT 32 - $C^{14}O_2$ UPTAKE IN LIGHT AND DARK FOLLOWED BY E

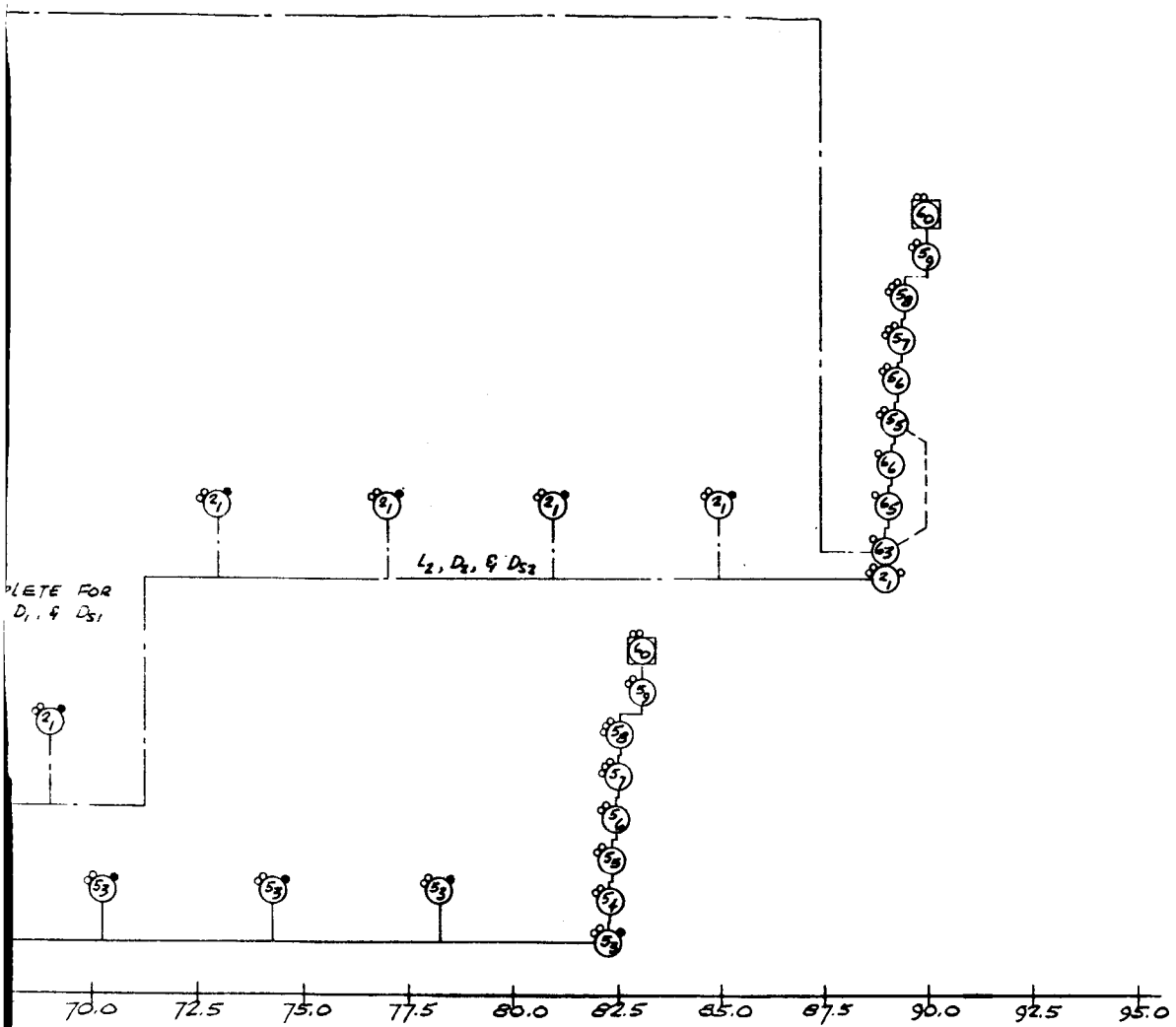


T / I

VOLUTION DUE TO METABOLISM



1E IN HOURS



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5.33 EXPERIMENT NO. 33: CULTURE EVALUATION AND GROWTH DETECTION

5.33.1 DESCRIPTION

This experiment is designed to investigate various types and concentrations of culture media, using both Earth-formulated media and media prepared from Martian soil extracts. Each culture plate will be prepared so that it incorporates a nutrient gradient to identify the optimum concentration. The procedure will be to inoculate a series of culture media preparations (10 to 12 types of formulations) from a common soil sample. These will be monitored to detect growth by increases in turbidity or optical density and pH changes. Localized changes will be determined by comparative imaging techniques or optical density surveys. If growth occurs, transfer plates will be made on successive culture plates for those culture media on which growth or changes have occurred most prominently. As many transfer cultures will be made as possible, up to a limit of 10. After growth has been established and the optimum media determined, life-detection experiments listed below will be repeated to detect increases in biological material.

Experiment No. 17	Soil Gas Exchange
Experiment No. 20	Detection of Porphyrins
Experiment No. 21	Detection of Flavins
Experiment No. 22	Detection of Nonsaponifiable Lipids
Experiment No. 23	Detection of Saponifiable Lipids
Experiment No. 24	Detection of Macromolecules by Absorption in the Visible Spectrum

5.33.2 APPARATUS USED

Chemical processing chambers as required, capable of preparing culture media of the required concentrations from Earth-originated and Mars-originated extracts and transferring these to plate containers which can be incubated at various temperatures with various controlled atmospheres. Turbidity or optical density monitors will be used to survey the surface of the plate for gross changes. Comparison imaging apparatus may be employed to detect changes such as colony growths and locations to be used with the optical density measurements. A pH meter is required. An inoculation apparatus such as a wire or rolling wheel to deposit a line of inoculum on the culture media of each plate is required.

5.33.3 RANGE OF PARA

Incubation

Incubation

Culture Med

Number of T

Turbidity o
Density:

pH Measurem

Incubation

Soil Sample

5.33.4 PROCEDURE

(1) On the
envirc
media
materi
acids,
and or
estima

(2) Deterr
the sa
temper

(3) Prepar
concer

(4) Prepar
using

(5) Store
transf

(6) Prepar
concer

METERS

Temperatures:	0° to $150^{\circ}C$
Atmospheres:	Native Martian; synthetic Martian, with and without labeled constituents
ia Formulations:	10
ransfer Cultures:	10
r Optical	4 decades of transmitted white light
ents:	Approximately 1 to 13
Times:	24 and 48 hours
Size:	1 gram (having particle diameters $\leq 50\mu$)

basis of results from life-detection and nmental experiments, determine types of to be formulated from Earth-originated als such as sugars, amino acids, fatty etc. Four types of Earth-originated e of Martian soil extract media are ted.

ine desirable incubation temperatures on me basis as in Step (1). Two incubation atures for each medium are estimated.

e Earth-originated media in varying trations. Assume 10 different concentrations.

e culture plates with nutrient gradient Earth-originated media.

remaining media until required to prepare er culture plates.

e Martian soil extract media in varying trations.

(7) Prepare culture plates with nutrient gradie using Martian soil extract media.

(8) Store remaining media until required to pre transfer culture plates.

(9) Inoculate each plate from a common soil sam graded to a particle size of 50μ or less. inoculation is deposited as a narrow line o normal to the nutrient gradient.

(10) Adjust and maintain incubation temperatures

(11) Record image of each plate in image-storing tube.

(12) Measure pH of culture.

(13) Control and maintain atmosphere over each plate.

(14) At 2-hour intervals, withdraw atmospheric g sample for each plate.

(15) Inject sample into gas chromatograph and perform analysis.

(16) Repeat Step (15) for each culture plate sam

(17) At 2-hour intervals, measure pH.

(18) At 2-hour intervals, record new image in th image-storing tube. Reverse polarization alternate images.

(19) Scan remaining difference image to detect changes.

(20) Repeat Step (19) for each culture plate.

(21) If no changes or growth is detected, contin Steps (14) through (20) for 48-hours and re cycle equipment for a new cycle.

(22) If growth or changes are detected, prepare additional culture plates for the media in which the change is detected.

(23) Inoculate transfer plate with inoculum obtained from preceding culture for those media.

(24) Repeat Steps (14) through (20).

(25) If growth is detected, repeat Steps (23) through (24) up to a maximum of four transfer cultures or until no growth is detected.

(26) If growth is detected, select optimum media and conditions; i.e., time of culture, temperature, etc.

(27) Prepare a larger quantity of culture media and inoculate with soil sample and/or inoculum from evaluation culture.

DATA OUT (28) Incubate under desired conditions and monitor for changes.

(29) When first detectable growth occurs, prepare samples for Life-Detection Experiments 17, 20, 21, 22, 23, and 24 and repeat these experiments. DATA OUT

(30) When growth culture changes have been amplified some predetermined amount, repeat Step (29) to

DATA OUT detect increase in biological material.

(31) Remove culture residue to waste storage.

DATA OUT (32) Heat (sterilize) equipment and recycle to initial conditions for future use.

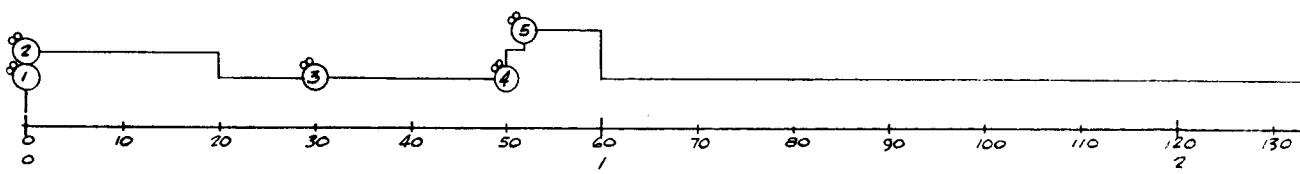
DATA OUT

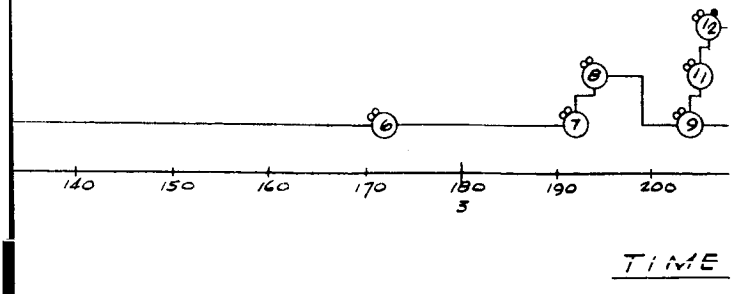
EXPERIMENT 33. CULTURE EVALUATION & GROWTH DETECTION

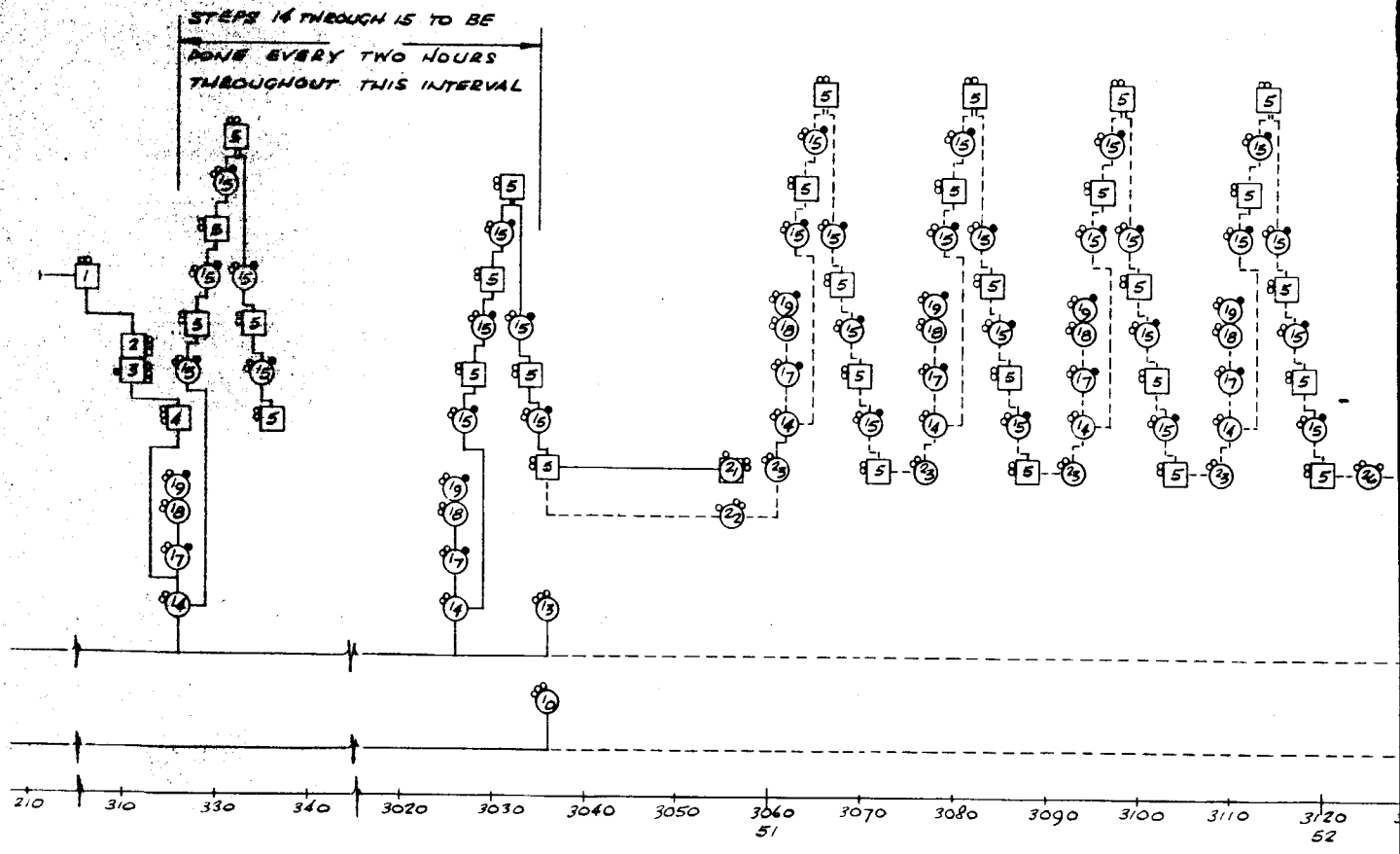
SYMBOLS

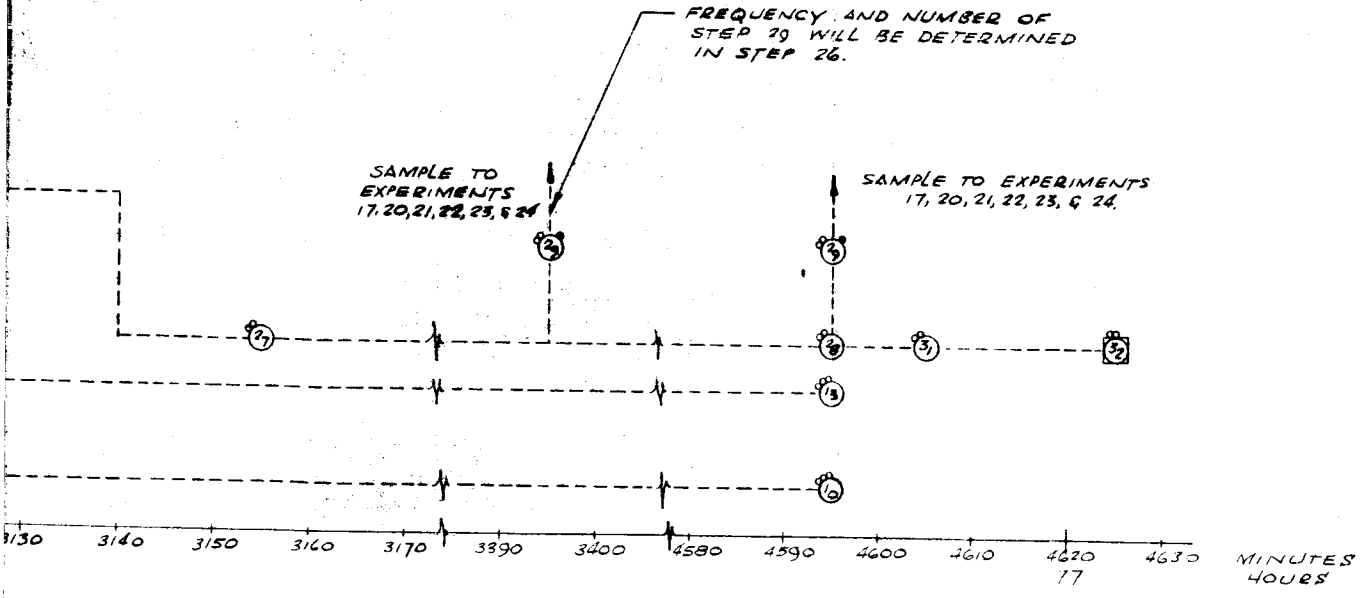
- EARTH COMMAND
- ABL COMMAND
- SELF CONTROLLED FUNCTION
- DATA OUTPUT
- CYCLE COMPLETION
- GAS CHROMATOGRAPH SUB. ROUTINE

NUMBERED CIRCLES REFER TO EXPERIMENT PROCEDURE




6
7
8
9
11
12
140
150
160
170
180
190
200
3
TIME


STEPS 14 THROUGH 15 TO BE
DONE EVERY TWO HOURS
THROUGHOUT THIS INTERVAL
210
310
330
340
3020
3030
3040
3050
3060
51
3070
3080
3090
3100
3110
3120
52


FREQUENCY AND NUMBER OF STEP 29 WILL BE DETERMINED IN STEP 26.
SAMPLE TO EXPERIMENTS 17, 20, 21, 22, 23, & 24
SAMPLE TO EXPERIMENTS 17, 20, 21, 22, 23, & 24.
27
29
29
31
32
13
10
3130
3140
3150
3160
3170
3390
3400
4580
4590
4600
4610
4620
4630
MINUTES
HOURS
17

5.34 EXPERIMENT NO. 34: MOTION DETECTOR

5.34.1 DESCRIPTION

Detection of motion, if it should occur (such as plant movement in the wind, movement of animal life, and motion because of geological movement).

5.34.2 APPARATUS USED

This motion detector consists of a telescope in the visible region of the spectrum focusing an image on a photosensitive matrix. Variations in output from the detector matrix can be interpreted as motion. A 360-degree lateral field of view can be achieved. Output data from Experiment 4 and this experiment will be coordinated.

5.34.3 RANGE OF PARAMETERS

Variation in Light Intensity:

Velocity of Travel: 1 to 50 fps

5.34.4 PROCEDURE

(1) Deploy lens and retract lens cover.

(2) Monitor variations in output voltages from detector matrix.

(3) When output voltage strength exceeds a predetermined critical value, record detector output and correlate with acoustical signals from Experiment 4. DATA OUT

EXPERIMENT 34- MOTION DETECTOR

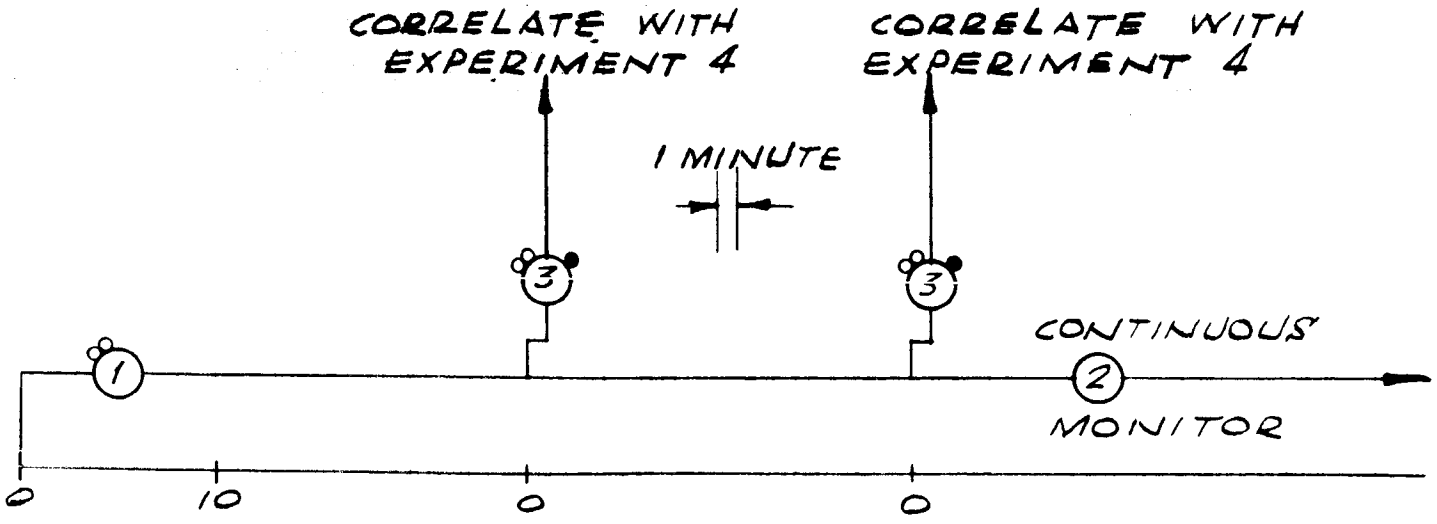
SYMBOLS

ABL COMMAND

DATA OUTPUT

NUMBERED CIRCLES REFER TO EXPERIMENT PROCEDURE

NOTE: NO EXTENDED TIME SCALE IS SHOWN. THIS EXPERIMENT IS EVENT, NOT TIME DEPENDENT.



TIME

5.35 EXPERIMENT NO. 35: MACROIMAGING AND INFRARED SCAN

5.35.1 DESCRIPTION

This experiment is designed to observe the morphological features of the surrounding terrain in the visible spectrum and, to a lesser degree, in the infrared spectrum. These scans are intended to detect macroscopic life forms, if they exist, and to detect thermal anomalies. From the low-resolution scan results, a higher-resolution scan of potentially desirable sampling sites will be made.

5.35.2 APPARATUS USED

A facsimile scanning device will be used to obtain the visual picture. An infrared radiometer is used to map the thermal features of the terrain. The infrared scan is made simultaneously with the visible, as well as at the Martian midnight.

5.35.3 RANGE OF PARAMETERS

Low-Resolution Visual Scan (Unity-Power)

Resolution: 0.3 degree, 13 gray levels

Field of View: 360 by 60 degrees

High-Resolution Visual Scan (10-Power)

Resolution: 0.03 degree, 13 gray levels

Field of View: 20 by 20 degrees

Infrared Scan

Resolution: 1.0 degree, 8 gray levels

Field of View: 360 by 60 degrees

5.35.4 PROCEDURE

(1) Detect sunrise with sun sensor and start timer.

(2) Monitor light level and correlate with elapsed time after sunrise.

(3) Deploy optical imaging system to scan position and remove optical covers.

(4) With data from Step (2), adjust aperture as required and orient instruments to begin scan

(5) Erect small identifiable target in field of view of visual scan instrument to check operation.

(6) Perform visual scan. DATA OUT

(7) Check infrared scanning instrument by viewing a sequence of calibrated blackbody raiators. DATA OUT

(8) Perform infrared scan. Synchronize with visual scans. DATA OUT

(9) During scan, monitor and adjust aperture as required.

(10) After scan is complete, replace lens covers and turn off power.

(11) Monitor timer started in Step (1) to determine start of midnight infrared scan.

(12) Remove lens cover on infrared scanning instrument.

(13) Repeat Steps (7) through (9), coordinating scanning points to correspond with daytime scan. DATA OUT

(14) Replace lens covers and turn off power.

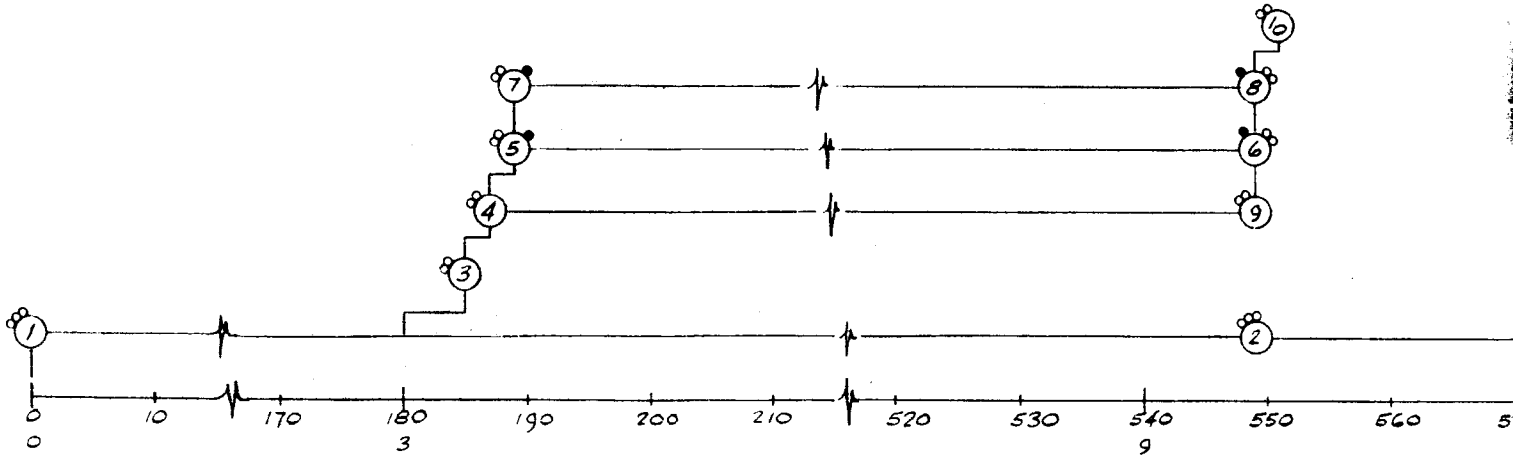
(15) Return scanning instruments to stowed position.

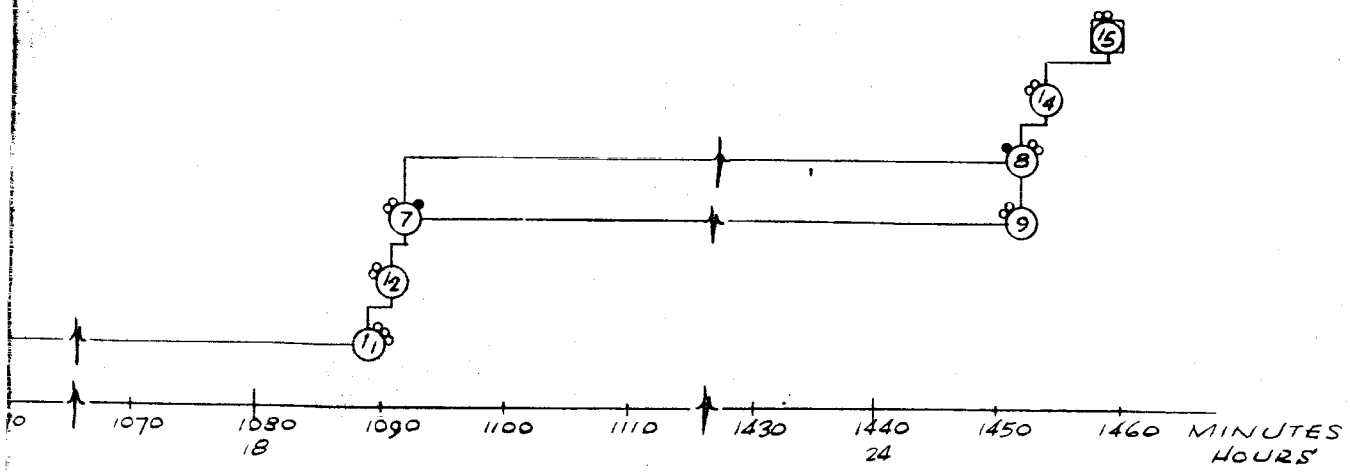
EXPERIMENT 35- MACROIMAGING AND INFRARED SCAN

SYMBOLS

- ABL COMMAND
- SELF CONTROLLED FUNCTION
- DATA OUTPUT
- CYCLE COMPLETION

NUMBERED CIRCLES REFER TO EXPERIMENT PROCEDURE AS DEFINED IN ABL-




15
14
8
7
9
12
11
1070
1080
18
1090
1100
1110
1430
1440
24
1450
1460
MINUTES
HOURS

5.36 GAS CHROMATOGRAPH SUBROUTINE

(1) Purge column with carrier gas for 5 minutes.

(2) Introduce slug of reference gas into carrier gas.

(3) Record detector output and compare with standard calibration.

(4) Backflush column for 5 minutes.

(5) After test, backflush column for a time t_1, equal to that used in analyzing the test samples.

5.37 SPECTRAL ANALYZER SUBROUTINE

(1) Set instrument mode switch and excite source. Allow warmup period of one minute.

(2) Check source output from filtered diode go/no-go signal.

(3) Introduce standard test and reference solutions.

(4) Record detector output and compare with instrument calibration.

(5) Remove test and reference solutions.

(6) Wash, rinse, and dry cuvettes.

5.38 GENERAL GROWTH CULTURE ROUTINE

(1) Acquire media from storage and add to culture chamber.

(2) Determine incubation temperature, C_1.

(3) Heat culture chamber.

(4) Monitor chamber temperature $\bar{T}_1$.

(5) If $C_1 < T_1$, go to Step (6).

If $C_1 > T_1$, go to Step (7).

If $C_1 = T_1 \pm K_1$, go to Step (8).

(6) Reduce heating

(7) Increase heatin

(8) Acquire inoculu

(9) Determine incub of aliquot remo

(10) Determine numbe

(11) Monitor gas com small sample to

(12) Remove aliquots Step (10).

(13) Discontinue hea

(14) Heat and pressu

(15) Determine prope

(16) Monitor tempera

(17) If $T_2 > C_2$, go

If $T_2 < C_2$, go

If $T_2 = C_2$, go

(18) Reduce heat by

(19) Increase heat

(20) Determine prop

(21) Monitor pressu

(22) If $P > C_3$, go

If $P < C_3$, go

If $P = C_3$, go

(23) Reduce pressur

fractional amount, ΔT.

by fractional amount, ΔT.

and add to media.

on interval and intervals

of aliquots to be removed.

sition above culture (valve
gas chromatograph).

for test, as specified in

ing.

ize culture chamber.

temperature, T_2.

ture, C_2.

to Step (18).

to Step (19).

to Step (20).

fractional amount, ΔT.

y fractional amount, ΔT.

r pressure, C_3.

e P.

o Step (23).

to Step (24).

to Step (25).

e by fractional amount, ΔP.

(24) Reduce pressure by fractional amount, ΔP.

(25) Discontinue heating.

(26) Cool until internal pressure equals external pressure.

(27) Remove sterile medium and transport to storage.

(28) Wash chamber.

(29) Rinse chamber.

(30) Dry chamber.

(31) Recycle to ready position.

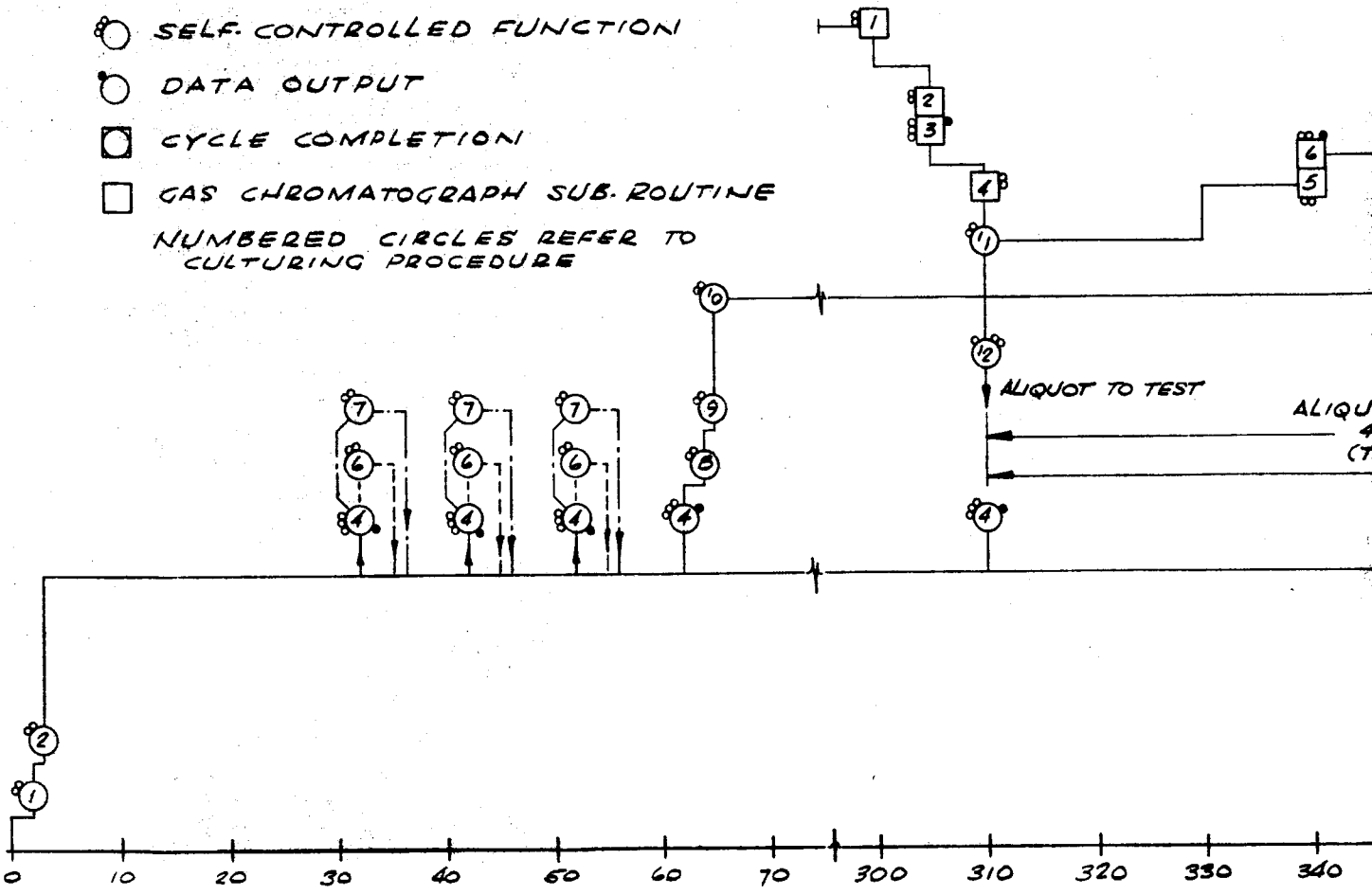
GENERAL GROWTH CULTURE ROUTINE

SYMBOLS

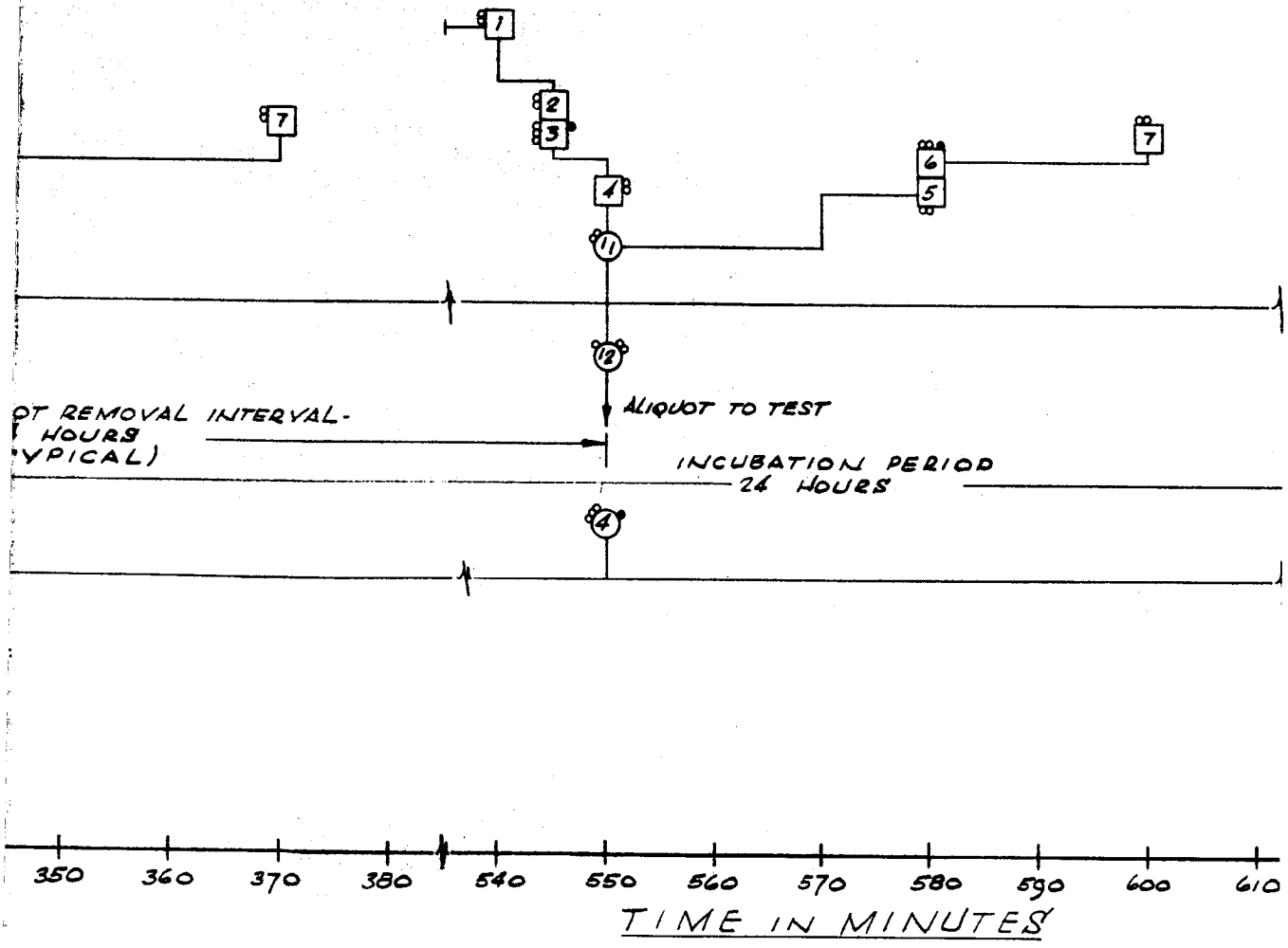
- EARTH COMMAND
- ABL COMMAND
- SELF-CONTROLLED FUNCTION
- DATA OUTPUT
- CYCLE COMPLETION
- GAS CHROMATOGRAPH SUB-ROUTINE

NUMBERED CIRCLES REFER TO CULTURING PROCEDURE

NOTE: ALIQUOT REM
MAY VARY, A
ABL COMMA



DVAL INTERVAL & INCUBATION PERIOD
ND/OR BE REVISED BY EARTH OR
VO.



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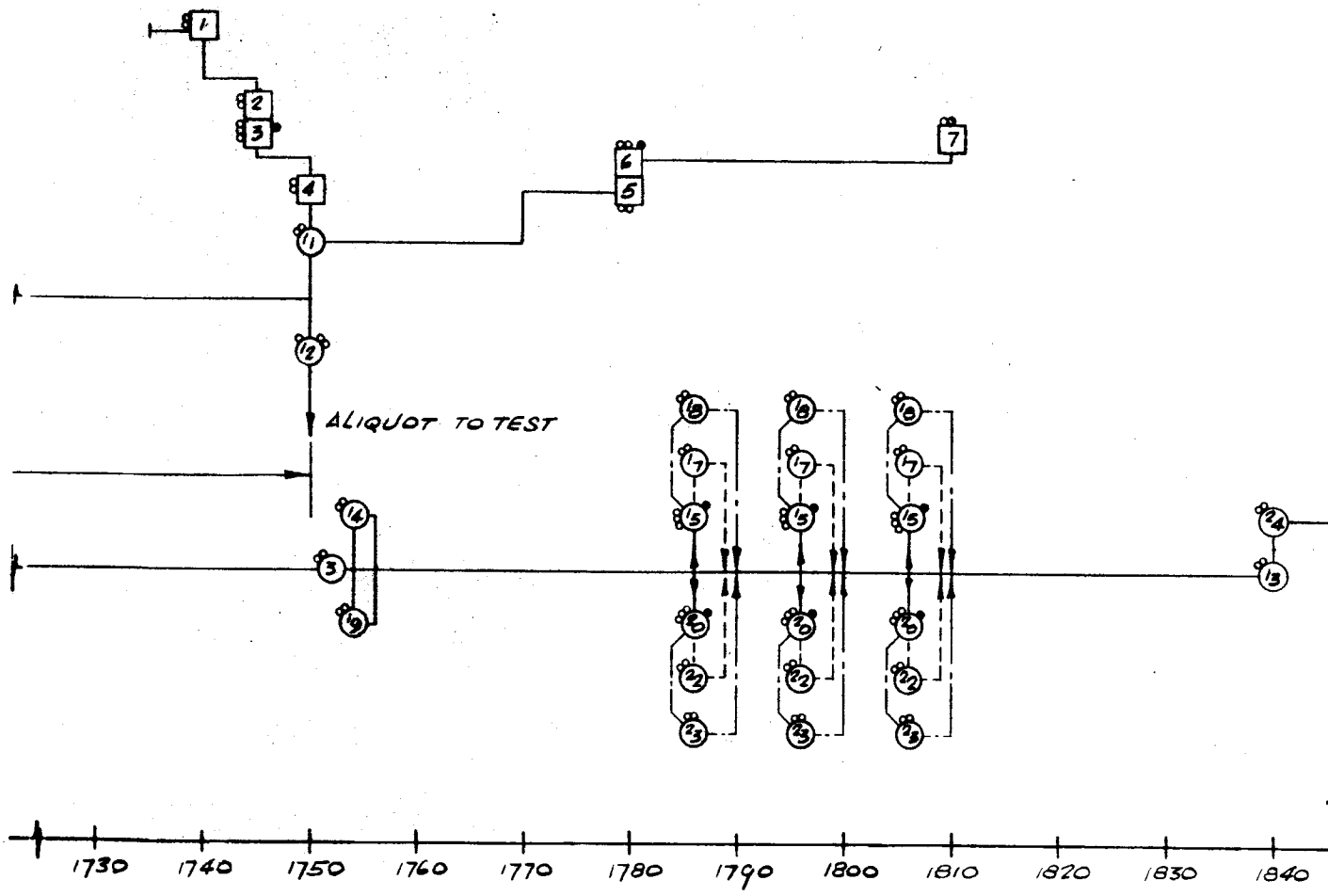
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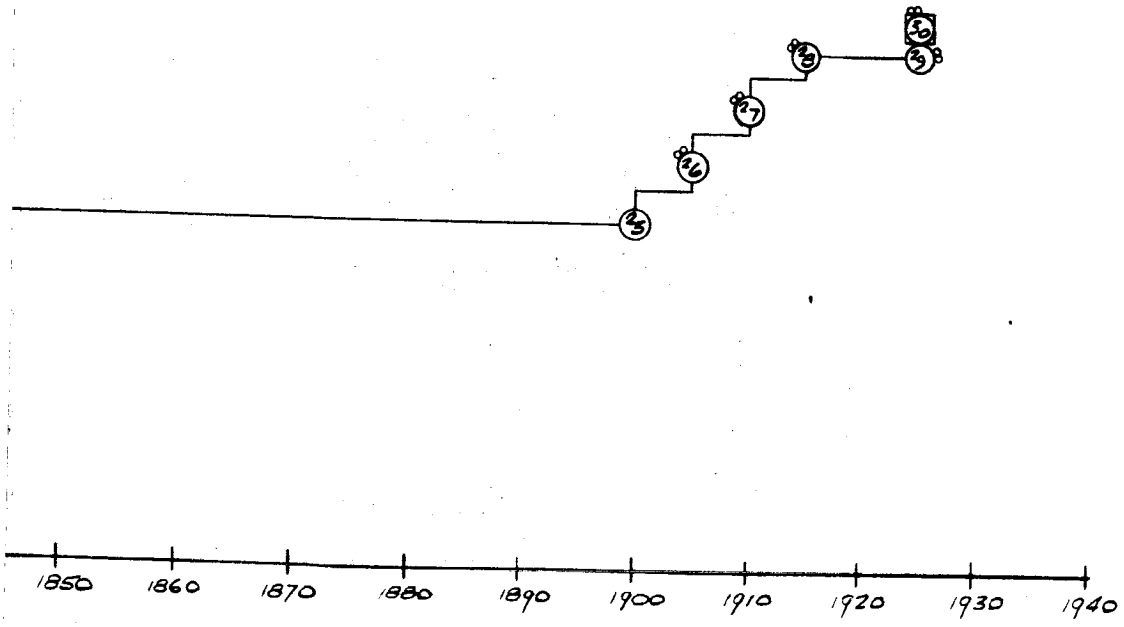
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APPENDIX 6

INSTRUMENT ANALYSES

6.1 INTRODUCTION

This appendix contains analyses performed on certain detailed aspects of instruments to establish their principal functional and physical characteriestics in sufficient detail to support the preliminary design study.

6.2 GAS CHROMATOGRAPHS REQUIRED FOR ABL

Two types of gas chromatographs are required for the ABL experiments. The atmospheric constituents and the soil gas analysis is performed with gas solid chromatography (GSC). This type of chromatograph uses a solid absorbent packing in the column to effect the separation of components. The analyses to detect more complex compounds associated with life detection require the use of gas liquid chromatography (GLC). This type of chromatograph uses a solid absorbent support such as diatomaceous earth, firebrick, glass beads, or teflon in the column to support a stationary liquid phase which performs the function of separating the components.

The following generalized requirements for a gas chromatograph have been drawn from the literature and will be used to define these instruments for the ABL.

(1) The sample should be introduced as a slug into the carrier gas flow without disturbing the flow. Sample sizes are usually in the range of 1 to 10 μliter.

(2) Most columns utilize programmed heating to aid in separating the components as well as reduce total operating time. Temperature programs up to 250 to 300°C are commonly used and may be either linear or step functions. For the ABL, a linear program is assumed.

(3) Carrier gases commonly used are helium, argon, nitrogen, and carbon dioxide. Helium is used in the ABL atmospheric and soil gas chromotograph so as not to mask the presence of argon. Argon is used in the remaining gas chromatographs in order to take advantage of the sensitivity of the argon β-ionization detector.

(4) Carrier gas flow rates typically vary from 10 ml to 100 ml per minute at the column exit. Most columns in general use tend to be most efficient at flow rates of 40 to 50 ml per minute. Since the actual optimum flow rate must be determined for the particular column and usage, an average of 50 ml per minute is assumed for the ABL gas chromatographs.

(5) Analysis times for fixed gases such as obtained in the GSC are usually in the order of minutes. For the ABL ten minutes will be assumed. Analysis times for GLC vary from 20 minutes to an hour with a half hour being fairly typical. Thus, a half hour will be assumed for these chromotograph analyses on ABL.

(6) The most commonly used detector is the thermal conductivity detector because it is nondiscriminating and has a linear response. The sensitivity of this detector is about half that achieved with glow discharge detectors, electron capture, and β-ionization detectors. The electron capture detector is discriminating in response and is limited to the types of compounds it can detect. Flame ionization detectors are sensitive and nonselective, but require the use of hydrogren gas presenting an explosive hazard as well as being less reliable in terms of the flame being extinguished. Thus, the atmospheric and soil gas chromatograph for ABL uses a glow discharge detector. The remaining ABL gas chromatographs will use the argon β-ionization detector.

(7) Column efficiency increases in proportion to column length and inversely proportional to column diameter. Capillary columns are most efficient but in general require high flow rates and very small samples. The extremely small sample size usually requires that sample splitting be resorted to obtain the proper sample in the column. Since column size does not strongly influence the final chromatograph size or weight, an 0.125 inch diameter column 6 to 8 feet in length is assumed for the ABL gas chromatographs. These dimensions are indicative of columns currently being used in the laboratory with success.

Using the preceding generalizations and specific data from the literature, five gas chromatographs are defined in the following paragraphs in terms of block diagrams, power requirements, and approximate configuration, giving weight and clearance envelopes.

6.2.1 GAS CHROMATOGRAPH NO. 1

This gas chromatograph is used to perform the analysis of soil gases and atmospheric gases. Table I presents in matrix form the various gases which can be separated with four different column packing materials. This table used in conjunction with information on research being performed at Ames and JPL was used to select the molecular sieve 5A and silica gel columns. The requirement for the low column temperature arises from the difficulty of separating argon and oxygen as well as NO and CO at higher temperatures. These two columns are housed in a single cooling jacket and oven since their thermal programs are the same.

The remaining two columns were selected on the basis of column packings suggested in Wilkens Instrument and Research, Inc., catalog of gas chromatograph supplies and accessories and also work done at Ames by Oyama. No temperature program is required for these columns so that they can be housed together in a single temperature control oven.

This gas chromatograph also uses a mass spectrometer as a detector. The effluent from the gas chromatograph is sampled when a peak is detected by the glow discharge detector and diverted to the mass spectrometer sample inlet chamber. The block diagram showing the elements of the atmospheric and soil gas analysis system is shown in Figure 1. Figure 2 is a more detailed schematic of the gas chromatograph column components indicating feed-back control and data output points.

TABLE I

GASES SEPARATED BY VARIOUS COLUMN PACKINGS

	Ne	He	HZ
Ar, etc	M 20° E	M 20° E C 20° E S,A 20° F/D S,A <0° E/F	M 20° E C 20° F S,A 20° F/D S,A< 0° E/F
Ne		M -78° F C -196° F	M 20° F M-20° E
He			M 20° F M-20° E

	Ar	O_2	N_2	CO	CH_4	CO_2	N_2O
O_2	M 20° D M-72° F						
N_2	M 20° E C 20° F S,A 20° D S,A-78° F	M 20° E C 20° F S,A 20° D S,A-78° F					
CO	M 100° E C 20° F S,A 20° F/D S -20° E	M 100° E C 20° F S,A 20° F/D S -20° E	M 100° E C 20° F S,A 20° F/D S -20° E				
CH_4	M 100° E/F C 20° E S,A 20° F/D	M 100° E/F C 20° F S,A 20° E/F	M 100° E C 20° E S,A 20° E/F	M 100° E C 20° E S 20° F S -78° E			
CO_2 (C&S ONLY EXCEPT >300°)	C,S 20-100° E	C,S 20-100° E	C,S 20-100° E	C,S 20-100° E	C,S 20-100° E		
N_2O	M,C,S,A 20-100° E	M,C,S,A 20-100° E	M,C,S,A 20-100° E	M,C,S,A 20-100° E	M,C,S,A 20-100° E	C 20° E S 20° F S-20° E	
NO (S ONLY)	S <0° E	S <0° E	S <0° E	S -20° F	S -20° F	S -20° E	S -20° E

M-MOLECULAR SIEVE 5-A, C-CHARCOAL, 5-SILICA GEL A-ALUMINA

E-EASY SEPARATION, TWO COMPLETELY SEPARATE PEAKS SHOULD BE OBTAINABLE IN A FEW MINUTES ON A COLUMN 1 METER LONG.

F-FAIR SEPARATION, MORE OR LESS COMPLETE SEPARATION IN A FAIRLY SHORT TIME ON A COLUMN OF ABOUT 2 METERS

D-DIFFICULT SEPARATION, COMPLETE SEPARATION WILL REQUIRE A LONG, CAREFULLY PACKED COLUMN OPERATED SO AS TO OBTAIN GOOD COLUMN EFFICIENCY.

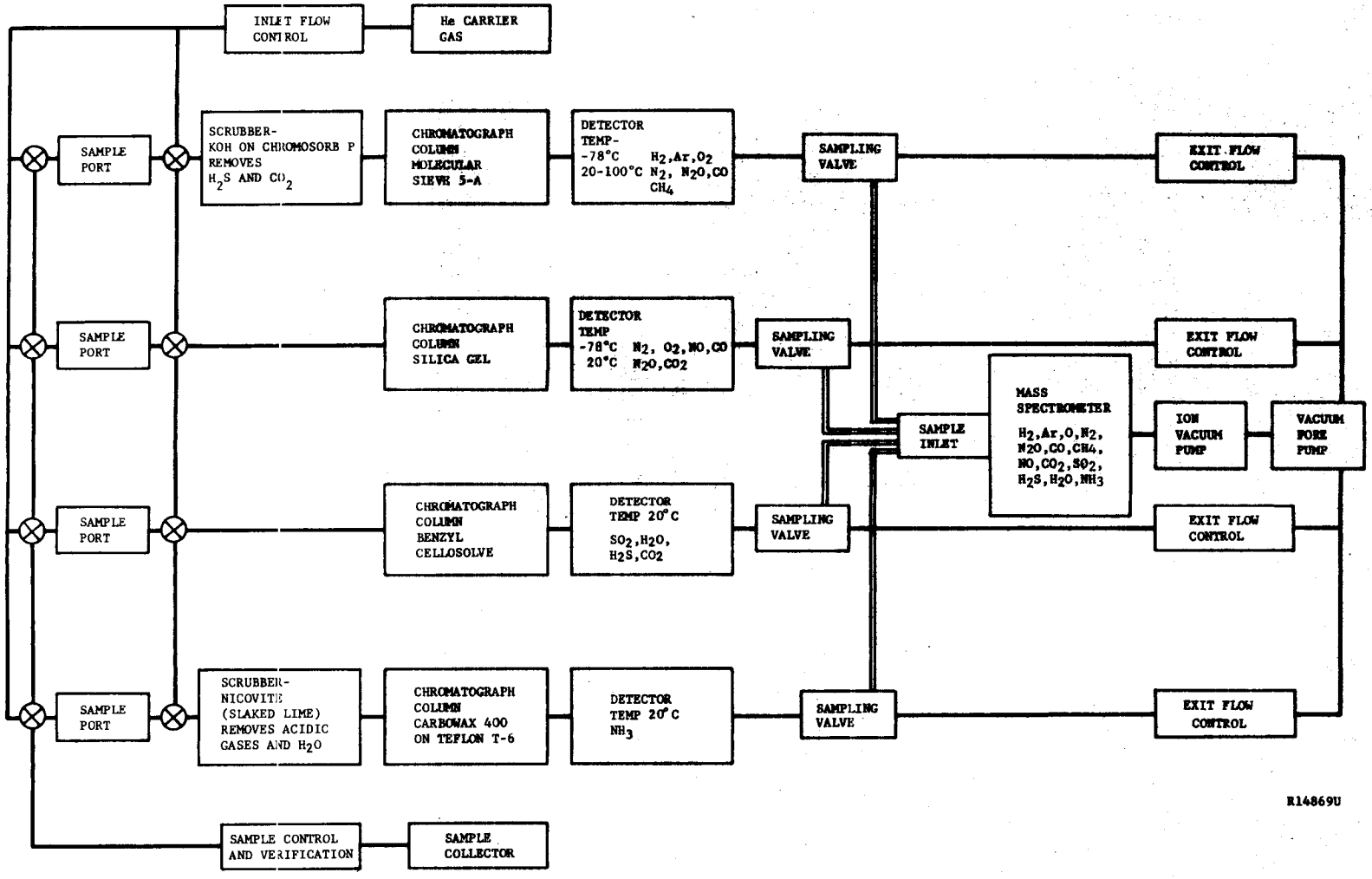


FIGURE 1. BLOCK DIAGRAM - GAS CHROMATOGRAPH NO. 1 ANALYSIS SYSTEM

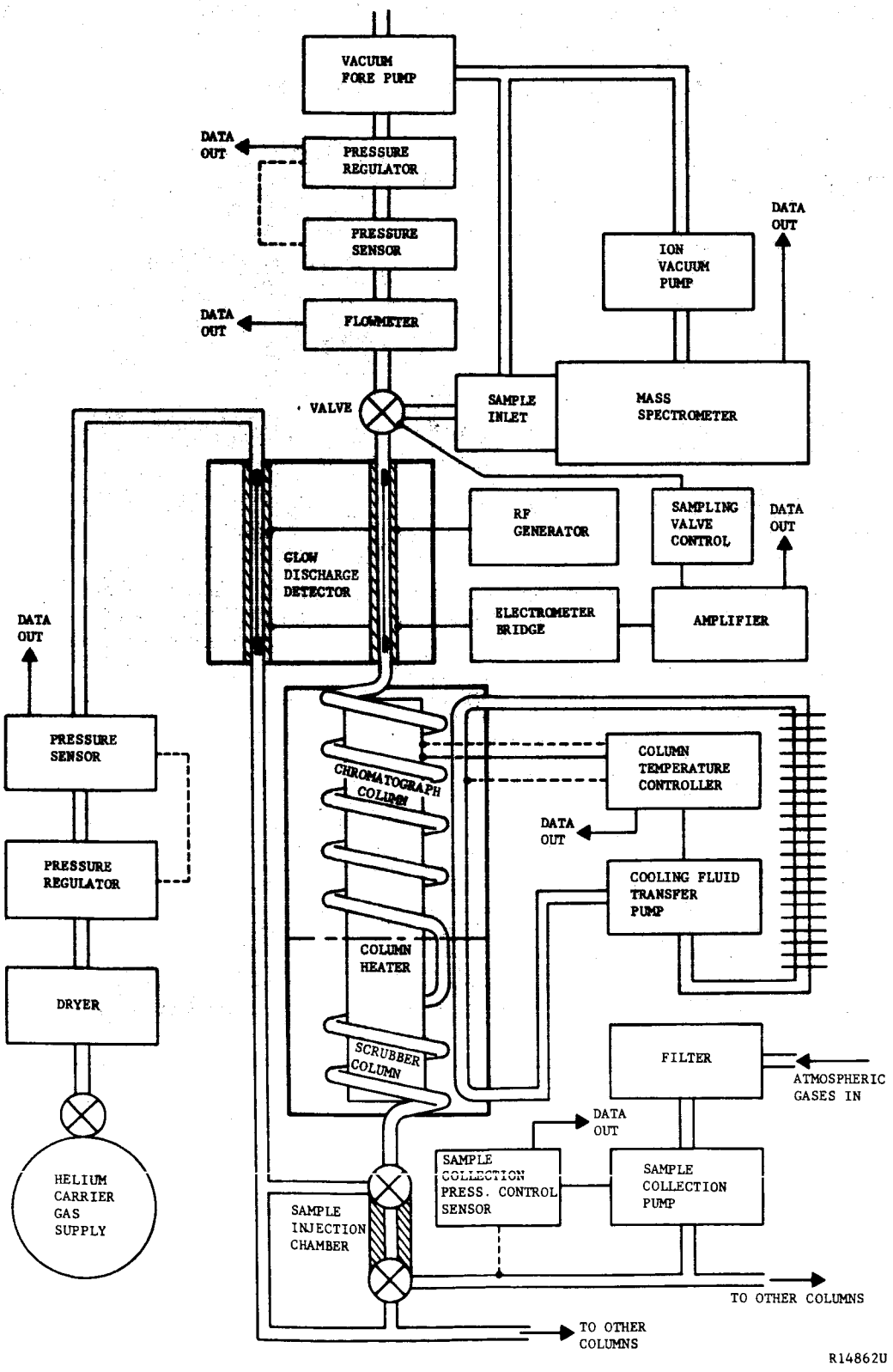


FIGURE 2. SCHEMATIC - ATMOSPHERIC ANALYSIS GAS CHROMATOGRAPH

The carrier gas requirements are estimated for gas chromatograph No. 1 as follows:

(1) Column exit pressure 10 psia

(2) Flow rate 50 ml/min (0.00177 ft^3/min)

(3) Temperature 5 minutes at -78°C (352°R)

5 minutes at 20°C (528°F)

(4) Helium carrier gas.

The flow density at -78°C is given by

$$\rho = \frac{P}{RT} = \frac{(10)(144)}{(386)(352)} = 0.0106 \text{ lb/ft}^3 \text{ and} \quad (1)$$

the flow rate per minute per column at -78°C is

$$\dot{w} = (0.0106)(0.00177) = 1.87 \times 10^{-5} \text{ lb/min.}$$

The flow density at 20°C is given by

$$\rho = \frac{(10)(144)}{(386)(538)} = 0.00707 \text{ lb/ft}^3 \text{ and} \quad (2)$$

the flow rate at 20°C is $\dot{w} = (0.00707)(0.00177) = 1.25 \times 10^{-5}$ lb/min.

The total carrier gas weight required for the chilled columns are

$$W_T = (1.87 \times 10^{-5})(5) + (1.25 \times 10^{-5})(5) = 1.56 \times 10^{-4} \text{ lb/cycle/column}$$

and for the 20°C columns are

$$W_T = (1.25 \times 10^{-5})(10) = 1.25 \times 10^{-4} \text{ lb/cycle/column.}$$

Total carrier gas consumed per operational cycle is $W = (1.56 \times 10^{-4})(2) + (1.25 \times 10^{-4})(2) = 5.62 \times 10^{-4}$ lb. The number of use cycles for this chromatograph are estimated as shown in Table II.

The total weight of helium carrier gas in the two year lifetime of ABL is $W = (5.62 \times 10^{-4})(1104) = 0.622$ lb.

Two types of tank storage are considered spherical and toroidal. Assuming at least two storage tanks for redundancy, the sizes of these tanks are given in Table III along with their weight and operating pressures. The maximum pressure is that encountered during sterilization and the normal pressure is that which occurs at the ambient laboratory temperature of 4°C.

TABLE II

ESTIMATED USE OF GAS CHROMATOGRAPH NO. 1

Experiment No.	Experimental Cycles	Cycles/ Experiment	Total Use Cycles
7	24	1	24
14	12	2	24
17	13	48	624
33	12	36	432
		Total	1104

TABLE III

ESTIMATED STORAGE TANK REQUIREMENTS FOR CARRIER GAS

Tank Type	Tank Dimension (inches)	Tank Weight (pounds)	Gas Weight (pounds)	Normal Pressure (psi)	Maximum Pressure (psi)
Spherical	14.4ID	1.71	0.311	4650	7000
Torroidal	7ID tube x 6ID	2.74	0.311	4650	7000

The density of helium at 7000 psia and 145°C (753°R) is

$$\rho = \frac{(7000)(144)}{(386)(753)} = 0.347 \text{ lb/ft}^3 \text{ and} \qquad (3)$$

the volume per tank required is

$$V = \frac{0.311}{0.347} = 0.896 \text{ ft}^3.$$

This yields a sphere with a radius of

$$R^3 = \frac{(0.75)(0.896)}{} = 0.214 \text{ and } R = 0.598 \text{ ft (7.2 inches).}$$

The mass of each gas chromatograph oven with columns is approximately 1.14 pounds with a total heat capacity of 0.15 Btu/lb^{o}F as tabulated in Table IV for the configuration shown in Figure 3.

These values are used to estimate the power requirements for chill-down warmup. Chill-down is estimated using a silicone oil (Dow Corning 331) as the fluid in the heat transfer loop. A pressure drop across the coolant pump of 1 psi is assumed. A quasi steady state heat transfer with a temperature drop of 10^{o}F is also assumed. The coolant flow rate is determined from

$$\dot{Q} = \dot{W}C\Delta T \text{ which gives } \dot{W} = \frac{\dot{Q}}{C\Delta T},$$

where

$\dot{Q}$ = heat transfer rate, Btu/min

C = specific heat of coolant, Btu/lb^{o}F

$\dot{W}$ = coolant flow rate, lb/min.

From this the pumping power can be determined and is given by

$$P = \frac{\dot{W}\Delta P}{\rho},$$

where

P = pumping power, ft-lb/min

Δp = pressure drop, psf

ρ = coolant fluid density, lb/ft^{3}.

Solving for the ideal pumping power yields

$$P = \frac{\dot{Q}\,\Delta p}{C\Delta T\,\rho} = \frac{\dot{Q}\,(144)}{(0.35)(10)(65)} = 0.633\,\dot{Q} \text{ ft-lb/min.}$$

In terms of electrical power this is $P = 0.15\,\dot{Q}$ (watts). The efficiency of the drive motor, pump, and gear train are estimated to be 50 percent, 40 percent, and 40 percent respectively. Total required power is the

$$P_T \eta_M \eta_P \eta_G = 0.15\,\dot{Q} \text{ or } P_T = 1.87\,\dot{Q} \text{ watts.}$$

TABLE IV

WEIGHT SUMMARY - GAS CHROMATOGRAPH NO. 1

Item	Unit Weight (lb)	Quantity	Weight Per Oven (lb)	Total Weight (lb)	Specific Heat (Btu/lb°F)	Heat Capacity/Oven (Btu)
Copper heat distributor	0.891	2	0.891	1.782	0.094	0.080
Heat transfer fluid	0.070	2	0.070	0.140	0.350	0.0245
Inlet pressure regulator	0.400	1	--	0.800	--	--
Column tubing	0.081	2	0.081	0.162	0.12	0.0097
Column packing	0.083	2	0.083	0.166	0.32	0.0265
Insulation	2.630	2	--	5.260	--	--
Outer shell	0.374	2	--	0.748	--	--
Detector	0.062	9	--	0.496	--	--
Exit flow control	0.100	4	--	0.400	--	--
Sample collection pump	1.100	1	--	1.100	--	--
Chill-down pump	1.100	1	--	1.100	--	--
Total			1.125	12.154	0.128 avg	0.1447

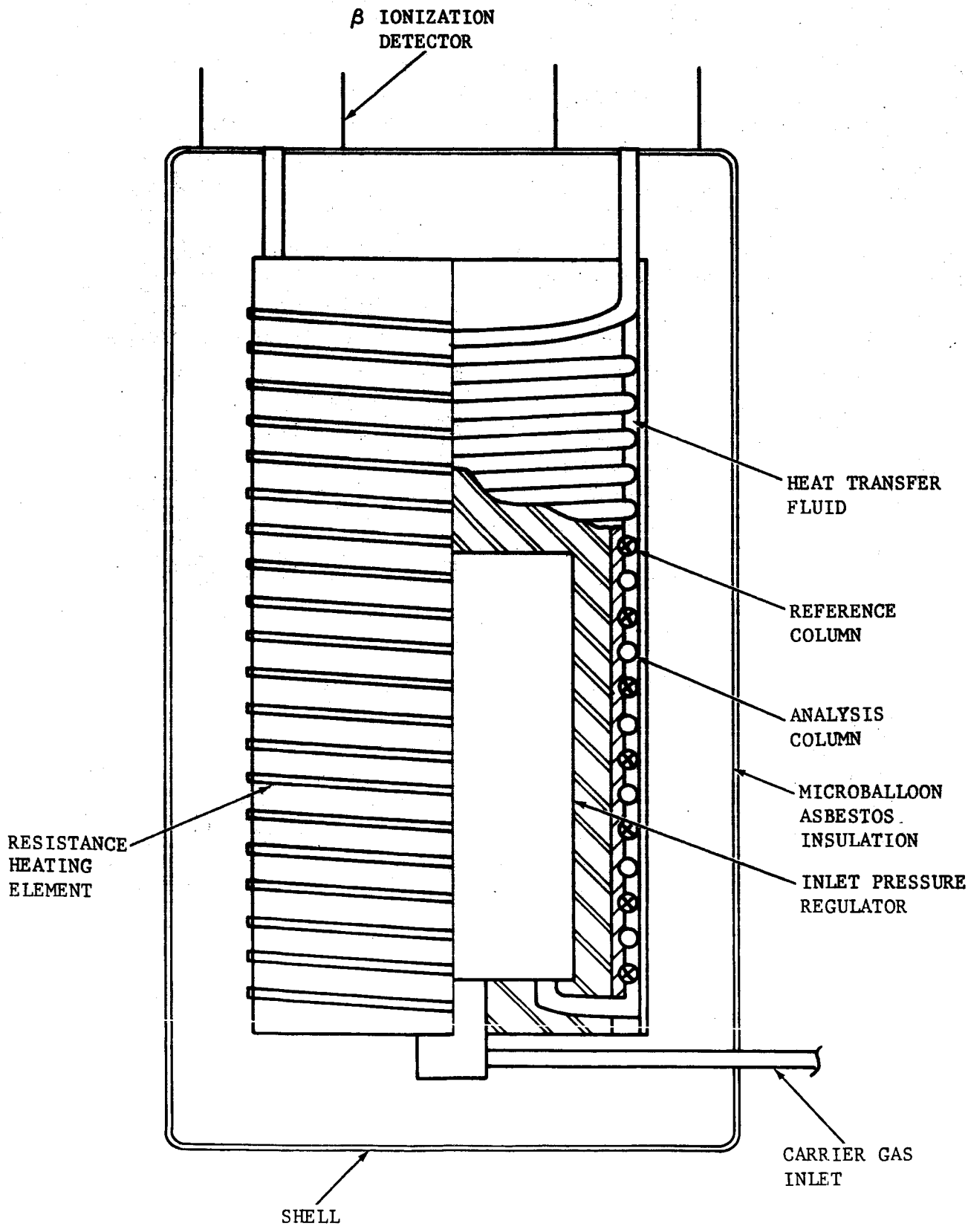


FIGURE 3. GAS CHROMATOGRAPH INTERNAL CONFIGURATION

The rate at which heat is removed depends on the total heat capacity of the column and the time required to cool,

$$\dot{Q} = \frac{Q}{T} = \frac{WC\Delta T}{t} .$$

A chill-down time of 10 minutes is assumed to be long enough to stabilize the column temperature at

$-78^{\circ}C$ ($-108^{\circ}F$). For this time, the required power is

$$P_T = \frac{(1.87)(0.15)(39 + 108)}{10} = 4.12 \text{ watts.}$$

The thermal program during the analysis is 5 minutes at $-78^{\circ}C$ followed by a linear increase to $100^{\circ}C$. The total heat required is

$Q = WC\Delta T$ and the heating rate is

$$\dot{Q} = \frac{WC\Delta T}{t} \text{ from which the power can be obtained}$$

$$\dot{Q} = \frac{(0.15)(212 - 108)}{S} = 9.6 \frac{\text{Btu}}{\text{min}} \text{ or } P = 169 \text{ watts.}$$

The benzyl cellosolve and carbowax columns are stabilized at $20^{\circ}C$. Assuming the column starts at the laboratory temperature of $4^{\circ}C$, the heat input to achieve $20^{\circ}C$ is

$$Q = (0.15)(68-39) = 4.5 \text{ Btu.}$$

Allowing 10 minutes for warmup gives a power requirement of P = 7.90 watts. The helium is capable of conducting away from the column heat at a rate of

$$\dot{Q} = \frac{(2)\ (125 \times 10^{-5})(1.25)}{60} = 5.22 \times 10^{-7} \text{ Btu/sec.}$$

This is about 0.007 percent of the power requirement to heat the column. Thus, the power required to maintain the column temperature during the analysis is determined by external conduction losses and is estimated to be 1 percent of that required for warmup.

The detector operates on the principle that when an RF discharge occurs between two electrodes between which the field is diverging, a dc potential appears between the electrodes (Reference 1). This dc potential is sensitive to the composition of gas through which the discharge is passing; thus, the output of the detector is a dc voltage. A typical peak may require one half a minute to a minute to pass. If the data is sampled at the rate of one point every 5 seconds, this gives 6 points to define a peak which should be adequate. A total of 120 data points are obtained

in 10 minutes. Assuming an output range of 4 decades for the detector voltage and an accuracy of one percent results in 19 bits per data point at an average rate of 4 bits per second per column. Total data output from the gas chromatograph detector is 8720 bits. The data output from the mass spectrometer is not included in this data output.

Figure 4 summarizes the thermal program, power requirements, and data output for gas chromatograph No. 1. Figure 5 shows the external configuration and dimensions for this gas chromatograph.

6.2.2 GAS CHROMATOGRAPH NO. 2

This gas chromatograph is used in experiment 18 to detect amino acids by pyrolyzing a soil sample and analyzing the evolved gases. This instrument differs from the other gas chromatographs in that it utilizes a solid soil sample and does not use the mass spectrometer as a detector in conjunction with the argon β-ionization detector. The column packing for this column consists of 15 percent Apiezon L, 4.5 percent Carbowax 20M, and 3 percent phosphoric acid on a chromosorb support. The block diagram in Figure 6 shows the elements of this system. The external configuration and size are shown in Figure 7. The internal configuration of the column and column oven is the same as was described for gas chromotograph No. 1. The weight is summarized in Table V.

Gas Chromatograph No. 3, No. 4, and No. 5.

These gas chromatographs are identical from an engineering and configurational viewpoint. The column packings are listed in Table VI for these gas chromatographs as well as the experiment in which it is used.

The block diagram in Figure 8 shows the elements of the analysis system. It is seen that the mass spectrometer is used in conjunction with the argon β-ionization detector. It should be noted here that gas chromatographs Nos. 2 through 5 use a dual column. The same packing is used in each column one of which is used to perform the analysis and the other is used as a reference. The purpose in this configuration is to reduce base line drift by reading the differential output of the detectors. Drift can be caused by column bleeding, vaporization, and polymerization of the liquid phase. The extra column also provides redundancy in the event that the analysis column fails. It can be used to perform the same analysis at some reduction in data reliability.

The external configuration and size for gas chromatographs Nos. 3, 4, and 5 are shown in Figure 9. The weight for these gas chromatographs are summarized in Table VII.

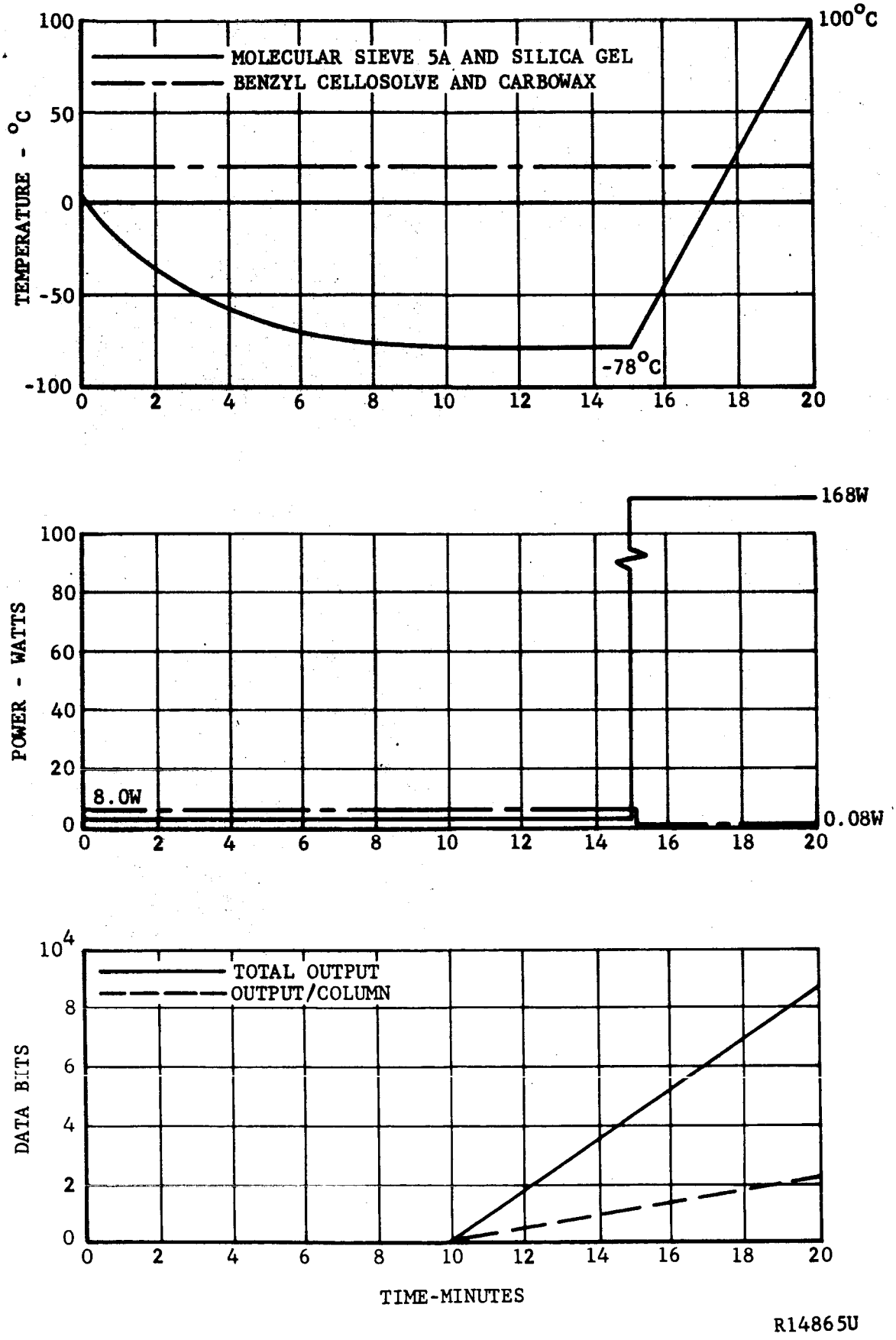


FIGURE 4. GAS CHROMATOGRAPH NO. 1 CHARACTERISTICS

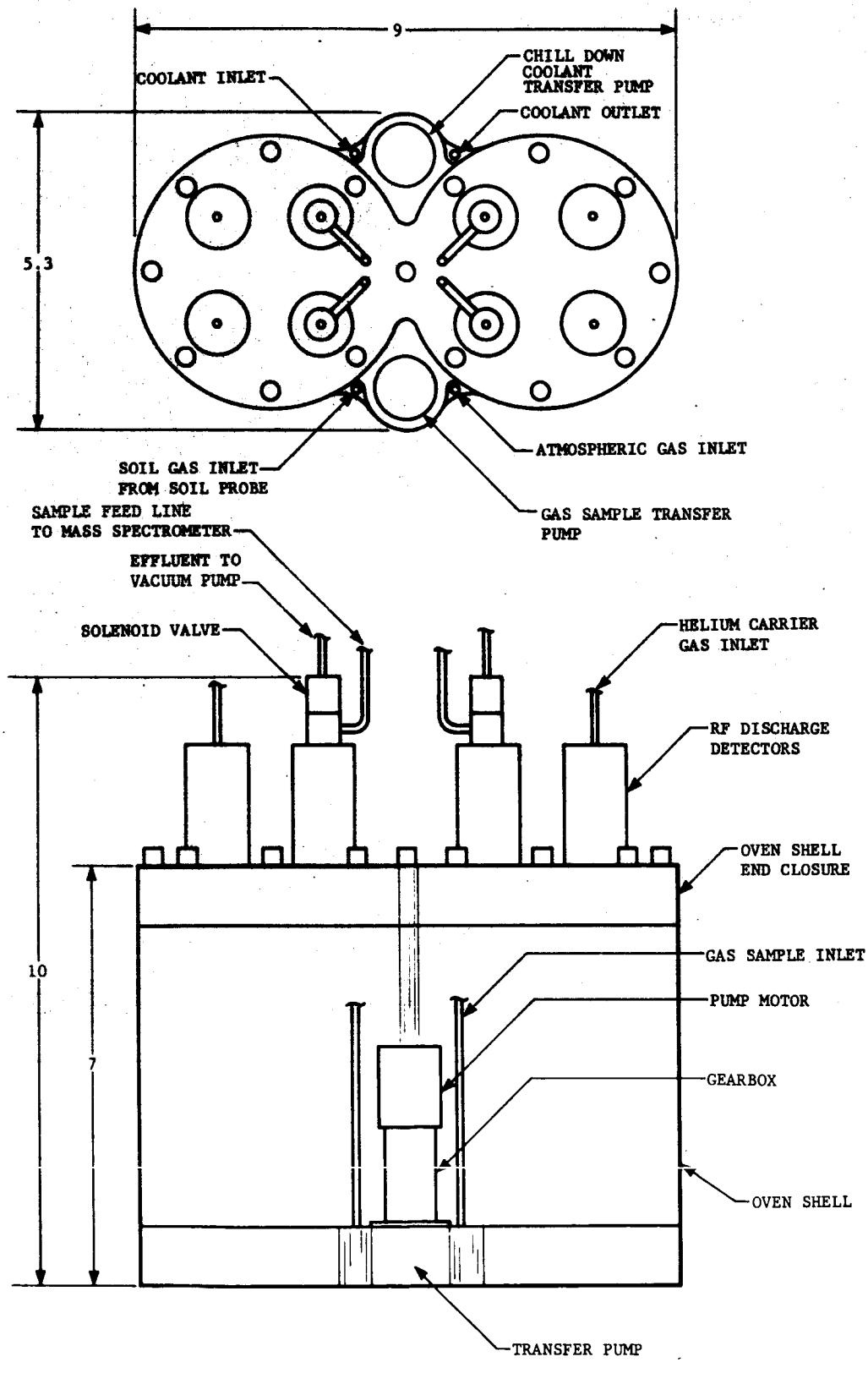


FIGURE 5. CONFIGURATIONAL ENVELOPE GAS CHROMATOGRAPH NO. 1

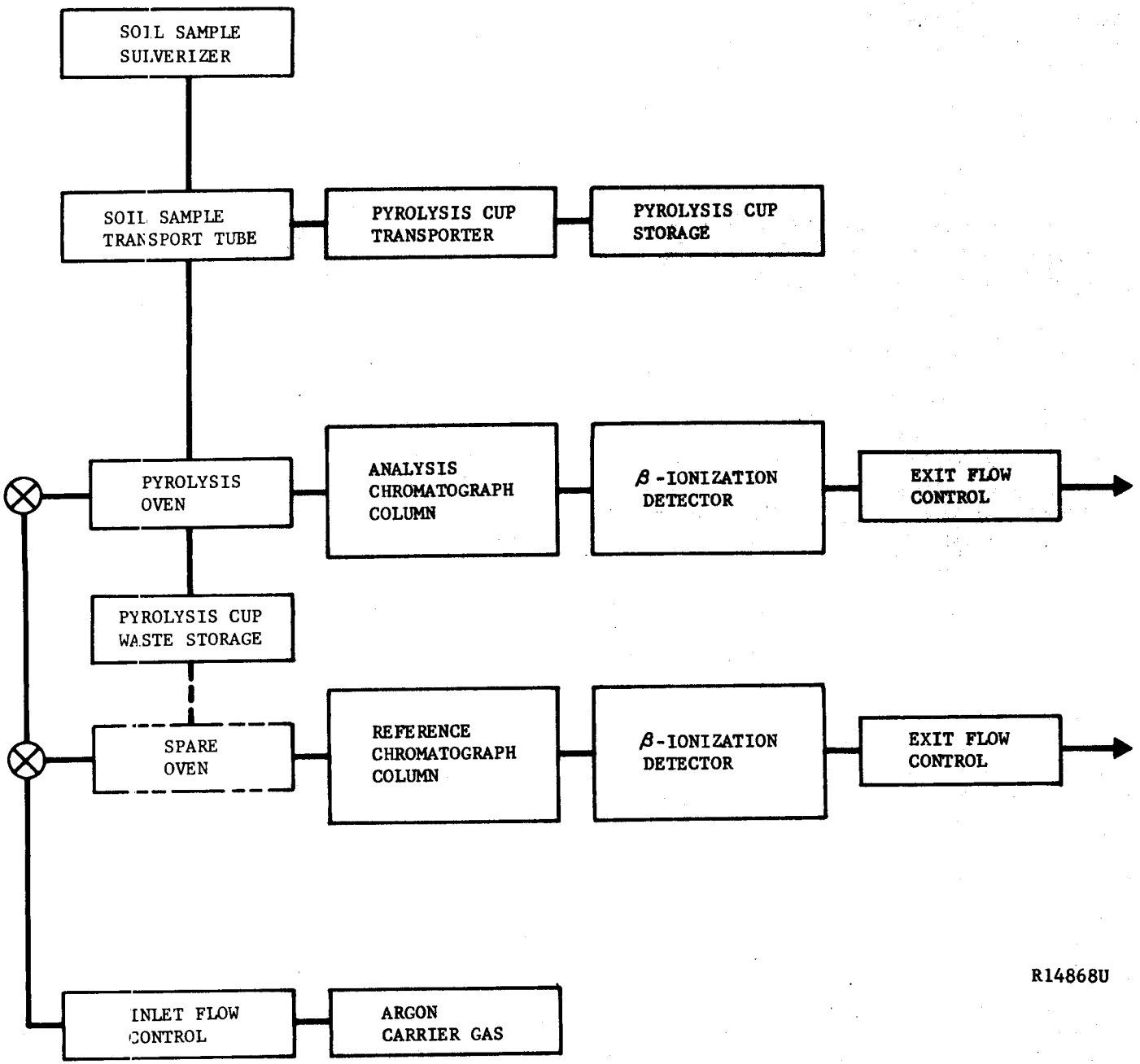
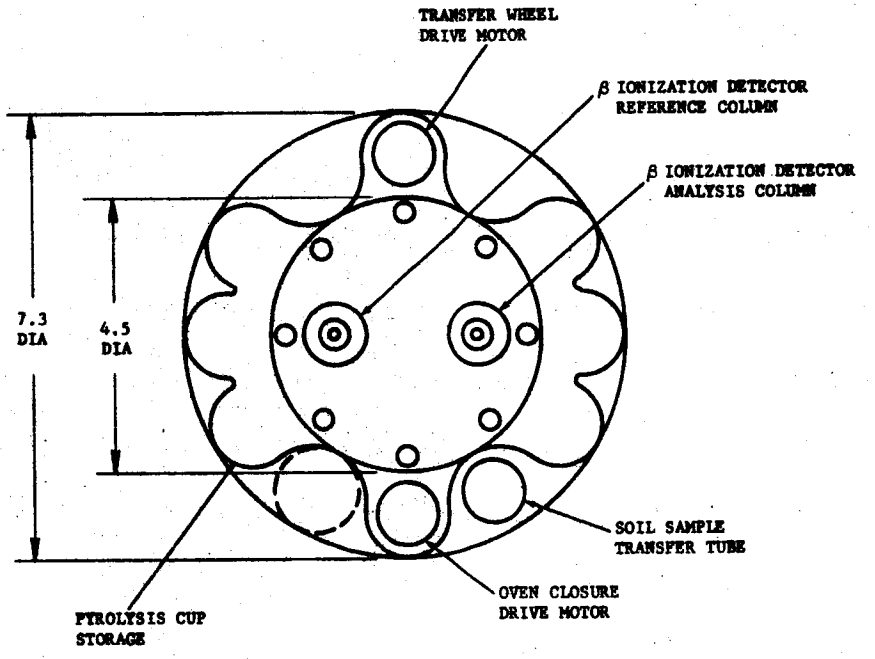


FIGURE 6. BLOCK DIAGRAM CHROMATOGRAPH NO. 2 ANALYSIS SYSTEM



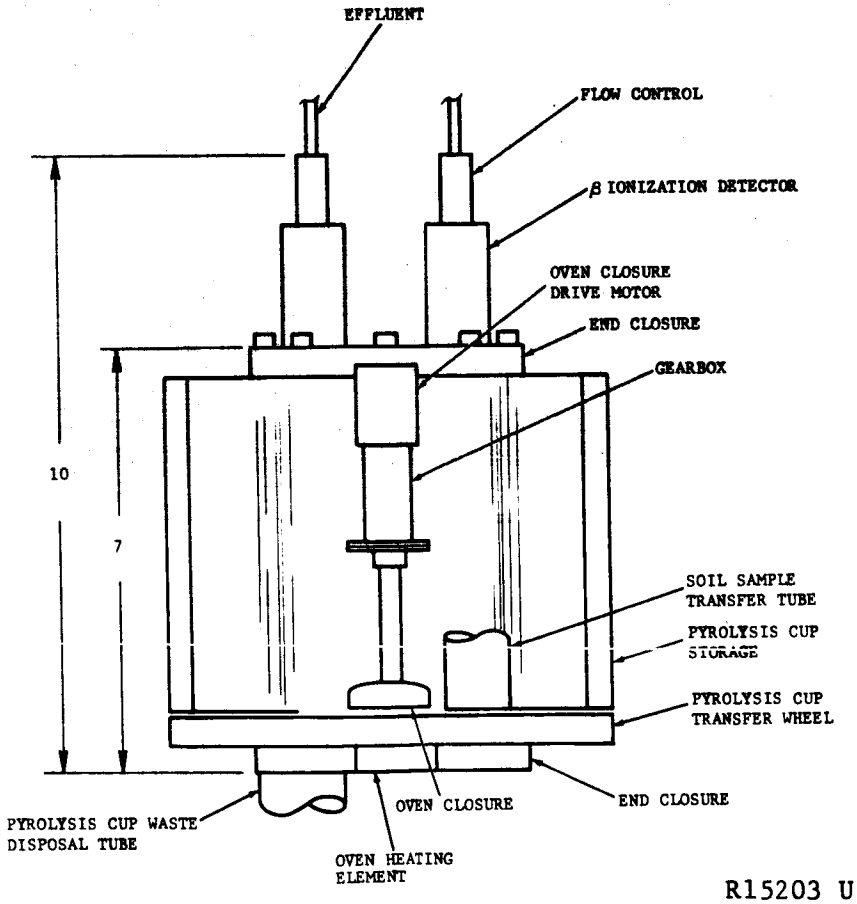


FIGURE 7. CONFIGURATIONAL ENVELOPE GAS CHROMATOGRAPH NO. 2

TABLE V

WEIGHT SUMMARY - GAS CHROMATOGRAPH NO. 2

Item	Weight (lb)
Copper heat distributor	0.891
Heat transfer fluid	0.070
Inlet pressure regulator	0.400
Column tubing	0.081
Column packing	0.083
Insulation	2.630
Outer shell	0.374
Detectors	0.124
Exit flow control	0.100
Pyrolysis oven cap and motor	1.300
Transfer wheel drive motor	1.100
Transfer wheel	0.250
Pyrolysis cup storage	0.160
Pyrolysis cups (66)	0.070
Total	7.733

TABLE VI

COLUMN PACKINGS FOR GAS CHROMATOGRAPHS NOS. 3, 4, AND 5

Gas Chromatograph	Packing	Experiment
No. 3	SE-30 Silicone polymer on a Chromosorb W support	22
No. 4	Acid washed glass bead support coated with 0.25 percent Carbowax and 4 percent isophthalic acid solution	23
No. 5	15 percent Apiezon L, 4.5 percent Carbowax 20M, and 3 percent phosphoric acid on a chromosorb support.	19 and 27

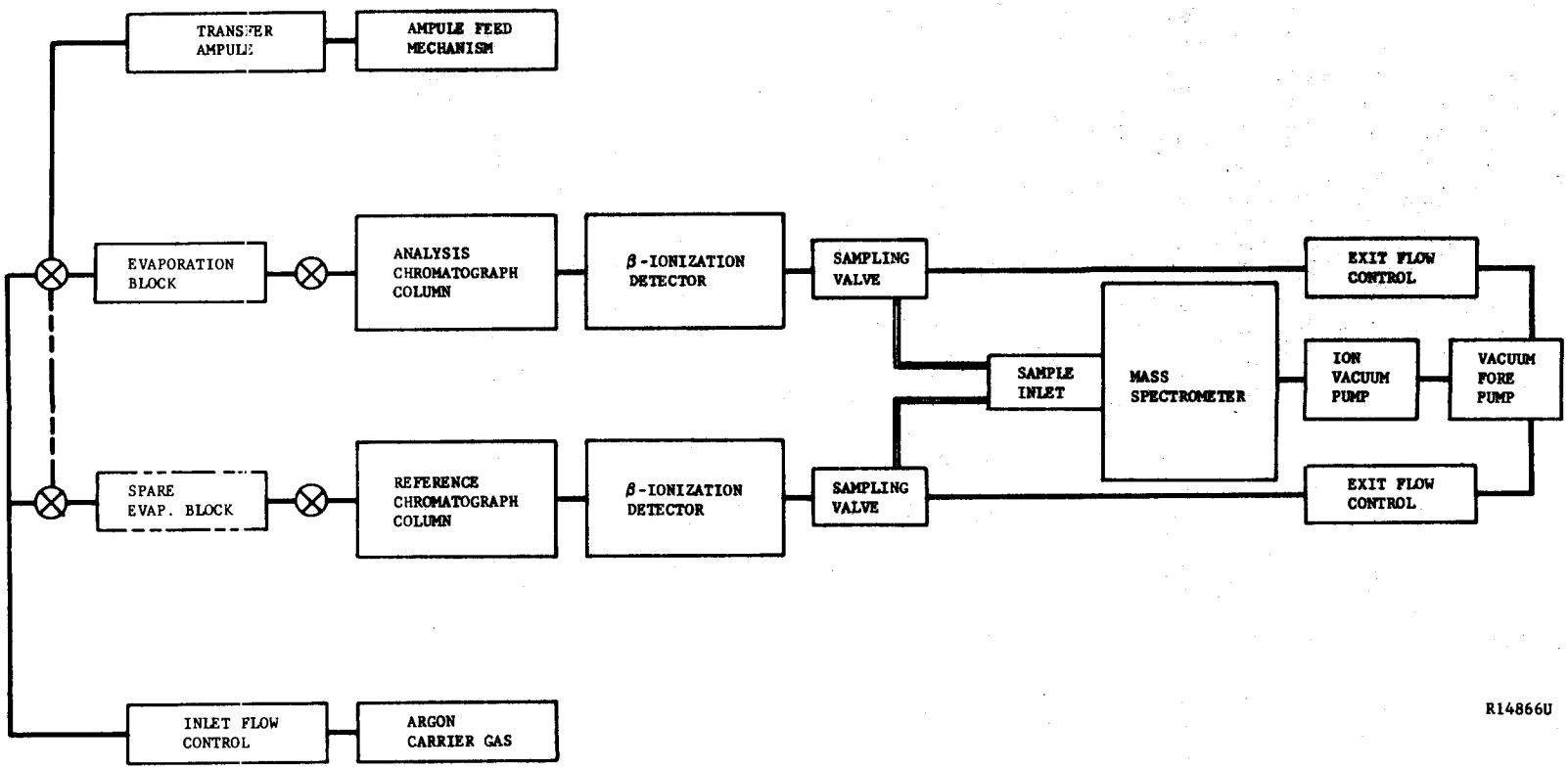


FIGURE 8. BLOCK DIAGRAM - GAS CHROMATOGRAPH NO. 3, NO. 4 AND NO. 5 ANALYSIS SYSTEM

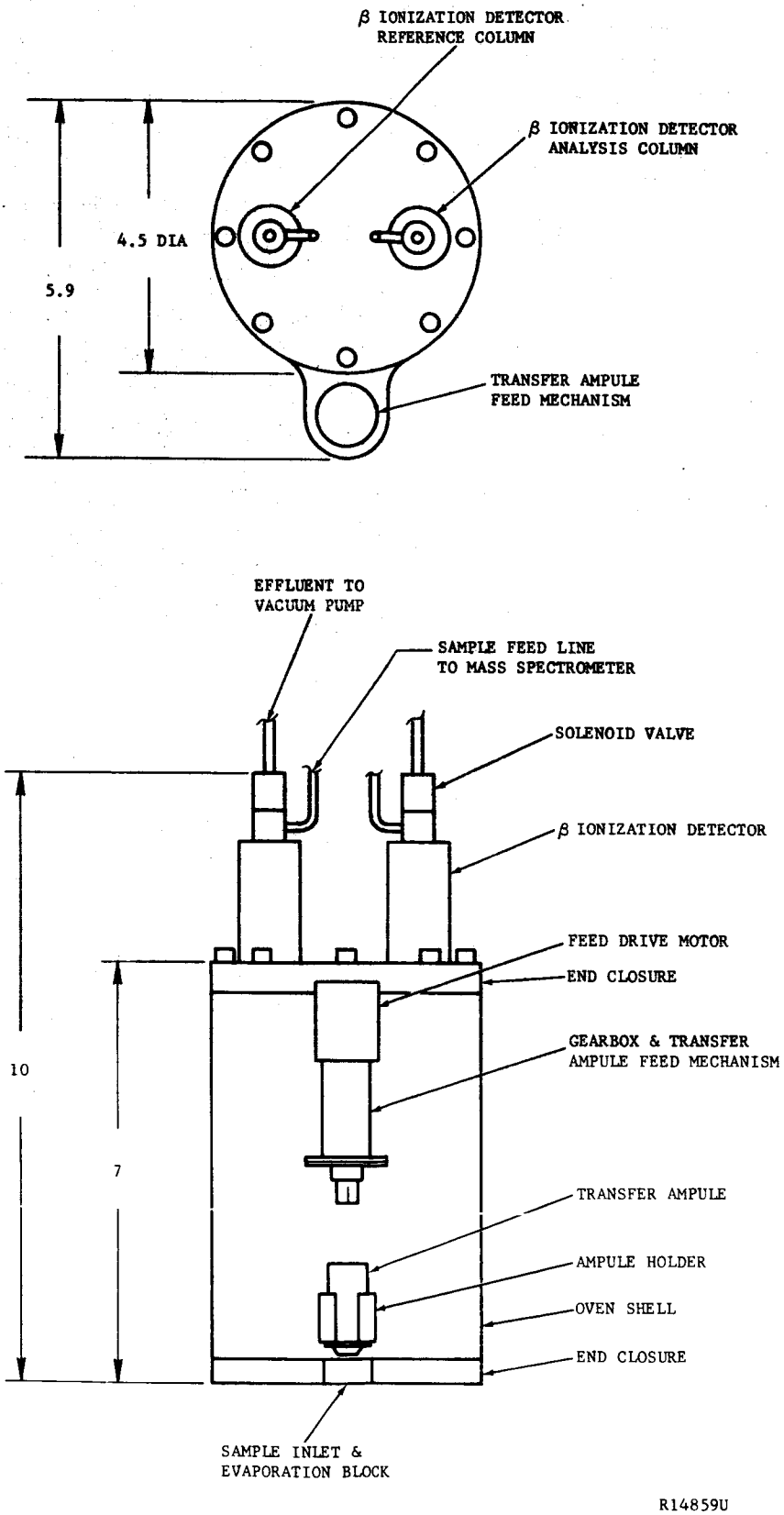


FIGURE 9. CONFIGURATIONAL ENVELOPE GAS CHROMATOGRAPH NO. 3, NO. 4 AND NO. 5

TABLE VII

WEIGHT SUMMARY - GAS CHROMATOGRAPHS NOS. 3, 4, and 5

Item	Weight (lb)
Copper heat distributor	0.891
Heat transfer fluid	0.070
Inlet pressure regulator	0.400
Column tubing	0.081
Column packing	0.083
Insulation	2.630
Outer shell	0.374
Detectors	0.124
Exit flow control	0.100
Transfer ampule feed mechanism	1.300
Total	6.453

The carrier gas requirements are estimated for gas chromatographs Nos. 2, 3, 4, and 5 as follows:

(1) Column exit pressure 10 psia

(2) Flow rate 50 ml/min (0.00177 ft^3/min)

(3) Column temperature 20°C (538°R) nominal

(4) Argon carrier gas.

The flow density is given by:

$$\rho = \frac{p}{RT} = \frac{(10)(144)}{(38.7)(538)} = 0.071 \text{ lb/ft}^3.$$

The flow rate per minute per column is

$$\dot{W} = (0.071)(0.00177) = 1.26 \times 10^{-4} \text{ lb.}$$

The total amount of argon consumed per 30 minute cycle is

$$W = (1.26 \times 10^{-4})(30) = 3.78 \times 10^{-3} \text{ lb.}$$

Each chromatograph uses a reference column which doubles the above weight. Then $W = 7.56 \times 10^{-3}$ lb/cycle.

The number of use cycles for each chromatograph are estimated as shown in Table VIII.

TABLE VIII

ESTIMATED USE OF GAS CHROMATOGRAPHS NOS. 3, 4, AND 5

Experiment No.	Gas Chromatograph No.	Experimental Cycles	Cycles/ Experiment	Total Use Cycles
18	2	60	1	60
19	5	60	1	60
22	3	84	1	84
23	4	84	2	168
27	5	60	1	60
			Total	372

Allowing 2 additional calibration cycles per season for each gas chromatograph, the total is then 372 + (8)(4) = 404 cycles, which results in a total weight of argon carrier gas of

$$W = (404)(7.56 \times 10^{-3}) = 3.06 \text{ lb.}$$

Assume argon storage at 7000 psia at 145°C (753°R).

The density is $\rho = \frac{(7000)(144)}{(38.7)(753)} = 34.6 \text{ lb/ft}^3$

and the total volume required is

$$V = \frac{W}{\rho} = \frac{3.06}{34.6} = 0.0885 \text{ ft}^3 \ (153 \text{ in.}^3).$$

Two types of tank storage can be used, either spherical or torroidal. Assuming two tanks for redundancy, the sizes of these tanks are given in Table IX with their normal ambient pressure and tank weights.

TABLE IX

ESTIMATED STORAGE TANK REQUIREMENTS FOR CARRIER GAS

Tank Type	Tank Dimension (inches)	Tank Weight (pounds)	Normal Pressure (psi)	Maximum Pressure (psi)
Spherical	5.3ID	0.92	4650	7000
Toroidal	2ID tube x 8ID	1.33	4650	7000

A temperature program for gas chromatographs Nos. 2 through 5 is assumed to be a linear variation from 4°C to 250°C during the 30 minutes of the analysis. This results in a heating rate of 8.2°C (14.8°F) per minute from which $\dot{Q}$ = (0.15)(14.8) = 2.22 Btu per minute or an electrical power of 39 watts. Gas chromatograph No. 2 has an additional requirement to pyrolyze the soil sample which has been determined to require 400 watts for one minute.

Data output is estimated in the same manner as for gas chromatograph No. 1. Sampling at the rate of one point every 5 seconds results in 360 data points. Assuming the dynamic range of 4 decades to one percent accuracy results in 21 bits per data point allowing 10 percent excess for error checks and losses in transmission.

The total data output for these gas chromatographs is then 7550 bits. Figure 10 summarizes the thermal program, power requirements, and data output for gas chromatographs Nos. 2 through 5.

6.2.3 REFERENCES

1. Gas Chromatography, Littlewood, A. B., Academic Press, 1962.

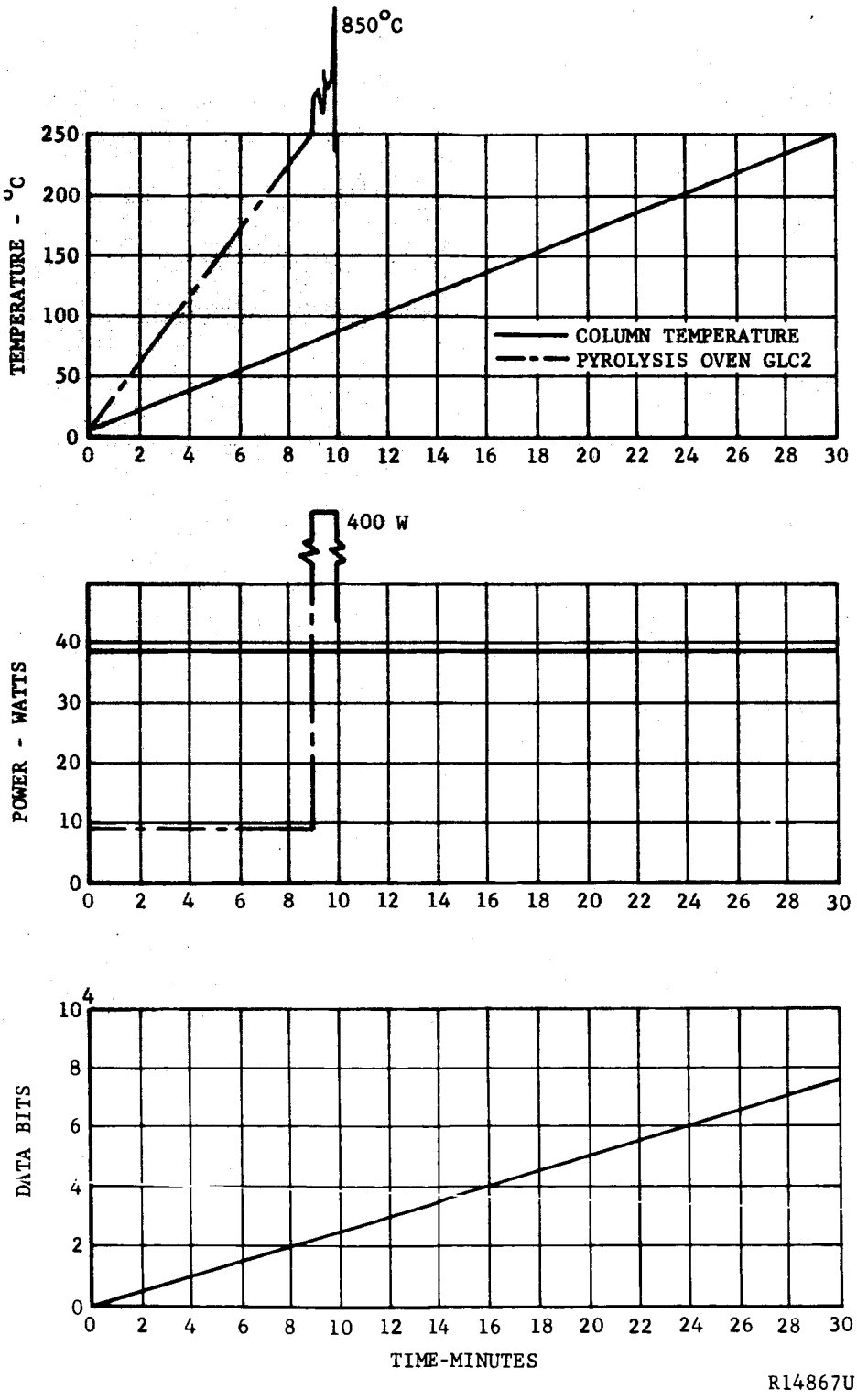


FIGURE 10. GAS CHROMATOGRAPH NO. 2 THROUGH 5 CHARACTERISTICS

6.3 DESIGN CONCEPT FOR ABL WEIGHT SCALE

6.3.1 DESIGN CRITERIA

(1) Weight range - 1 mg to 10 grams.

(2) Accuracy - 10 percent of 1 mg.

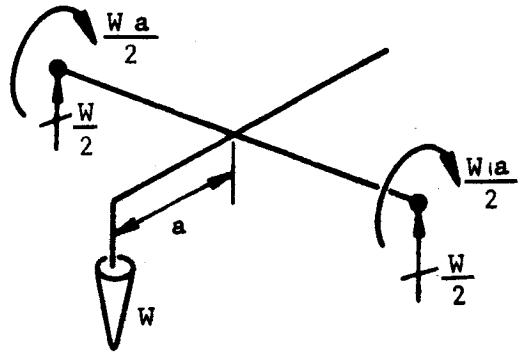
(3) Components capable of sterilization cycles.

(4) Capable of withstanding 500 g landing impact.

6.3.2 DESIGN PHILOSOPHY

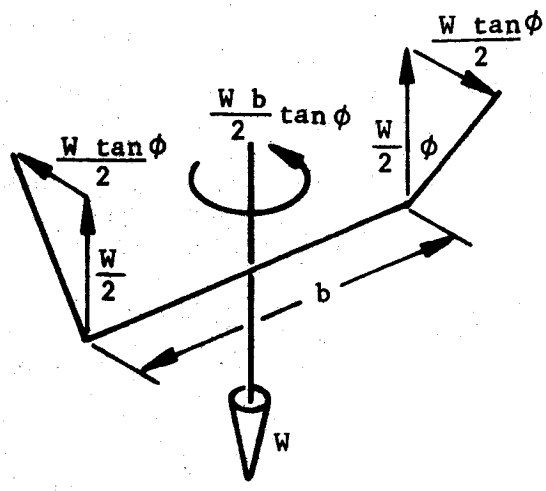
A number of beam balance techniques were considered, each having the basic disadvantages of requiring delicate knife edge supports, and requiring variable geometry to accommodate the wide range of weights to be evaluated. It was felt that some technique of utilizing the torsional deflection of a solid shaft would allow the use of simple and accurate support techniques, and had the capability of providing measurements over a broad range of deflections.

Initially the torsional system shown schematically below was considered.



Torsional deflections were calculated as a function of torsion bar length and weight moment arm. It was found that to maintain reasonable arm deflections, the arm length or the torsion bar length had to be variable during use. It was felt that this was an unnecessary and unreliable complication.

Finally, a torsion reaction system was examined, using an approach shown schematically below.



The weight to be measured is placed in a pan at the center of the arm. The weight is reacted by canted wires at the arm extremity. The horizontal reactions are equal in magnitude, and because of the cant produce horizontal components of equal magnitude resulting in a pure torsional moment. If a single vertical torsion bar is employed it will provide the stabilizing torque by deflecting torsionally. This deflection can be measured to calibrate the force required to produce such a deflection.

6.3.3 ANALYSIS

a. Torsional Deflection. The torsional deflection of a solid shaft is defined by:

$$\theta = \frac{M_t \ell}{G I_p}$$

where

θ = deflection in radians

M_t = applied moment; inch lb

G = modulus of rigidity, psi

I_p = polar moment of inertia, $in.^4$

$= \frac{\pi d^4}{32}$, for a solid circular shaft

$$\theta = 10.2 \frac{M_t \ell}{G d^4}$$

To minimize the variables under examination, the material was selected as 4340 steel. Hence,

$G = 11.5 \times 10^6$ psi

The equation for torsional deflection was presented graphically by separating it into two steps, thus

$$(1) \quad K_1 = \frac{M_t}{d_4} \quad \text{for} \quad 10^{-6} < M_t < 10^{0} \text{ inch lb}$$

$$0.001 < d < 0.10 \text{ inch}$$

and

$$(2) \quad \theta = \frac{10.2 \ \ell K_1}{G}$$

$$\theta = 0.887 \times 10^{-6} \ell K_1$$

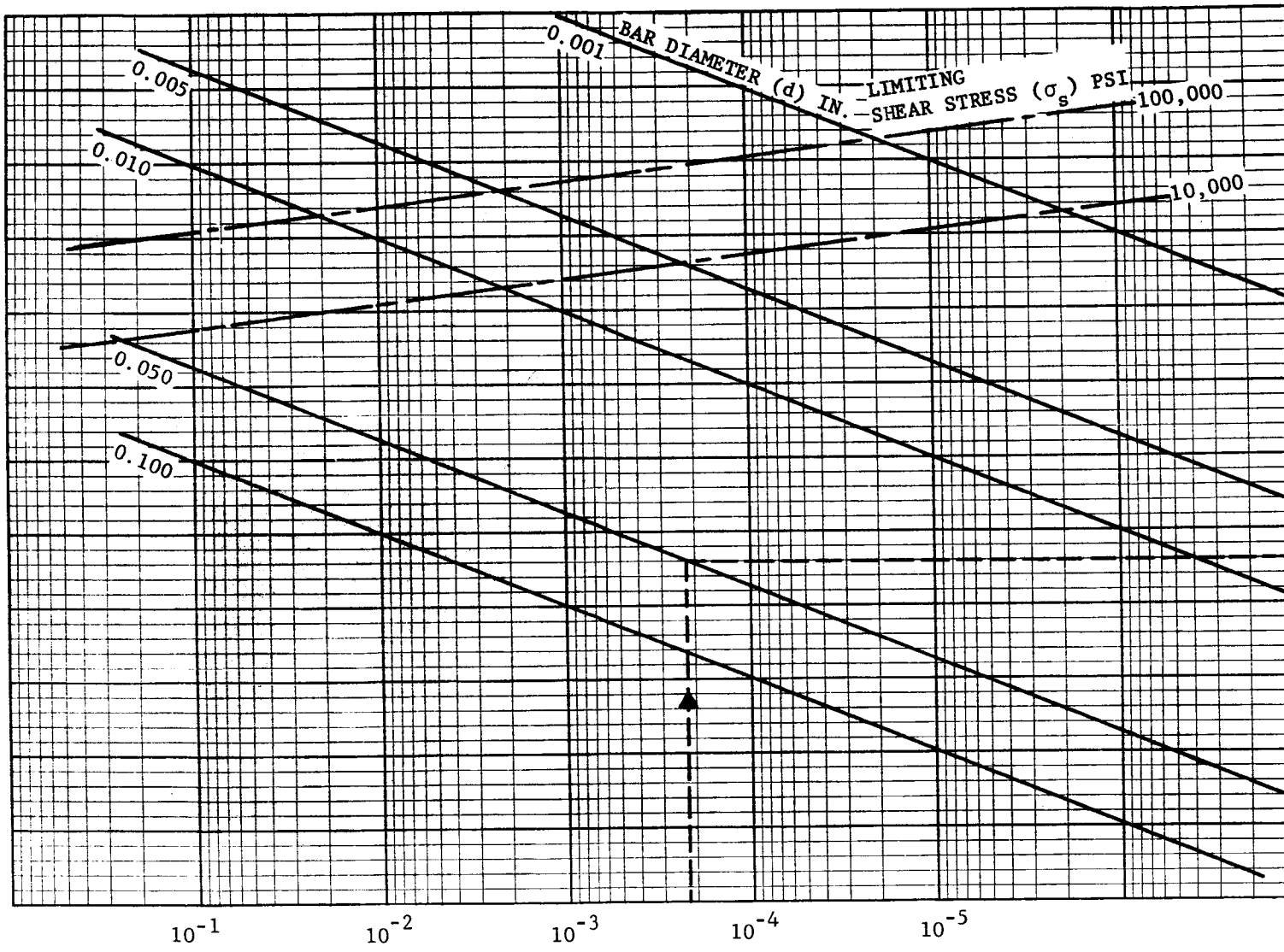
These data are presented graphically in Figure 11, along with a sample solution.

b. Torsional Shear Stress Level. To ensure that the geometric constraints for any range of interest do not result in excessive stress levels, the limiting torsional shear stress in terms of the variables of Figure 11 was defined.

$$\sigma = \frac{16 M_t}{\pi d^3}$$

$$\sigma_{allow} = \frac{16 M_{t_{max}}}{\pi d^3}$$

$$M_{t_{max}} = \frac{\pi}{16} \sigma_A d^3$$


BAR DIAMETER (d) IN.
LIMITING SHEAR STRESS (σ_s) PSI
0.001
0.005
0.010
0.050
0.100
100,000
10,000
10^{-1}
10^{-2}
10^{-3}
10^{-4}
10^{-5}
TORSIONAL MOMENT (M_t) INCH-POUNDS

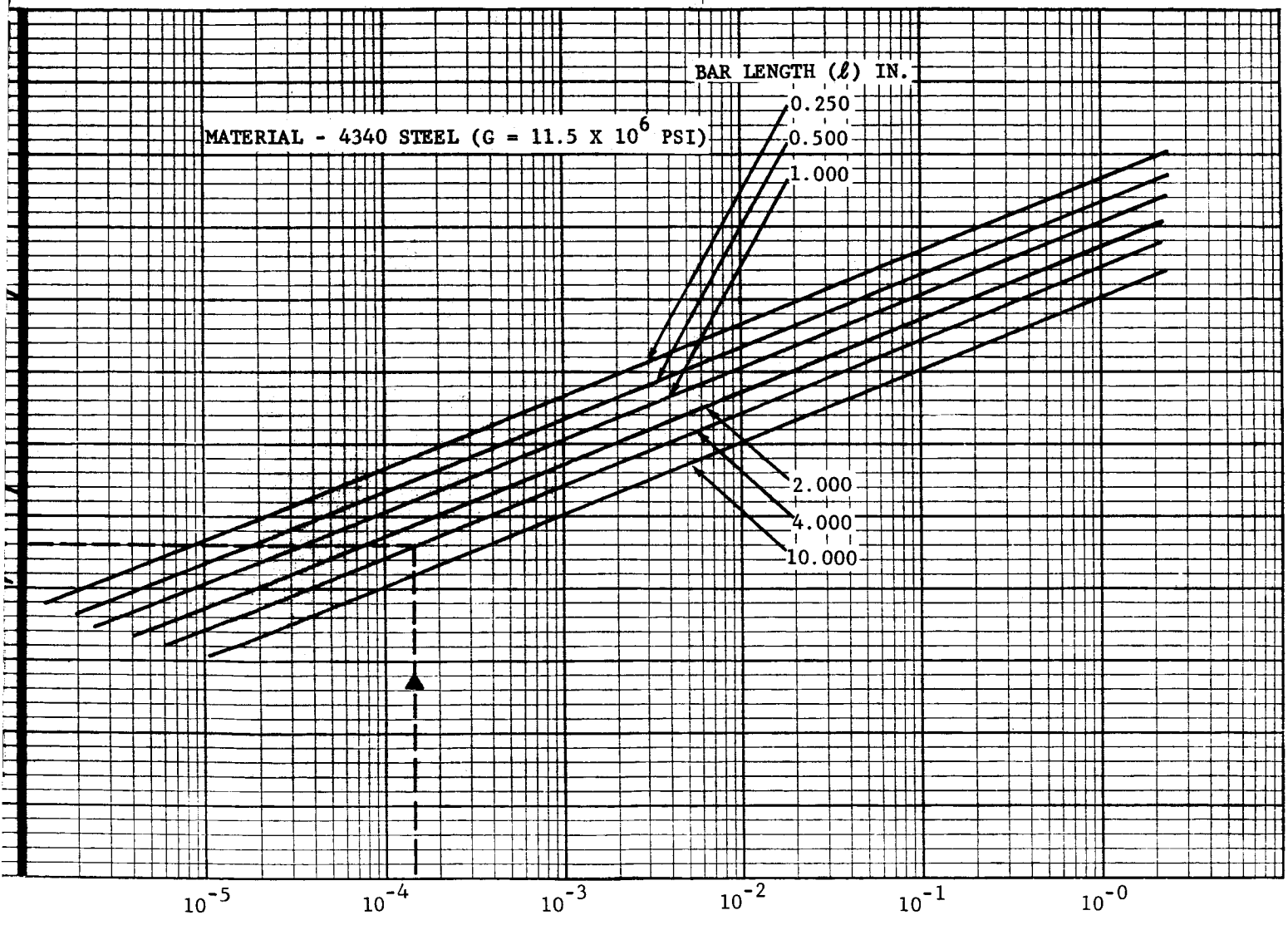


FIGURE 11. TORSIONAL DEFLECTION OF A SOLID CIRCULAR BAR AS A FUNCTION OF MOMENT, DIAMETER AND LENGTH

The allowable shear stress was defined as follows:

(1) To guarantee the repeatibility of the measurements, the geometry of the scale would be such that the stress level would never exceed 75 percent of the proportional limit in shear.

(2) The proportional limit in shear is expressed by:

$$F_{sp} = 0.550\ F_{tp}$$

where

F_{tp} = proportional limit in tension.

$$\therefore\ \sigma_{allow} = 0.413\ F_{tp}$$

(3) Values of F_{tp} were conservatively approximated from the room temperature stress strain data presented in MIL-HDBK-5, for 4340 steel.

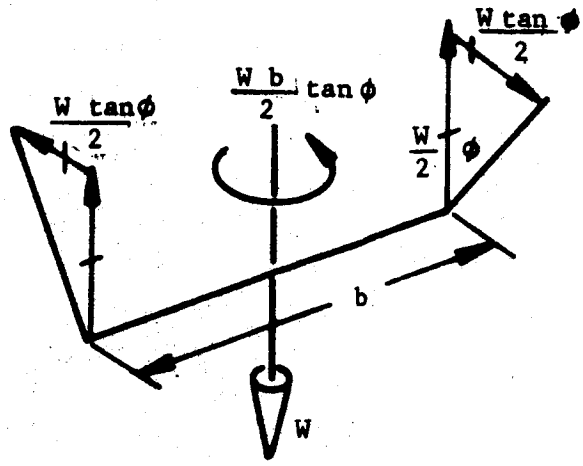
F_{tu}	F_{tp}	σ_{allow}
140,000	128,000	52,700
180,000	148,000	61,000
200,000	160,000	66,000

(4) Values of the maximum moment for 4340 steel, heat treated to 140,000 psi were calculated from:

$$M_{t_{max}} = 52{,}700\ \frac{\pi}{16}\ d^3 = 10{,}350\ d^3$$

These values are shown on Figure 11.

c. Torsional Moment Analysis. To enhance the use of Figure 11, the variation of torsional moment with scale geometry was investigated. The geometric approach in sketch form is shown on the following page.



Thus, the moment is a function of the weight applied, the scale arm length, and the support wire pulloff angle. Range of variables used were:

$2 \leq b \leq 10$ inches

$0 \leq \phi \leq 30$ degrees

$\omega = 1$ mg $= 2.21 \times 10^{-6}$ lb

The weight applied was selected at the lower value because the sensitivity of the scale was felt to be the most critical at that value.

The moment variation was calculated and is presented graphically in Figure 12.

6.3.4 DESIGN CONCEPT

In order to present a conceptual arrangement, and investigate the feasibility of system details, the scale geometry was arbitrarily selected as:

$\phi = 20^{\circ}$, $b = 6$ in.

From Figure 12, $M_t = 2.5 \times 10^{-6}$ in. lb

Assuming a torsion bar diameter of 0.032 inch and a length of 10 inches:

$\theta = 0.211 \times 10^{-4}$ radians $= 4.35$ minutes of arc.

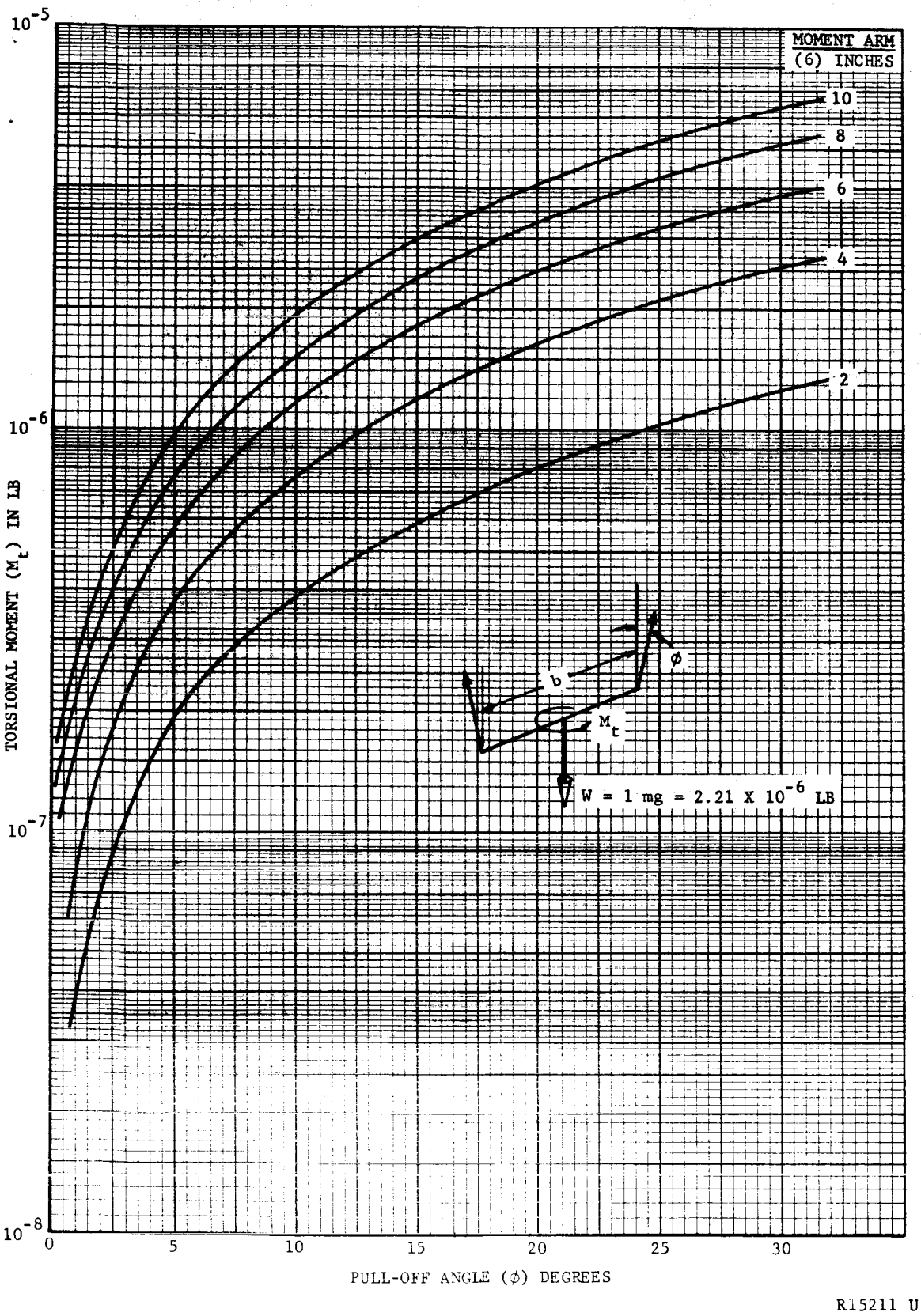


FIGURE 12. TORSIONAL MOMENT AS A FUNCTION OF SCALE GEOMETRY

The scale, with a 10 inch weight bar and 10 inch torque rod, could be reduced by decreasing the diameter of the weight bar and increasing the support wire pulloff angle. For $\phi = 45^{o}$, b = 2 in.

$$M_t = \frac{(2.21 \times 10^{-6})}{2} \quad (2)\ (1)$$

$$M_t = 2.21 \times 10^{-6} \text{ in.lb,}$$

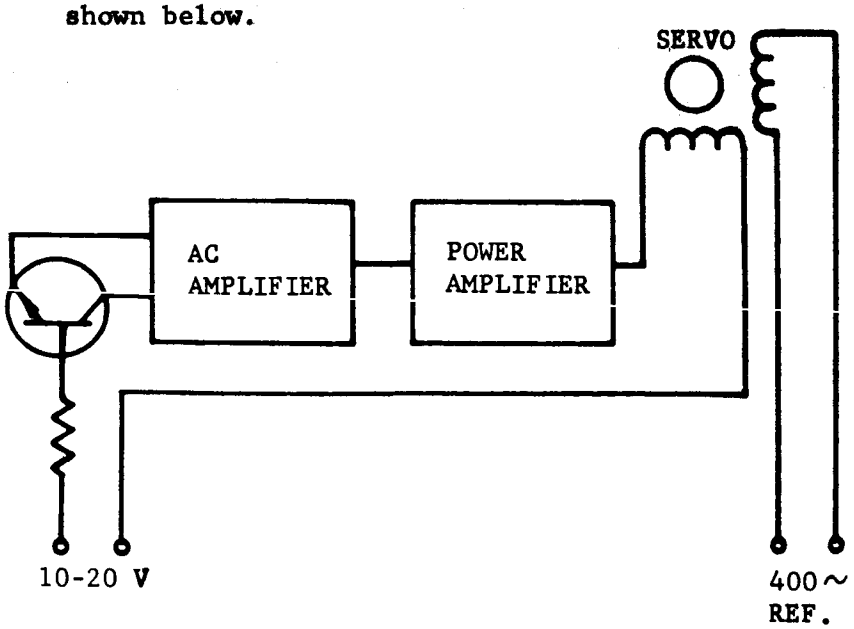
and

$$\theta = 0.187 \times 10^{-4} \text{ rad} = 3.70 \text{ seconds of arc.}$$

a. Deflection Sensing. The torsional deflection of the weight pan is calibrated as follows:

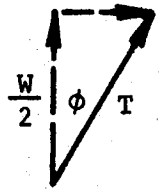
(1) A neon strobe, slaved to a 400 cycle ac power source, is directed toward a front surface mirror affixed to the reaction arm. The light is, in turn, reflected to a turning mirror, and directed at a bank of photo resistive detectors.

(2) The unbalanced voltage generated within the photocells drives a servomotor, supplied by the same 400 cycle source as the neon strobe. The function of the servo is to rotate the fixed end of the torsion bar to return the system to a null position. The servo shaft deflection read by a shaft encoder indicates the weight applied to the weight pan. A preliminary schematic is shown below.



b. Support Wire Sizing. The wire supporting the reaction arm was sized as follows:

(1) The tension in the wire was calculated for the 10 gram applied weight.



$$T = \frac{W}{2\cos\phi} = \frac{2.21 \times 10^{-2}}{(2)\ (0.707)}$$

$$T = 1.56 \times 10^{-2} \text{ lb}$$

(2) The maximum stress level was assumed to be 75 percent of the proportional limit in tension. For 4340 steel, heat treated to 140,000 psi,

$$F_A = 0.75\ (128{,}000) = 96{,}000 \text{ psi}$$

(3) The minimum wire diameter, then:

$$F_A = \frac{4T}{\pi d^2}$$

$$d = \sqrt{\frac{4T}{\pi F_A}} = \sqrt{\frac{(4)\ (1.56)\ (10^{-2})}{(\pi)\ (96{,}000)}} = \sqrt{20.4 \times 10^{-6}}$$

$$d = 0.0045 \text{ inch}$$

Number 36 gage wire is 0.005 inch in diameter.

c. Shaft Encoder Sensitivity Requirements. To determine the feasibility of using a shaft encoder to detect the small deflections involved at the lower range, a gearing system was investigated using commercially available sizes. A worm-worm gear combination was selected because it is self-locking under external load conditions. From the Pic Corporation catalog, a 64 pitch antibacklash worm-worm gear combination was selected with a worm gear pitch diameter of 1.875 inches, and 120 teeth.

(1) Axial pitch of the worm

$$P = \frac{D\pi}{n} = \frac{1.875\pi}{120}$$

$$P = 0.049 \text{ q in for both worm and worm gear}$$

(2) Axial rotation of worm - to sense a shaft rotation of 3.70 seconds of arc

$$a = (0.938)(0.187)(10^{-4}) = 0.176 \times 10^{-4} \text{ in.}$$

$$\therefore \text{ Angle of worm rotation } (\beta) = \frac{(0.176)\ (10^{-4})(2\pi)}{0.04\ q}$$

$$= 22.6 \times 10^{-4} \text{ radians}$$

$$\beta = 7.8 \text{ minutes of arc}$$

For the 10 g weight β = 22.6 radians

Several encoders in the literature have the capability of providing the sensitivity and accuracy. These deflections can be increased, however, by spur gearing the worm shaft between the worm gear and the encoder.

d. Conceptual Arrangement. The scale, as defined throughout this analysis, is shown in conceptual form in Figure 13. The solenoids shown are used to cage the reaction arm and weight pan throughout the mission until landing has been successfully achieved and the ABL experimental program has been initiated.

6.3.5 DYNAMICS OF THE PROPOSED TORSION BALANCE

a. Introduction. A preliminary design concept for an automatic rugged, analytical balance for use in planetary scientific exploration has been presented in the preceding paragraphs. This balance concept is based on torsion pendulum. The motions of the device under perturbation are those of a compound torsion pendulum. This section of Appendix 6 provides an analysis of pendulum oscillation frequencies as a function of damping, weight in the balance pan, and pendulum dimensions and geometry and contains a mechanical analysis of the proposed balance.

In summary, the principal results are:

(1) The frequency of oscillation is very sensitive to the diameter of the torsion bar.

(2) The frequency is proportional to the reciprocal of the square root of the applied weight.

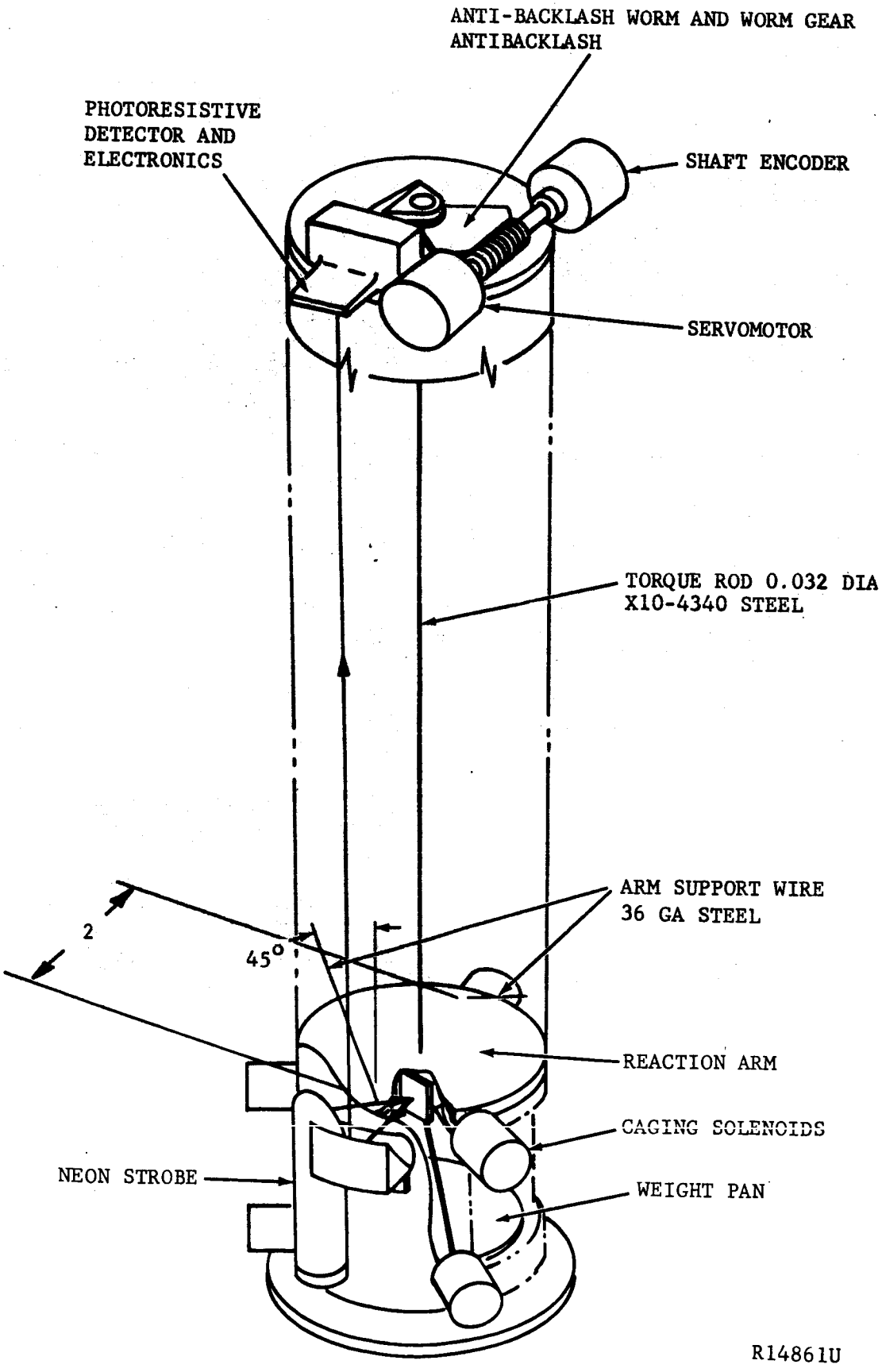


FIGURE 13. WEIGHT SCALE CONCEPT

(3) Damping also affects the fundamental frequency of oscillation. Further specification of the damping factor magnitude is required to evaluate its effects. Experimental determination of the air damping in a model device is warranted.

b. Normal Modes of Torsional Pendulum. Definitions of symbols used:

P - Period of oscillations ($2\pi/\omega$)

ω_o - 2π times the natural frequency

M_t - Applied moment

I_p - Polar moment of inertia of the oscillating system

The moment of inertia of a plane area with respect to an axis perpendicular to the plane is called the polar moment of inertia with respect to the point where the axis intersects the plane. If a case of circular cross section is considered, then(1)

$$I_p = \frac{\pi}{2}\left(\frac{d}{2}\right)^4 \qquad (1)$$

d - diameter of the cross section

G - magnitude of the shear modulus

G^* - complex shear modulus

I - moment of inertia of the oscillating system

N - number of oscillations about the equilibrium position

k - shape constant

$$k = \frac{I_p}{\ell} \qquad (2)$$

ℓ - length of the pendulum

α - attenuation constant or damp factor

j - $\sqrt{-1}$

δ - logarithmic decrement

$$\delta = \alpha_p \tag{3}$$

$$= \ell n\ (\theta_i / \theta_{i+1}) \tag{4}$$

θ - twist angle

P - period of oscillation

$$P = \frac{2\pi}{\omega} = \frac{1}{f} \tag{5}$$

The problem can be divided into three sections.

(1) Torsional Pendulum with Free Oscillation and No Damping. Using the general formulation of classical mechanics, the equation of motion of the torsional pendulum when the damping factor is set to zero, and when free oscillation is considered, may be written as:

$$I\ddot{\theta} + k\,G\,\theta = 0 \tag{6}$$

Let us assume that the angle of twist θ as a function of time has the form of:

$$\theta = \theta_o \exp (j\ \omega_o\ t) \tag{7}$$

where θ_o is the amplitude of the oscillation at $t = 0$. If at $t = 0$ it is assumed that the torsional pendulum is at equilibrium and the maximum twist angle is obtained at $t = t_o$, then, Equation (7) should be replaced by

$$\theta = \theta_o \exp \left[j\ \omega_o\ (t - t_o)\right] \tag{8}$$

In any case, substituting the solution into the equation of motion we obtain:

$$\dot{\theta} = j\ \omega_o \theta$$

$$\ddot{\theta} = -\omega_o^2 \theta$$

$$\therefore = I\ \omega_o^2 + k\,G = 0 \tag{9}$$

or

$$\omega_o = \left(\frac{k\,G}{I}\right)^{1/2} \tag{9-1}$$

$$= 2\pi f_o \tag{9-2}$$

Thus, the natural frequency of the torsional pendulum is given by:

$$f_o = \frac{1}{2\pi} \sqrt{\frac{k\,G}{I}} \tag{10}$$

Replacing the constants with their values obtained above, the natural frequency then is:

$$f_o = \frac{d^2}{2\pi} \sqrt{\frac{G\pi}{P\ell^2 \pi d^4 + 8\,Wb^2 \tan\phi}} \tag{11}$$

In the above equation the moment of inertia has been calculated for both the circular cylinder wire (I_1) and the pendulum pan which carried the weight W, defined by I_2. Thus,

$$I = I_1 + I_2$$

$$I_1 = mr^2 g$$

$$I_2 = 2\left(\frac{W}{2}\right)\left(\frac{b}{2}\right)^2$$

where rg is the radius of gyration of the wire and is equal to $d/2\sqrt{2}$, m is the mass of wire and $W/2 \tan\phi$ is the weight applied at each corner of the pan which has a length b.

$$I = \frac{P\ell\pi d^2}{4}\left(\frac{d}{2\sqrt{2}}\right)^2 + \frac{Wb^2}{4}\tan\phi$$

$$I = \frac{P\ell\pi d^4}{32} + \frac{Wb^2}{4}\tan\phi \tag{12}$$

Notice that for numerical calculation of f_o, the range of the diameter d has to be known accurately. This is because in the range of 1 mg to 10 gm, the value of d determines the approximate form of the natural frequency formula. In order to show this, let us choose the following two examples:

(a) $b = 6$ in.

$\ell = 10$ in.

$G = 1.15 \times 10^7$ psi

$P = 0.284$ lb in.$^{-3}$

$$\phi = 45^{o}$$

$$\omega = 1 \text{ mg } 2.2 \times 10^{-6} \text{ lb}$$

$$W = 0.01 \text{ in.}^{*}$$

Then, from Equation (11) we obtain

$$f_o \simeq \sqrt{\frac{1.8 \times 10^{-3}}{W}}$$. which is shown in Figure 14.

In the second example we choose 0.1 for the value of the diameter, then,

$$f_o = 0.18/\sqrt{W + 3.10 \times 10^{-6}} \qquad W \text{ in lb}$$

The maximum torsional deflection is defined by:

$$\theta_o = \frac{M_t \ell}{G I_p} \tag{13}$$

Thus, the time variation of the torsional pendulum, when free of damping, is given by:

$$\theta(t) = \frac{M_t \ell}{G I_p} \exp (j \omega_o t) \tag{14}$$

substituting for I_p, ω_o and M_t, which is given by

$$M_t = \frac{Wb}{2} \tan\phi \ , \tag{15}$$

in the Equation (14) we obtain:

$$\theta(t) = \frac{16 \text{ Wb } \ell \tan\phi}{G d^4 \pi} \exp \left[j \left(d^2 \sqrt{\frac{G\pi}{P \ell^2 \pi d^4 + 8 \ell Wb^2 \tan\phi}} \ t \right) \right] \tag{16}$$

---

* $f_o \simeq \frac{0.04}{\sqrt{W}}$ W in grams [Example 1]

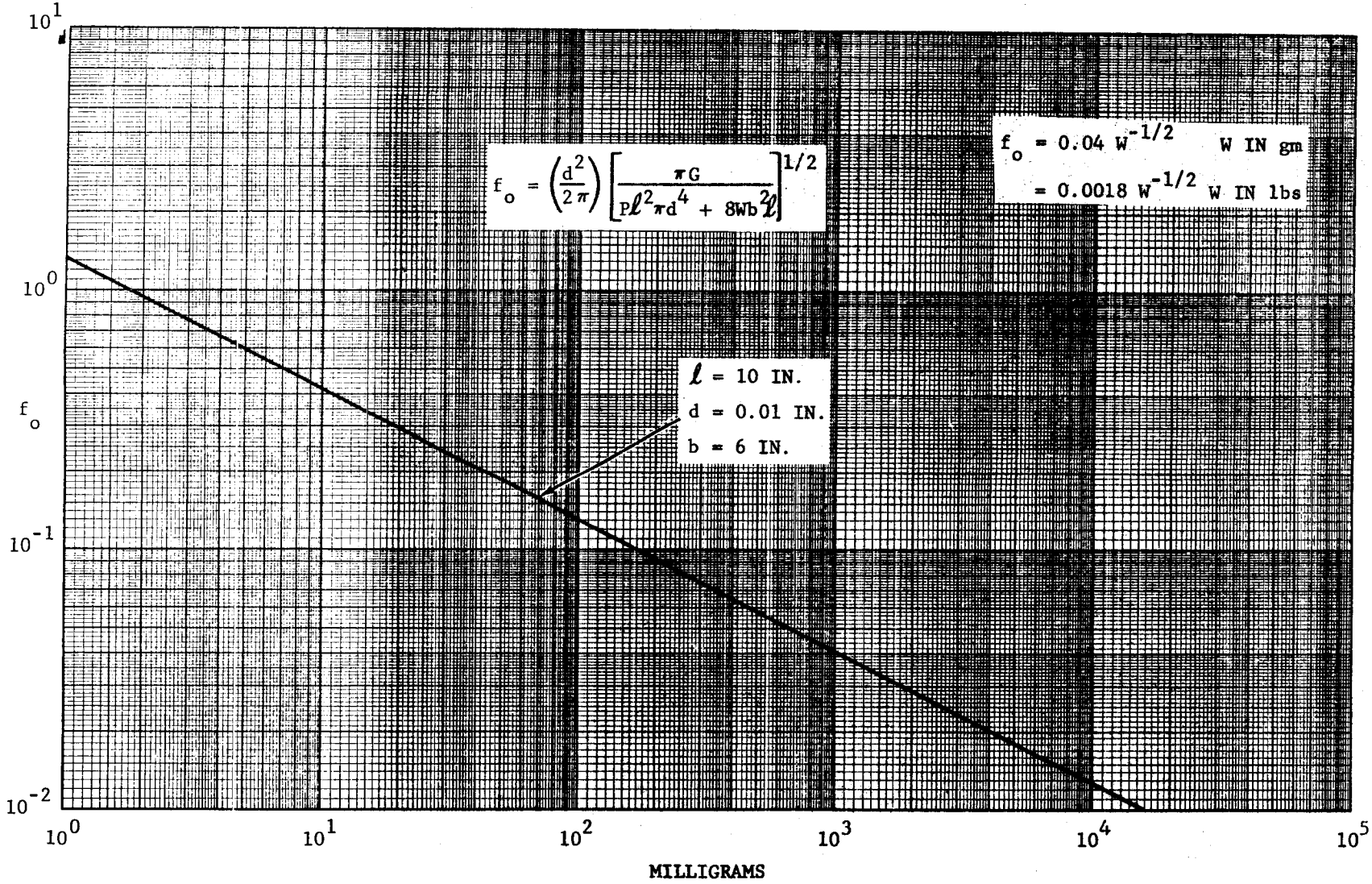


FIGURE 14. NATURAL FREQUENCY - PLOT OF EQUATION (11)

Equation (16) indicates that at a given time t, the twist angle is linearly proportional to W and is inversely proportional to the fourth power of the diameter.

(2) Oscillation With Attenuation. The general equation of motion of the freely oscillating torsional pendulum is given by the following differential equation:

$$\ddot{\theta} + 2\alpha\dot{\theta} + \omega_o^2\,\theta = 0 \tag{17}$$

when $\theta = \theta_o$ at $t = 0$, the solution is

$$\theta = \theta_o \exp\left[(j\omega - \alpha)\,t\right] \tag{18}$$

In order to obtain a relationship between ω and ω_o, we may replace Equation (17) above by the following equation:

$$I\,\ddot{\theta} + k\,G^*\,\theta = 0 \quad \text{where } G^* = (G' + I\,G'') \tag{19}$$

This equation is identical to Equation (6). In this case, the shear modulus is considered to be a complex number.

Substituting Equation (18) into Equation (19), we obtain:

$$(j\omega - \alpha)^2 + \frac{k}{I}\,G^* = 0 \tag{20}$$

Thus, by separating real and imaginary parts of the above equation, the following two equations will be obtained:

$$-\omega^2 + \alpha^2 + \frac{k}{I}\,G' = 0 \tag{21}$$

$$-2\alpha\omega + \frac{k}{I}\,G'' = 0 \tag{22}$$

where G' and G'' are the real and the imaginary parts of the shear modulus, respectively. Thus, the solutions for the damped frequency in terms of G' and G'' are

$$-\omega^2 = \alpha^2 - \frac{k}{I}\,G' \tag{23}$$

$$\omega = \frac{k}{2\alpha I}\,G'' \tag{24}$$

It should be noted that the sign of the left hand side of Equation (23) is taken (-) rather than (+). This is due to the argument given by N. N. Tschaegl.[2]

Eliminating ω in the above equations, we obtain a single equation relating G' and G'', namely:

$$G''^2 = \frac{4\alpha^2 I^2}{k^2}\left(\frac{k G'}{I} - \alpha^2\right) \tag{25}$$

The natural frequency ω_o, then, is

$$\omega_o^2 = \frac{k}{I} G = \frac{k}{I}\sqrt{G'^2 + G''^2}$$

$$= \frac{k}{I}\sqrt{G'^2 + \frac{4\alpha^2 I^2}{k^2}\left(\frac{k G'}{I} - \alpha^2\right)} \tag{26}$$

or

$$G'^2 + \frac{4\alpha^2 I}{k} G' - \frac{4\alpha^4 I^2}{k^2} - \frac{I^2 \omega_o^4}{k^2} = 0 \tag{27}$$

$$\therefore\ G' = \frac{2 I \alpha^2}{k}\left[\left(2 + \frac{\omega_o^4}{4\alpha_4}\right)^{1/2} - 1\right] \tag{28}$$

Notice that only the positive square root in the solution of Equation (27) is acceptable because the shear modulus must be a positive number. From this statement, we conclude that the restriction of $\omega_o > \alpha$ must also be satisfied.

Substituting this value of G' back into the Equation (23) we obtain

$$\omega^2 = 2\alpha^2\left[\left(2 + \frac{\omega_o^4}{4\alpha_4}\right)^{1/2} - 1\right] - \alpha^2 \tag{29}$$

This equation relates the damped frequency to the natural frequency ω_o and the attenuation factor α. Since ω_o is greater than α, therefore,

$$\omega^2 = \omega_o^2 - 3\,\alpha^2$$

or

$$\omega = \omega_o \left(1 - \frac{3\,\alpha^2}{\omega_o^2}\right)^{1/2} \tag{30}$$

On the other hand if $\omega_o^2 >> \alpha^2$, then, from Equation (28) we get:

$$G' = \frac{I\,\omega_o^2}{k} \tag{31}$$

and thus

$$\omega^2 = \omega_o^2 - \alpha^2$$

or

$$\omega = \omega_o \left(1 - \alpha^2/\omega_o^2\right)^{1/2} \tag{32}$$

Substituting for ω_o, from Equation (9), the oscillating frequency of the torsional pendulum may be given by

$$\omega = \sqrt{\frac{k\,G}{I} - \alpha^2} \tag{33}$$

$$= \sqrt{4\,\pi^2\,f_o^2 - \alpha^2}$$

$$= 2\,\pi f_o \sqrt{1 - \left(\frac{\alpha}{2\,\pi f_o}\right)^2} \tag{34}$$

where f_o is given in Equation (11).

The twist angle, then is:

$$\theta(t) = \frac{16\ Wb\ \ell \tan \phi}{G\ d^4 \pi} \exp\left[(j\,\omega - \alpha)\ t\right] \tag{35}$$

(3) Experimental Evaluation of the Damping Factor. The damping factor is given in Equations (3) and (4). Namely,

$$\delta = \alpha p$$

$$= \frac{\alpha}{f}$$

$$= \sqrt{\frac{2\pi\alpha}{\omega_o^2 - \alpha^2}} \qquad (36)$$

Thus, the logarithmic decrement must be measured experimentally. The amplitude measurement of δ is accomplished by the measurement of the twist angle after N oscillation. If θ_1 and θ_2 are the twist angles at t_1 and t_2, then,

$$\delta = \frac{1}{N} \ln (\theta_1/\theta_2) \qquad (37)$$

where N is the number of oscillations for the amplitude to decay from θ_1 to θ_2.

The velocity measurement of δ is also accomplished by considering the following boundary conditions.

Let us assume at $t = 0$, $v = v_o$ and at $t = np$, $v = v_n$ then

$$\delta = \frac{1}{n} \ln (v_o/v_n) \qquad (38)$$

If the times to traverse the distance 0 to S are t_o and t_n, then, by using average velocities over distance, the above equations become,

$$\delta = \frac{1}{n} \ln (t_n/t_o) \qquad (39)$$

for which a good approximation when δ is small is

$$\delta = \frac{1}{n} \frac{\Delta t}{t} \qquad (40)$$

where $\Delta t = t_n - t_o$ and $\bar{t} = 1/2\ (t_o + t_n)$. The accuracy of the approximation[3] is about 1 percent for values of t_n/t_o up to 1.5.

If a higher order term of the above approximation is desired, one can use the expanded form of the logarithmic decrement, that is,

$$\delta = \frac{1}{n} \frac{\Delta t}{t} \left(1 - \frac{\omega^2}{3} \bar{t}^2 + \cdots\right) \tag{41}$$

(4) Forced Oscillation. In the case of forced oscillation, the equation of motion is much more complex than above. The solution of such differential equations involves tensor analysis of the shear modulus.

A much more simplified version is the case of oscillation with the equation of motion of the following type:

$$I \ddot{\theta} + k G^* \theta = \Theta \exp (j \omega' t) \tag{42}$$

where ω' is the impressed radian frequency and Θ is the torque. The solution of this differential equation may be written as

$$\theta = \theta'_o \exp \left[j (\omega' t - \phi)\right] \tag{43}$$

The complete integral containing the transient solution cannot be derived from Equation (42).

6.3.6 REFERENCES

1. Timoshenko, Strength of Materials, page 420, 1955.

2. M. E. DeMorton, S. A. Lott and D. F. Stainsby, J. of Sci. Inst. 40, 441, 1963.

6.4 OPTICAL PROPERTIES OF TRANSPARENT AND REFLECTIVE MATERIALS

Three instruments of the ABL equipment complement utilize optical techniques operating at wavelengths outside the visible range. The instruments and the wavelengths at which they must function are summarized in Table X.

TABLE X

SUMMARY - SPECTRAL RANGE OF ABL INSTRUMENTS

Instrument		Wavelength
Macroimaging System	Macroimaging scan	250 mμ - 2μ
	IR radiometer	2μ - 14μ
	Solar isolation	250 mμ - 14μ
Infrared Spectrophotometer		
Spectral Analyzer		

The broad range of wavelengths handled by the instruments shown indicated that a careful appraisal of optical component materials was required. Preliminary optical train layout indicated that mirrors were required to restrict the size of the instrument. In addition it was necessary to determine whether mirrors or lenses offered the least attenuation in source intensity. Various attractive mirror and lens materials were investigated in order to evaluate and compare their properties.

Mirror materials noted for high reflectance in the low visible range (circa 350mμ) were examined to determine their performance in the far UV range required. Figures 15 through 19 indicate the reflectance characteristics variation with wavelength for several materials. It can be seen by comparing these data that solid aluminum maintain higher reflectance into the UV than any other material investigated. Aluminum does not exhibit the same high reflectance as gold in the visible and IR ranges, however, increased source intensity at those higher wavelengths is more readily supplied that in the ultraviolet.

Reflectance characteristics are based upon use of the equation

$$R = \frac{\left(n_o - n_1\right)^2 + k_1^2}{\left(n_o + n_1\right)^2 + k_1^2} \qquad (1)$$

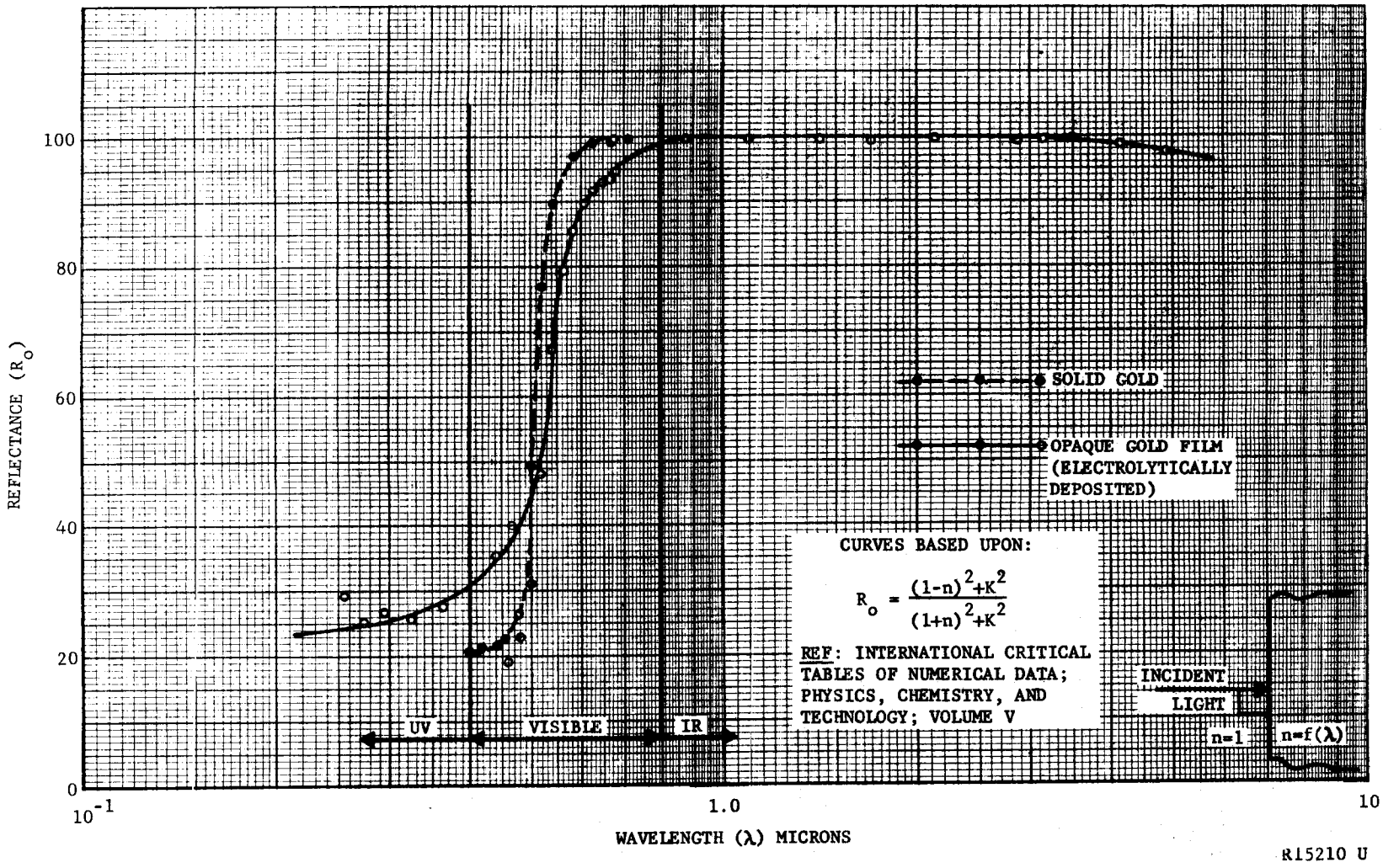


FIGURE 15. REFLECTANCE CHARACTERISTIC VARIATION WITH WAVELENGTH

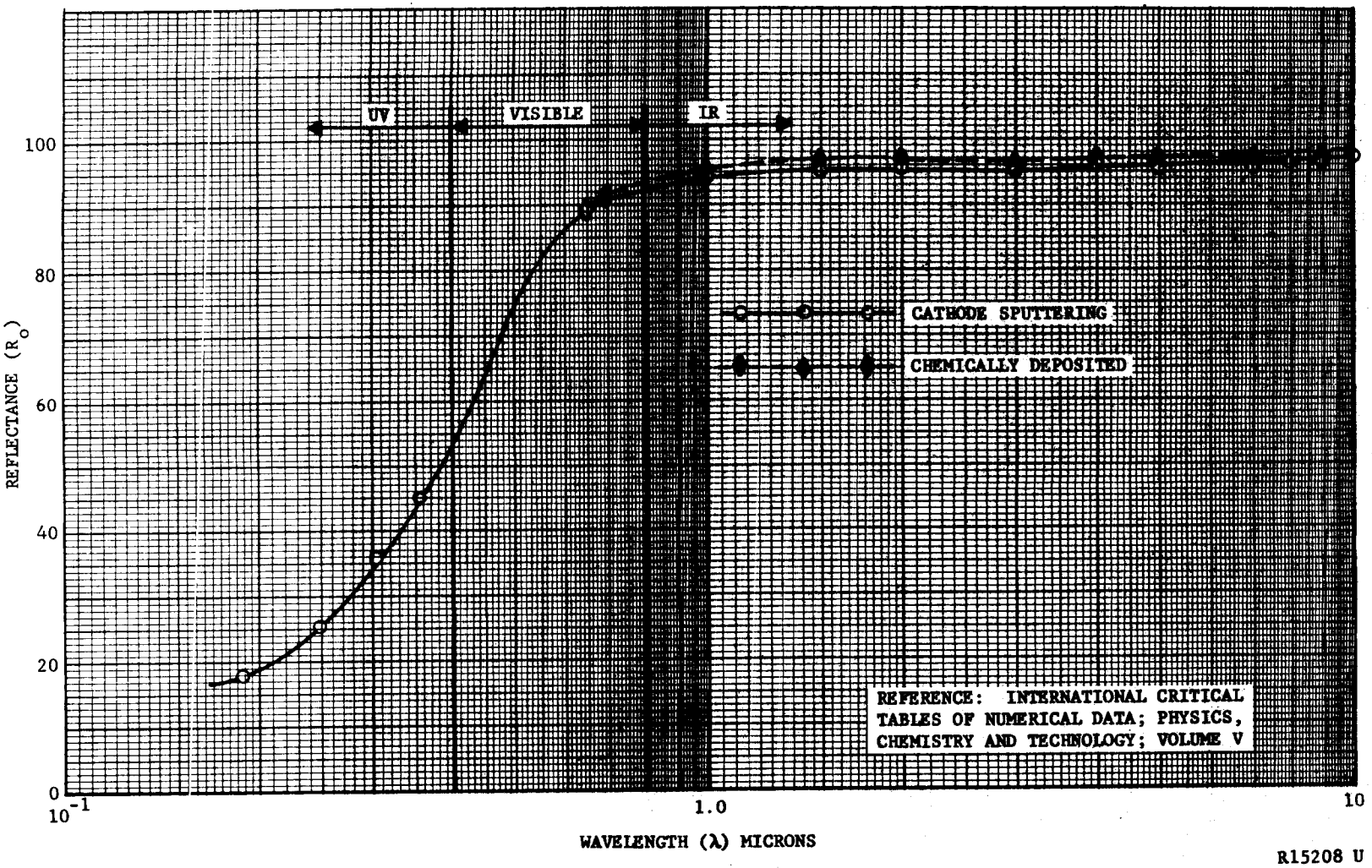


FIGURE 16. REFLECTANCE CHARACTERISTICS - OPAQUE GOLD FILM

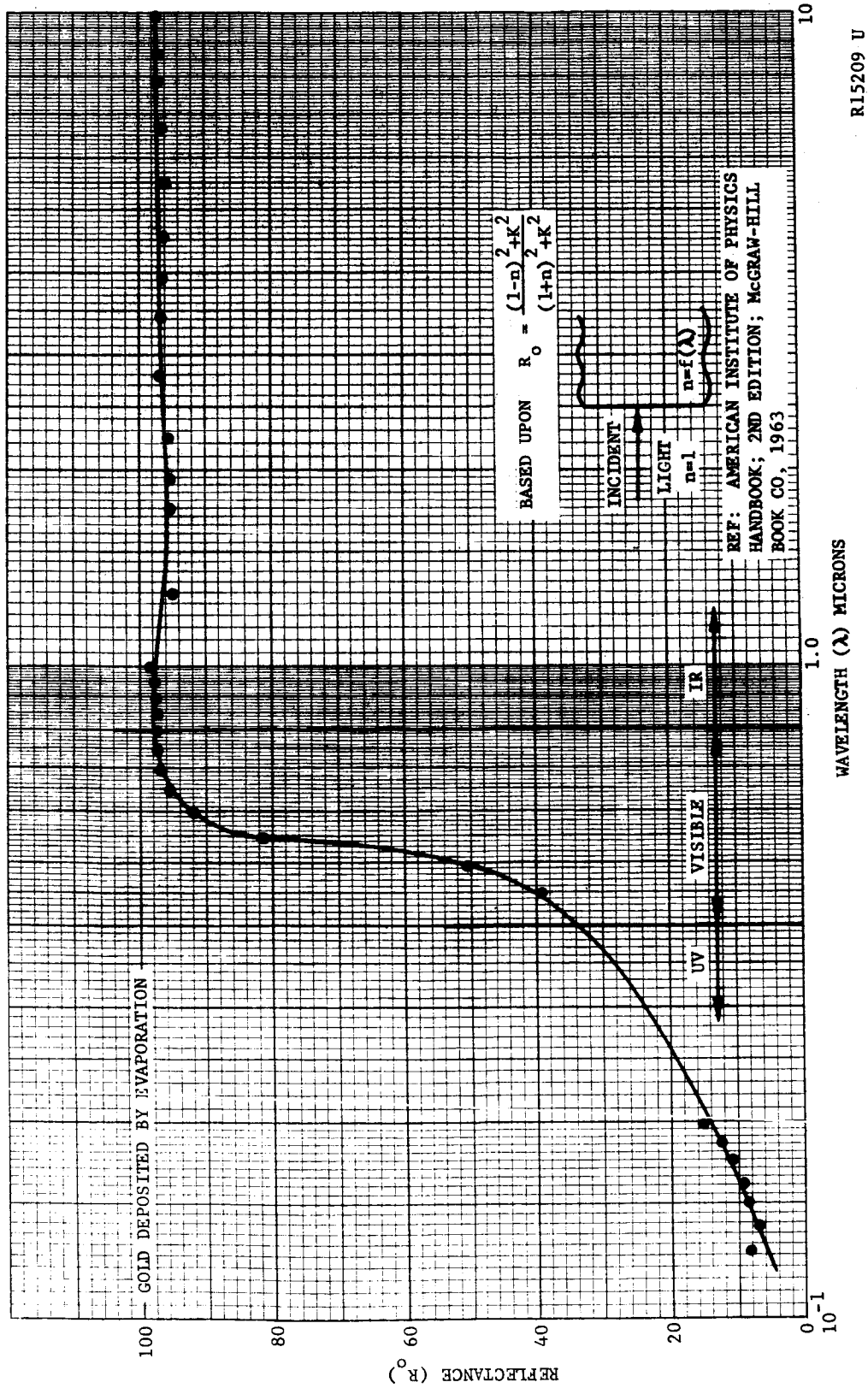


FIGURE 17. REFLECTANCE CHARACTERISTICS VARIATION WITH WAVELENGTH

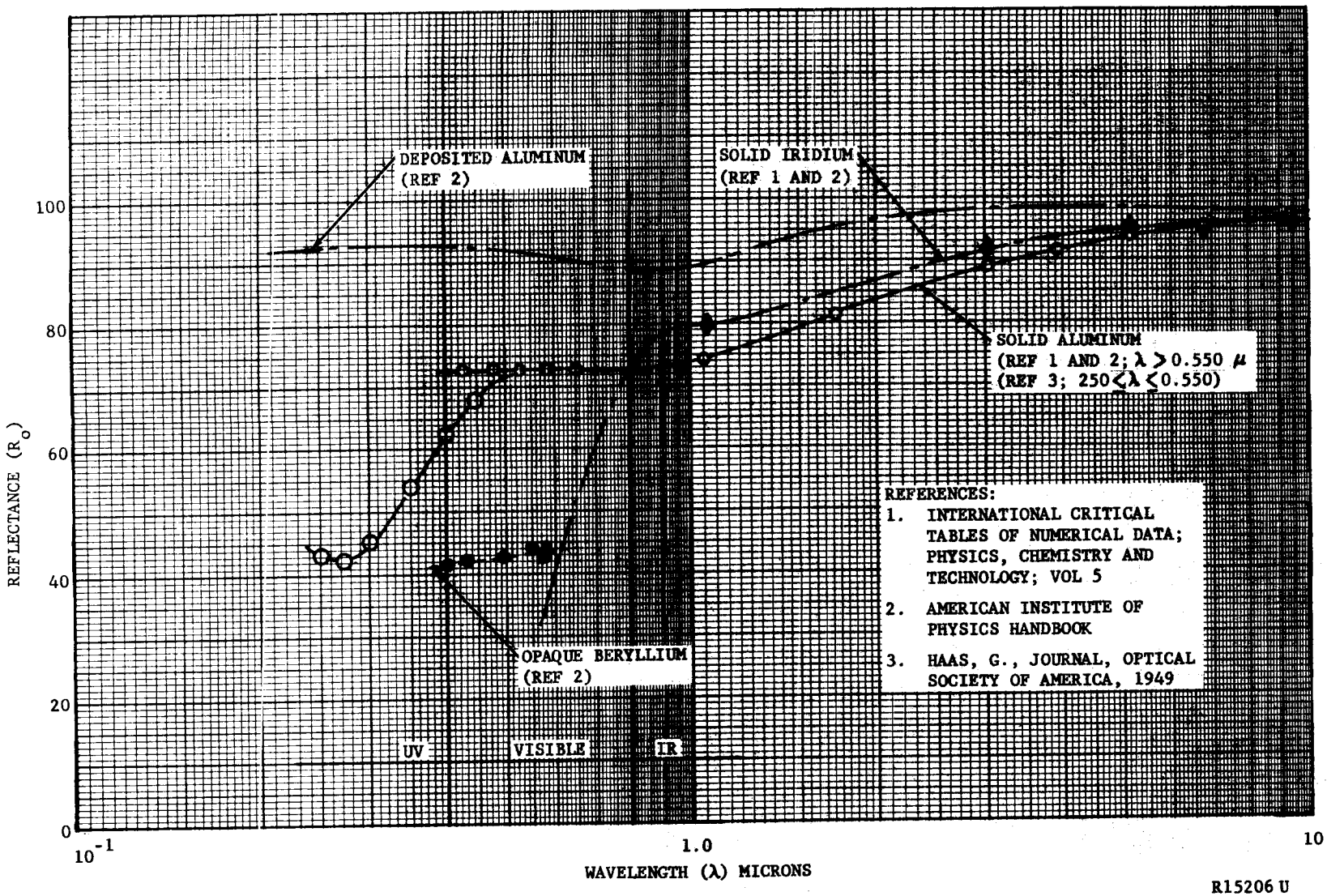


FIGURE 18. REFLECTANCE CHARACTERISTICS OF METALS

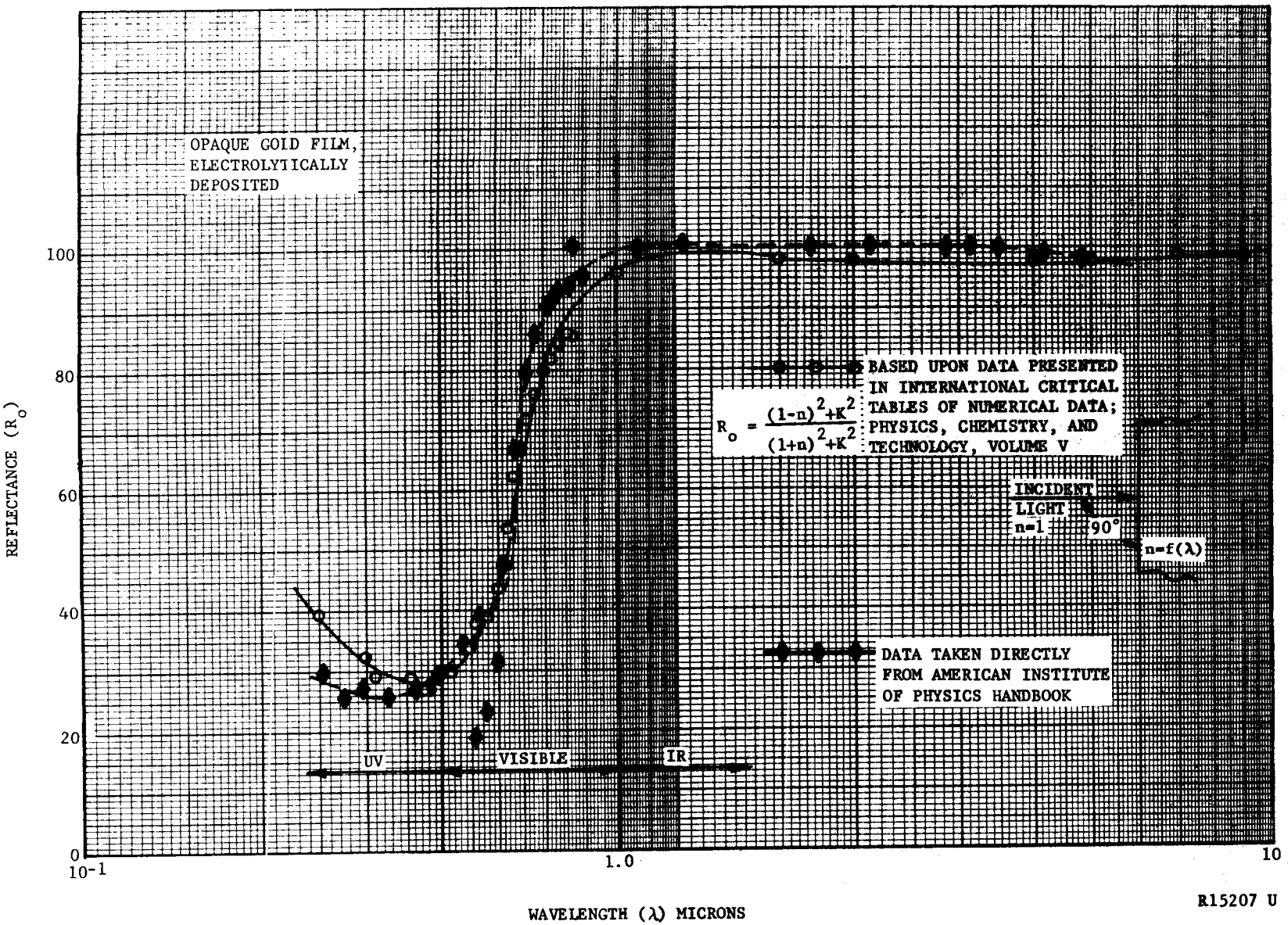


FIGURE 19. COMPARISON OF REFLECTANCE CHARACTERISTICS

where

n = index of refraction

k = index of absorption

subscripts indicate media

If one media is assumed to be a primarily nitrogen filled atmosphere where $n_o = 1$, Equation (1) reduces to

$$R = \frac{(1-n_1)^2 + k_1^2}{(1+n_1)^2 + k_1^2}$$

Values of n and k were listed in the reference data for some materials, while values of reflectance were listed in others. To verify the validity of using the normal incidence equation shown above, values of $R = f(\lambda)$ using given values of n and k were compared with explicit values of R. This comparison is shown in Figure 19 for electrolytically deposited opaque gold film.

Based upon the data presented, all ABL instruments requiring optical elements that function in the ultraviolet were assumed to use aluminum deposited front surface mirrors. It is also shown that this material would also be applicable for use in IR instruments where nontransparent optical elements are required.

Transparent elements are required for sample cuvettes, inlet windows, focusing lenses, and diffraction prisms. The transmission characteristics of various transparent materials are presented in Figures 20 and 21. It can be seen that for a range of wavelengths from the low UV to about 2.5μ, Suprasil[1] (fused silica) exhibits satisfactory transmission, Irtran[2] (synthetic sapphire) appears to offer the best performance throughout the required wavelength range.

6.4.1 REFERENCES

1. Elliott and Dickson, Laboratory Instruments, Chemical Publishing Co., 1960, 2nd edition.

2. Eastman Kodak - Data Sheets - Irtran.

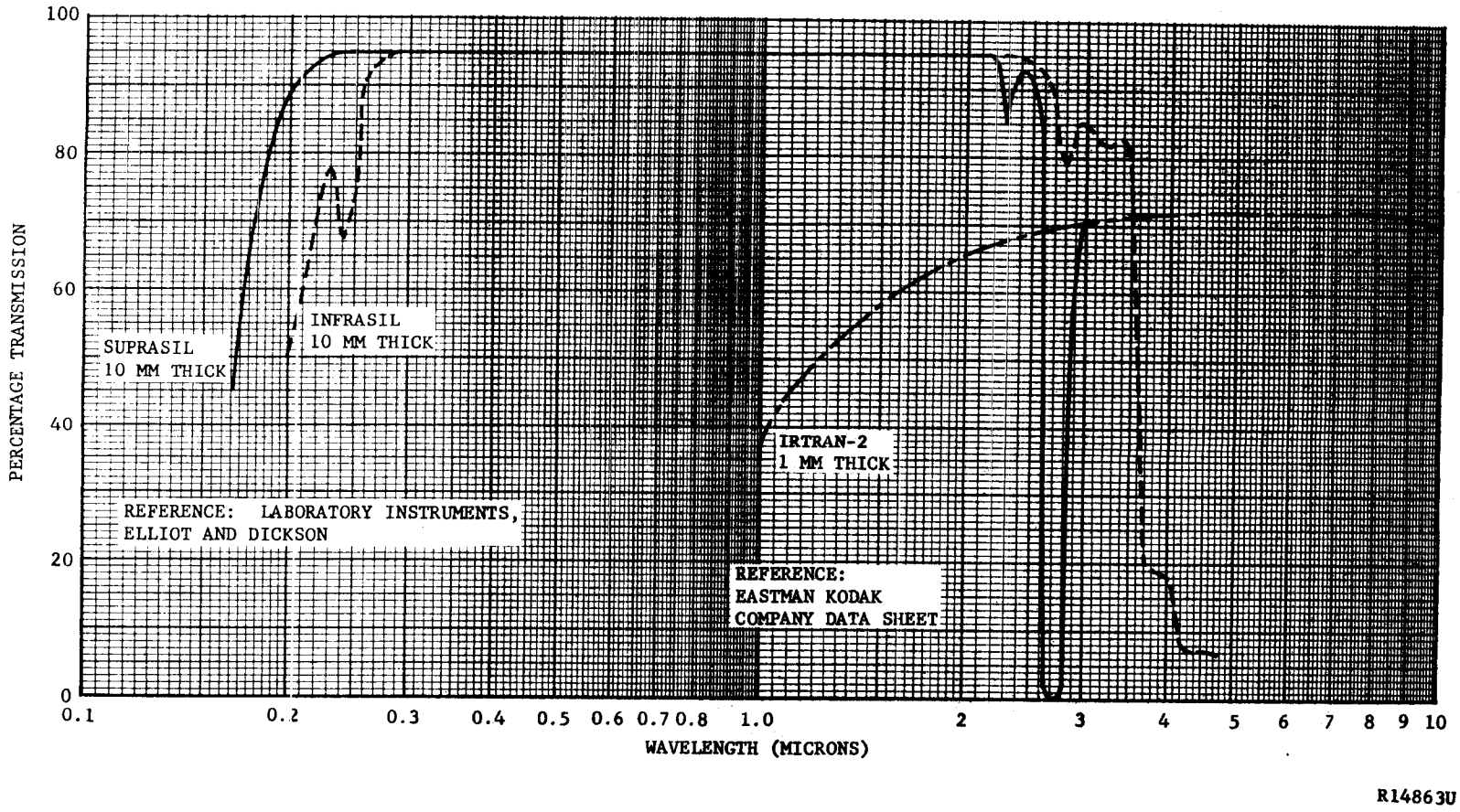


FIGURE 20. TRANSMISSION CHARACTERISTICS OF FUSED SILICA AND SYNTHETIC SAPPHIRE

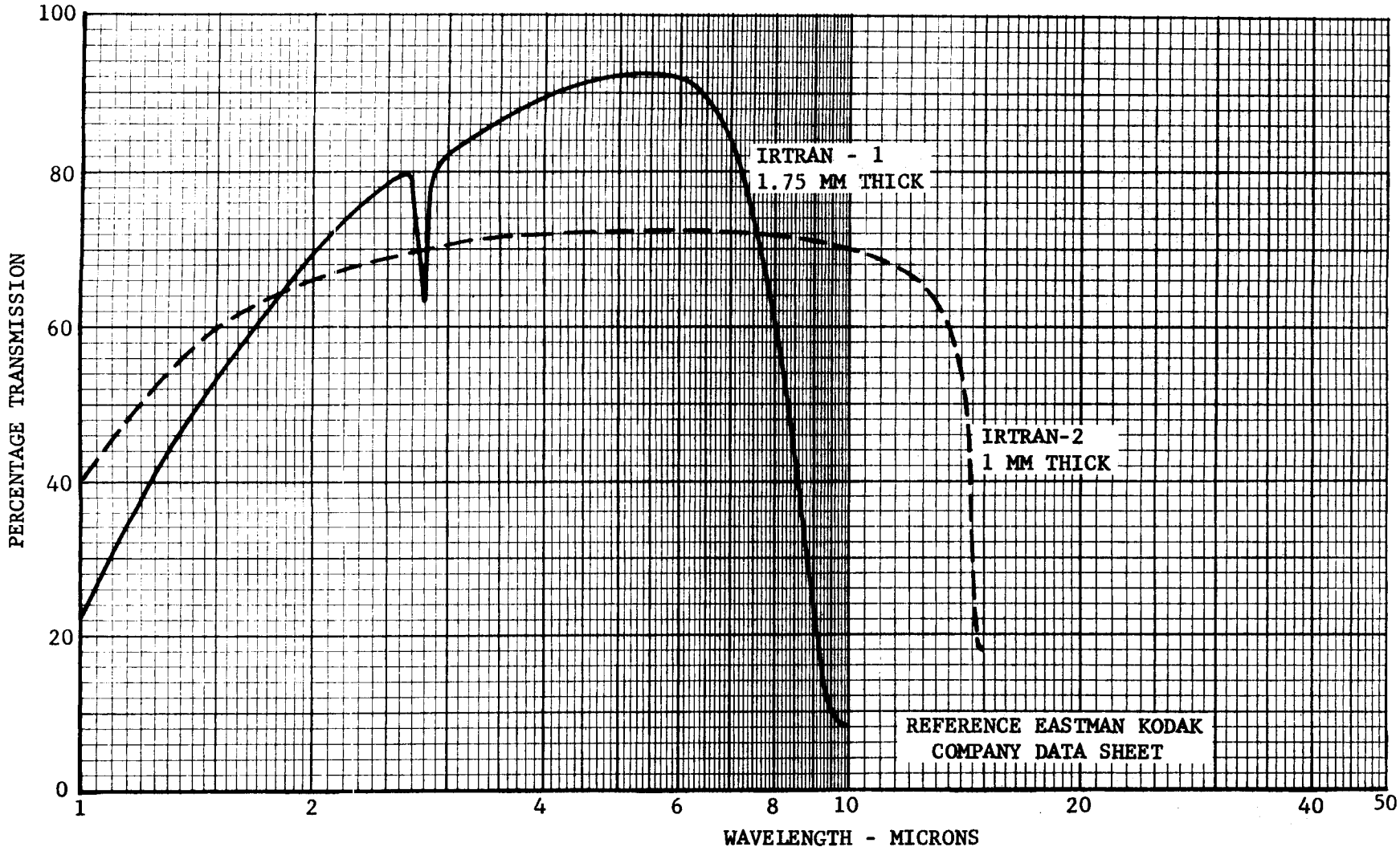


FIGURE 21. TRANSMISSION CHARACTERISTICS OF SYNTHETIC SAPPHIRE