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Title Page

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Significant Achievements in

SPACE BIOSCIENCE

1965-1966

NATIONAL AERONAUTICS AND SPACE ADMINISTRATION U.S.A.

NATIONAL AERONAUTICS AND SPACE ADMINISTRATION

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FOREWORD

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PREFACE

The significant achievements of certain aspects of the bioscience program for the period 1958-1964 were reviewed in NASA SP-92. The significant achievements for the period 1965-1966 summarized here reflect increased and new interest in space biology and planetary biology.

The strong endorsement of space biological objectives by the President's Science Advisory Committee and by the National Academy of Sciences and its Space Science Board has given vigorous impetus to this program. Increased priority in the search for extraterrestrial life and for study of the effects of the space environment on living organisms has increased the scope and responsibilities of this program.

The results of the biological research supported by the Bioscience Programs Office from 1958-1966 has resulted in publication of 1,894 scientific publications in 257 different journals throughout the world.

This summary was written and compiled as a joint project by members of the Bioscience Programs Office, NASA, with the staff and consultants of the Biological Sciences Communication Project, The George Washington University. The report was edited by Bruce Berman, BSCP, and Dale Jenkins, Assistant Director (Science), Bioscience Programs, NASA. Chapters 1, 8, 9, and 11 were written by Bruce Berman, Chapters 2 and 4 by Gregg Mamikunian, Biometrics Research Institute, Chapter 3 by Dale W. Jenkins, Chapters 4 and 6 by Gilbert Levin, Biospherics Research Institute, Chapters 6 and 9 by Joseph F. Saunders, Chief, Environmental Biology, NASA, Chapter 7 by Morton Werber, BSCP, Chapter 5 by Donald Wright, BSCP, Chapter 8 by Thomas P. Dallow, Biosatellite Program Manager, NASA, and Chapter 10 by Charles Shilling, Director, BSCP. Other staff members of the Bioscience Programs Office reviewed specific chapters.

1. INTRODUCTION

The bioscience program of the National Aeronautics and Space Administration consists of space biology and planetary biology together with their associated flight missions. Space biology deals with (1) the effects of space and planetary environments on living Earth organisms and adaptations of organisms to these environments; (2) exploitation for biological research of the unique conditions accessible through space flight: weightlessness and effective disassociation from factors related to the Earth's rotation; (3) determination of the range of physical and chemical environmental factors for growth and existence of Earth life; and (4) development of life support and protective measures for enabling long-duration manned flights. Planetary biology involves (1) the detection of the existence of extraterrestrial life, study of its origin and nature, and assessment of its level of complexity; (2) the formulation of fundamental theories and theoretical models relative to the origin and development of life; and (3) the prevention of transfer of indigenous organisms by spacecraft between planetary bodies.

In 1962, the Bioscience Program Office of NASA's Office of Space Science and Applications was organized. Before its formal organizational recognition, the space biology program had been the object of study of a small life sciences group, started in 1958, which dealt with life support and use of primates for system and vehicle testing for the Mercury program. That group's efforts bore fruit in three small suborbital flights of biological materials.

Long-range bioscience goals in space exploration have received much attention from the biological community. The following objectives have been advanced as especially germane to the goal of maintaining U.S. leadership in space and developing the science of space biology (ref. 1):

(1) To detect the presence of extraterrestrial life within the solar system, to study its origin and nature, and to assess its level of development.

(2) To study effects of the space environment on the indigenous life process of biological organisms.

(3) To contribute to the development of required reliable life-support systems, protective measures, operational medical support, and effective man-machine integration.

(4) To determine and extend the capabilities for effective utilization of man in the exploration and exploitation of space and the planets.

These concerns of the bioscience program--bearing directly on fundamental considerations of the origin and nature of life, and man's survival and performance potential in space--are in consonance with an early 1965 review and recommendation of NASA's Space Science Board. The board's Working Group on Biology urged that planetary biological exploration enlarge its search for life to a quest for any observable stage of biological

evolution--this to include evidence of early stages in the chemical evolution toward organisms and remains of extinct life. The evidence from biological evolution on Earth argues for traces of some organic evolutionary state--even if only simple organic molecules--as well as the possibility that life on another planet may be extinct.

The first of the above objectives is at present focused on the planet Mars. A feasibility study has been successfully completed of a possible Automated Biological Laboratory (now known as Voyager Biological Laboratory) for life detection on Mars. Great scientific and philosophical impact would, of course, derive from achieving this goal. Individual aims embodied under this head include: (1) determinination of the physical and chemical conditions of the Martian surface as a potential environment for life; (2) identifying and characterizing any existing life as well as any fossil remains or chemical precursors of life which may be found; (3) studying the origin and nature of any such life, and assessing its developmental level; and (4) developing fundamental theories and theoretical models relative to the origin and development of life.

Also of paramount interest is the aim of determining effects of both individual and combined space, lunar, and planetary environmental conditions on living organisms, including man. These conditions involve atmospheric requirements, subgravity, weightlessness, electromagenetic spectrum, hard vacuum, altered magnetism, radiation from solar flares, and trapped radiation. Achievement of this goal would give insight into metabolic and physiological mechanisms, and into the compensatory devices in adaptation. Indeed, all of space biology's goals, seeking as they do understanding of the probable human response in space, constitute the pacing item for manned interplanetary flight. Only with this understanding can suitable spacecraft, life support, and protective systems be designed (ref. 2).

At appropriate intervals, NASA compiles and presents in compact form significant results deriving from its research endeavors. The predecessor volume in this series (ref. 3) covered a period (1958-64) of space bioscience research largely seeking baseline information preparatory to space-flight experimentation. In that period, the few space biological tests undertaken sought to evaluate life-support systems, and to determine, before putting man in space, animal survival capability. Extraterrestrial life detection, up to 1964, was limited to groud-level research and planning for planetary and lunar landings. However, many life-detection experiments were conceived, new instrumentation was tested, a biosatellite program was begun, and essential work preliminary to space embarkation was completed.

In the past two years, the scientific community's interest in space biology has dramatically widened, and the NASA program for this field, with which this volume is concerned, had made notable progress in overcoming its comparatively late start. NASA research prgram results are reflected in this volume and demonstrate significant advances in capability for manned space flight. Important biological space-flight experiments have been defined, flown in various Gemini flights, and packaged in readiness for launch in biosatellites and Apollo craft. The frontier of this challenge-filled program has indeed been pushed forward.

This volume presents some of the results of research and developments stemming from the bioscience program during 1965-66. It points up the many disciplines that must be drawn on to yield data crucial for understanding space, revealing its secrets, and equipping man for its exploration. The spacecraft in which man explores requires solution of

endless bioengineering problems before human safety can be predicted. The spacecraft must be sterile; its human occupants must be equally so, if they are not to convey contaminants to celestial bodies. The humans must be protected against the hazards of weightlessness, radiation, and other conditions of space, and much research on both animals and man, has sought knowledge of what physiological changes, what disturbances in metabolism, what derangements in biorhythms, what nervous, behavioral, and cardiovascular anomalies must be anticipated and provided against.

Man's quest in space seeks knowledge of whether some form of life exists beyond Earth. To detect signs of life requires ever profounder knowledge of life's fundamental consituents. Research has sought penetration of life's molecular make-up, of the protein structure of its ultimate units, of the strangely subtle quality of its genetic codification. Wherever any traces of organic evolution exist in space, wherever primordial chemical systems may evolve into life, research must prepare for their recognition and return of samples to Earth for analysis and application.

Biology now faces the opportunity to broaden its grasp through comparisons with life processes that may exist elsewhere in the universe. It will need rigorous development and continued theoretical refinement in its search of the solar system for exotic life, in its need to modify planetary environments and solve problems of communication among various biosystems.

2. CHEMICAL EVOLUTION AND MOLECULAR BIOLOGY

A. Chemical Evolution

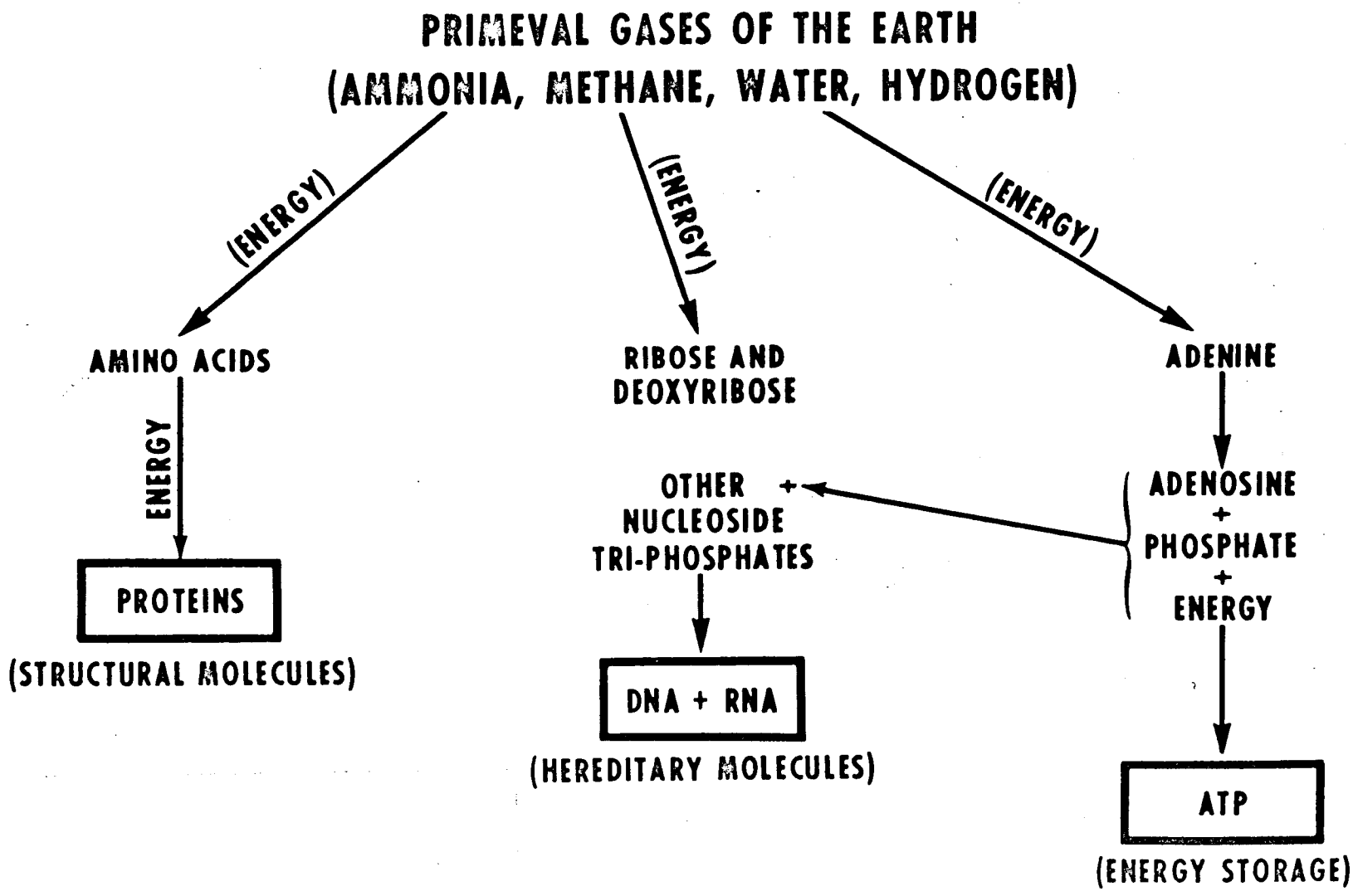
Doctrines on the origin of life go back to the ~~sixth~~ 6th-century philosophic speculations of Anaximander, who taught that the first animals arose from sea slime. After Louis Pasteur, in the 19th century, showed that bacteria in a decaying organic medium were produced by pre-existing micro-organisms, it became widely accepted that spontaneous generation of life was not possible.

It is possible today to speak of the spontaneous origin of life, although the idea is quite different from that of the time of Pasteur (ref. 4). Today's concept is that of organic compounds generating spontaneously over millions of years, evolving into the first primitive living system, which gradually assumed the form of today's micro-organisms. ~~(ref. 1)~~ (fig. 1).

(Insert fig. 1)

Man's personal involvement has, without doubt, always led him to considerations of life's origin and evolution, but only in recent years has the experimental approach been used with any notable effect. Today, it is generally accepted that life can develop spontaneously, provided abundant organic compounds be concentrated in one or more specific zones (ref. 5). The sequence is thus defined: simple molecules from these compounds spontaneously modify to greater structural complexity, and finally emerge as a

SYNTHESIS OF KEY MOLECULES OF LIFE



NASA SB 64-526

Figure 1.—

"living" organism.

Urey has made clear the possible role of a reducing atmosphere in synthesizing prebiological organic molecules. Indeed, a variety of organic compounds have been synthesized by action of various energy sources upon reducing atmospheres, and — by extension of this method — various nucleic-acid components have been synthesized. The ease with which organic compounds can be synthesized under reducing conditions has led to the belief that planetary surfaces may contain an abundance of similar organic matter. Modern laboratory technology has already permitted attempts to simulate the events (chemical evolution) on a primitive Earth that must have preceded abiogenic synthesis. One difficulty, however, in the postulated easy progression lies in the probable variations among planets in chemical evolution of the original organic matter beyond the prebiotic stage.

Individual organic molecules — essential to dynamic stability in cellular functions and stoichiometry of molecular reactions — are readily synthezised in the laboratory. The progenitors of all such molecules are the "organic" elements of H,C,N, and O, whose cosmic abundance and distribution has been well documented. Advances in both the theoretical and experimental aspects of chemical evolution and abiogenic synthesis have been made at several laboratories within the last few years.

Considerable emphasis has been placed on the synthesis of amino acids. Experimentally, many variations of gas mixtures simulating primitive atmospheres and a variety of energy sources have been used. Miller (ref.6) synthesized amino acids from a gaseous mixture approximating the "primitive" environment. Glycine, alanine, B-alanine, aspartic acid, and glutamic acid resulted following his exposure, for about a week, of a mixture of methane, ammonia, and water to an electric discharge from tesla coils. The gases used in Miller's first experiment were the same as those Urey (ref.7) proposed for the reducing environment of the primitive Earth, namely, methane, ammonia, and water.

In recent experiments performed at Ames Research Center by Ponnamperuma and Flores (ref.8), a 95-percent conversion of methane, from a gaseous mixture of methane, ammonia, and water, into organic compounds was achieved when this mixture was subjected to an electric discharge for 24 hours. Among the amino acids that were identified following the hydrolysis of the reaction products were: glycine, alanine, threonine, aspartic acid, valine, glutamic acid, leucine, isoleucine, and phenylalanine. The presence of alpha aminonitriles was confirmed ~~by Ponnamperuma and Woeller~~ (ref.9) when equimolar mixtures of methane and ammonia alone were exposed to an electric arc. These alpha aminonitriles, upon hydrolysis, give rise to amino acids. This type of synthesis

may well be going on today in the atmosphere of Jupiter.

The effect of ionizing radiation on mixtures of "primitive" gases related to chemical synthesis has been well documented(ref. 10). In an early experiment, Palm and Calvin (ref. 11) subjected a mixture of methane, ammonia, and water to 5 MeV electrons from a linear accelerator and found alanine and glycine in the reaction product. Exposure of N-acetylglycine as a starting material to gamma rays led to identification of a wide range of amino acids, again indicating the plausibility of the ease of organic synthesis by the interaction of various energy sources upon simple molecules (ref. 12).

Thermal energy used on a mixture of glucose, urea, alpha-hydroxy glutaric acid, and ammonia, yielded glycine, glutamic acid, and alanine (ref. 13). In varying the experimental procedure, heat was applied to a mixture of methane and ammonia alone, and amino acids were synthesized (ref. 14). In this experiment methane was bubbled through a solution of concentrated ammonium hydroxide and passed over quartz sand in a glass reaction tube which was heated between 900° to 1000° C. The end products were absorbed in aqueous ammonia and hydrolyzed for subsequent amino acid analysis.

Formation of amino acids by pyrolysis of ammonium formate or foramide has been reported (Harada, ref. 15) ~~(ref. 12)~~. Thermal decomposition of ammonium formate or foramide resulted in several amino acids: glycine, alanine, and aspartic acid. Seven to nine other ninhydrinpositive materials were observed on the amino acid analyzer and in two-dimensional paper chromatography.

Thermal energy has been used as the main source in the production of various amino acids (refs. 16, 17). Up to this period, the absence of sulfur-containing amino acids has been conspicuously apparent from the long list of the already synthesized amino acids. The presence of cysteic acid, cysteine, and taurine was demonstrated by irradiation of a mixture of methane, ammonia, water, and hydrogen sulfide (ref. 18), thus advancing significantly this segment of abiogenesis.

The search for organic matter in Precambrian sediments (and eventually in returned lunar samples) requires unambiguous criteria for distinguishing abiogenic material from the biological. With this in mind, unusual deposits of hydrocarbons have been analyzed and compared with abiogenic hydrocarbons synthesized in the laboratory and biological hydrocarbons extracted from rocks containing marine fossils. The analysis of the Mountsorrel hydrocarbons and the aliphatics from the Trinidad Lake asphalt has shown there are deposits in nature which may be abiogenic in origin. The results on the Mountsorrel hydrocarbons were the first case of such an identification by careful analytical techniques such as gas chromatography and mass spectrometry (fig. 2).

(insert fig. 2)

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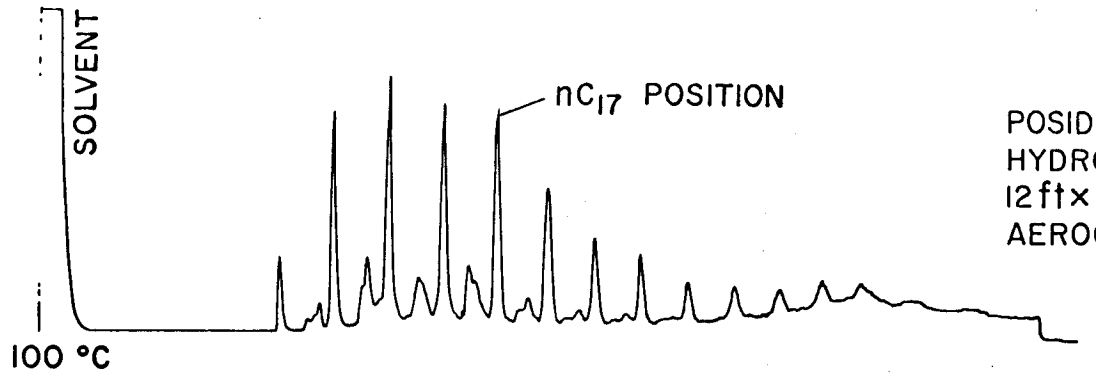
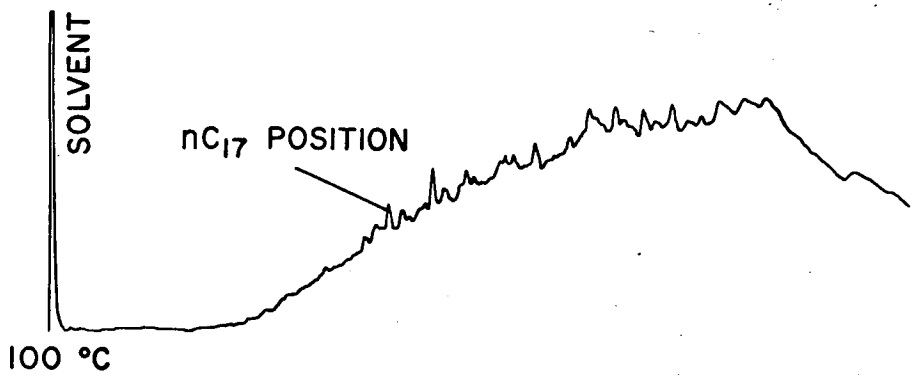


POSIDONIAN SHALE ALIPHATIC HYDROCARBONS TEMP PROG 2°/min; 12ft×1/8in., 3% SE-30 COLUMN; AEROGRAPH 660



MOUNT SORREL BITUMEN ALIPHATIC HYDROCARBONS TEMP PROG 2°/min; 12ft×1/8in., 3% SE-30 COLUMN; AEROGRAPH 660

METHANE DISCHARGE ALIPHATICS TEMP PROG 2°/min; 12ft×1/8in., 3% SE-30 COLUMN; AEROGRAPH 660

Figure 3 – Abiogenic Hydrocarbons

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AMES RESEARCH CENTER, MOFFETT FIELD, CALIFORNIA

The synthesis of adenine has been extended from the original work done by Oro(ref.19), who used a concentrated solution of ammonium cyanide as starting material. The base-catalyzed reaction of hydrogen cyanide has also been confirmed by Ponnamperuma et al. (ref. 20), who synthesized adenine by the electron irradiation of methane, ammonia, and water. The synthesis of both adenine and guanine was accomplished when a dilute solution of hydrogen cyanide was exposed to ultraviolet light (ref. 21).

The ease of synthesis of amino acids, nucleic-acid bases, and certain sugars under the postulated primitive Earth conditions has led to further experimental approaches to the abiogenic synthesis of nucleotides and nucleosides. Adenosine was obtained (ref. 22) when ribose and phosphate were exposed to ultraviolet light. Deoxyadenosine has also been synthesized in a similar manner by exposing adenine and deoxyribose to ultraviolet light in the presence of hydrogen cyanide. This reaction also proceeds without the presence of the ultraviolet irradiation (ref.23).

Phosphorylation of the nucleotides has been achieved both at moderate and low temperatures (ref. 24). Waenheldt and Fox (ref. 25) phosphorylated the nucleosides, adenosine, cytidine, guanosine, uridine, deoxycytidine, and thymidine with polyphosphoric acid at low temperatures, ranging from 0° to 22° C. A drawback of this experimental approach is the presence of high concentrations of the polyphosphoric acid, since such concentrations may not be compatible with its geochemical abundance in the early history of the Earth (ref.26).

The achievement of condensation reactions incorporating amino acids to yield peptides and polypeptides helpd demonstrate a possible pathway for the evolution of the "protoprotein" molecule in the organic milieu. Studies of thermal copolymerization of amino acids have yielded evidence for peptide-bond formation as determined by infrared analysis (ref. 13). Synthesis of peptides from amino acids has been reported (ref. 27) upon irradiation of a dilute aqueous solution of glycine and leucine. The formation of the di- and tri-peptides of alanine was reported when a dilute solution of amino acid was treated with dicyanamide(ref. 28).

The presence of small peptides in experiments starting with methane, ammonia, and water has been reported (ref. 8). Subjecting N-acetyl glycine to gamma rays and beta rays, investigators have demonstrated the formation of a number of peptides (ref. 12). In the synthesis of polynucleotides, with use of 85-percent polyphosphoric acid, it has been shown that polymerization of cytidylic acid to di- and tri-nucleotides is achieved (ref. 29).

B. Pre-Biology

Proteinoids have been formed which produce "microspheres" (ref. 30). These are very similar to bacteria, and sections have been made which show much morphological similarity (fig. 3). In recent experiments, Fox has incorporated geological factors into the (insert fig. 3) synthesis of the proteinoids. Variation in the composition of the proteinoids has been found when different crustal materials were employed.

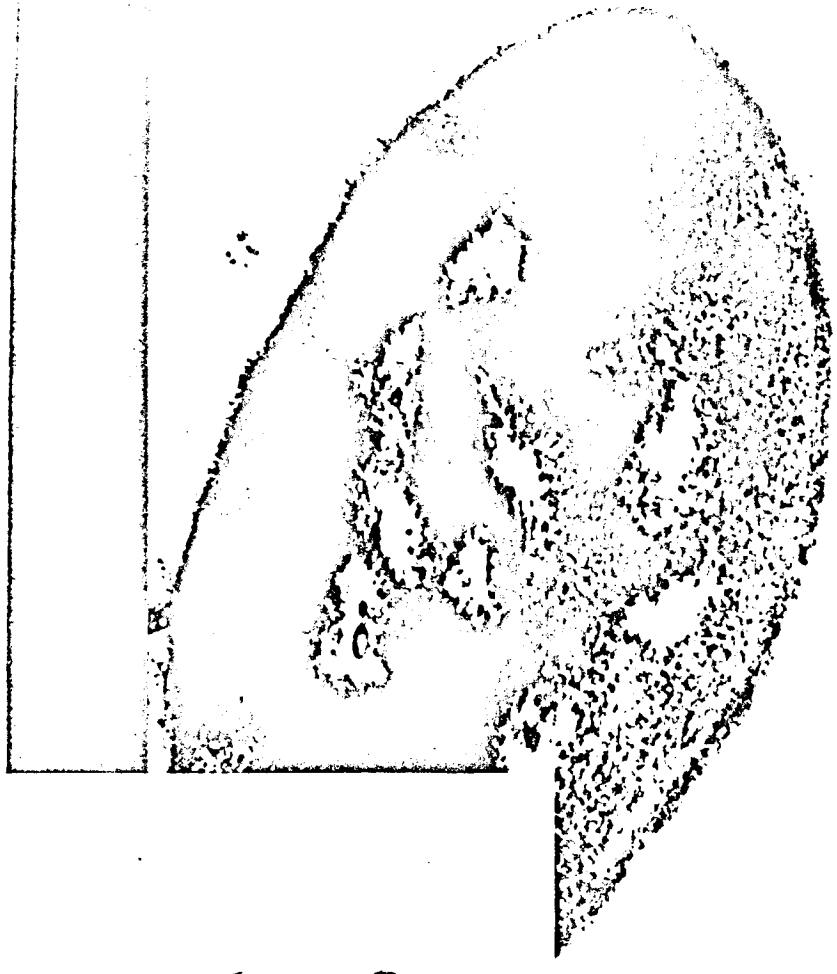

Figure 3

These results are significant in two respects. One, they demonstrate the feasibility of laboratory reactions under a greater variability of geological conditions, and the consequent utilization of geochemical vectors as essential moderators in abiogenic synthesis. Secondly, the analysis reveals amino acid compositions more like those of naturally occurring proteins rather than those obtained in pure "glassware synthesis."

Contrary to earlier indications of a somewhat limited heterogeneity of the thermal proteinoids has come the astonishing finding that this heterogeneity is sharply limited. In the latest and most extensive study reported by Fox, 1:1:1- proteinoid was first converted to proteinoidamide in liquid ammonia (ref. 26). This treatment was designed to convert imide groups to amides with the intention of avoiding

variation in structure due to a theoretical variation in mode of opening of imide rings. The obtained polymer proved to be neutral. The proteinoidamide was distributed on DEAE-cellulose and five major peaks were obtained on elution. Several of these peaks have a high degree of symmetry, and the entire pattern is far removed from being a random smear. The material represented in each peak was next purified by partial dialysis. Upon further purification of fractions 3, 4, and 5, each was found to be homogeneous. When hydrolyzed for 24 hours at 105° ~~degrees~~ C. and then subjected to an automatic analyzer, fractions 3, 4, and 5 displayed remarkably similar amino acid compositions. The results show that all fractions contain all amino acids with the exception of serine and threonine.

~~Upon~~ Partial hydrolysis of fractions 3, 4, and 5 by 12 N HCl at 37° ~~degrees~~ C. for 72 hours yielded "fingerprints." ~~were obtained.~~ The number of peaks observed was 39-41; no more than 17 of these peaks can be free amino acids (tryptophan being destroyed with mineral acid hydrolysis). The remaining peaks, which are assumed to be peptides (as indicated by the reactivity to ninhydrin), were distributed also in a fashion similar to that of the total hydrolyzates of fragments 3, 4, and 5 from the DEAE-cellulose elution. The inevitable conclusion is that both the composition and sequence within the polymers are sharply limited by internal processes, and

that the information within the polymers is derived from information within the monomers only.

Thus, the observed order is present even at this rudimentary molecular level. Other phenomena which have emerged from the studies of these thermal poly-alpha-amino acids are more readily understood in the light of this degree of order. Evolution at the molecular level, therefore, in a prebiological phase is visualized as sharply self-limiting (ref. 30).

The indication is that systems containing such information can arise so easily from simpler systems having information at another level, and that none of the information has been imparted from outside the macromolecular system itself. In this model of original protoprotein, for instance, no information has been obtained from nucleic acid. The premise that nucleic acids had to appear before protein has been advanced by several authors. Rigid acceptance of such a hypothesis would have precluded the experiments described by Fox (ref. 30).

Ultraviolet photomicrographs and electron microscopy have offered evidence for a membrane in the proteinoid microspheres (ref. 31). The selective nature of the boundary of the proteinoid microsphere has been demonstrated experimentally (fig. 4). Changes in pH can cause the migration (insert Fig. 4) of the polymer from the interior of the

PROTEINOID MICROSPHERES SHOWING "BUDDING"
STAINED SECTION BELOW SHOWS DIVISION

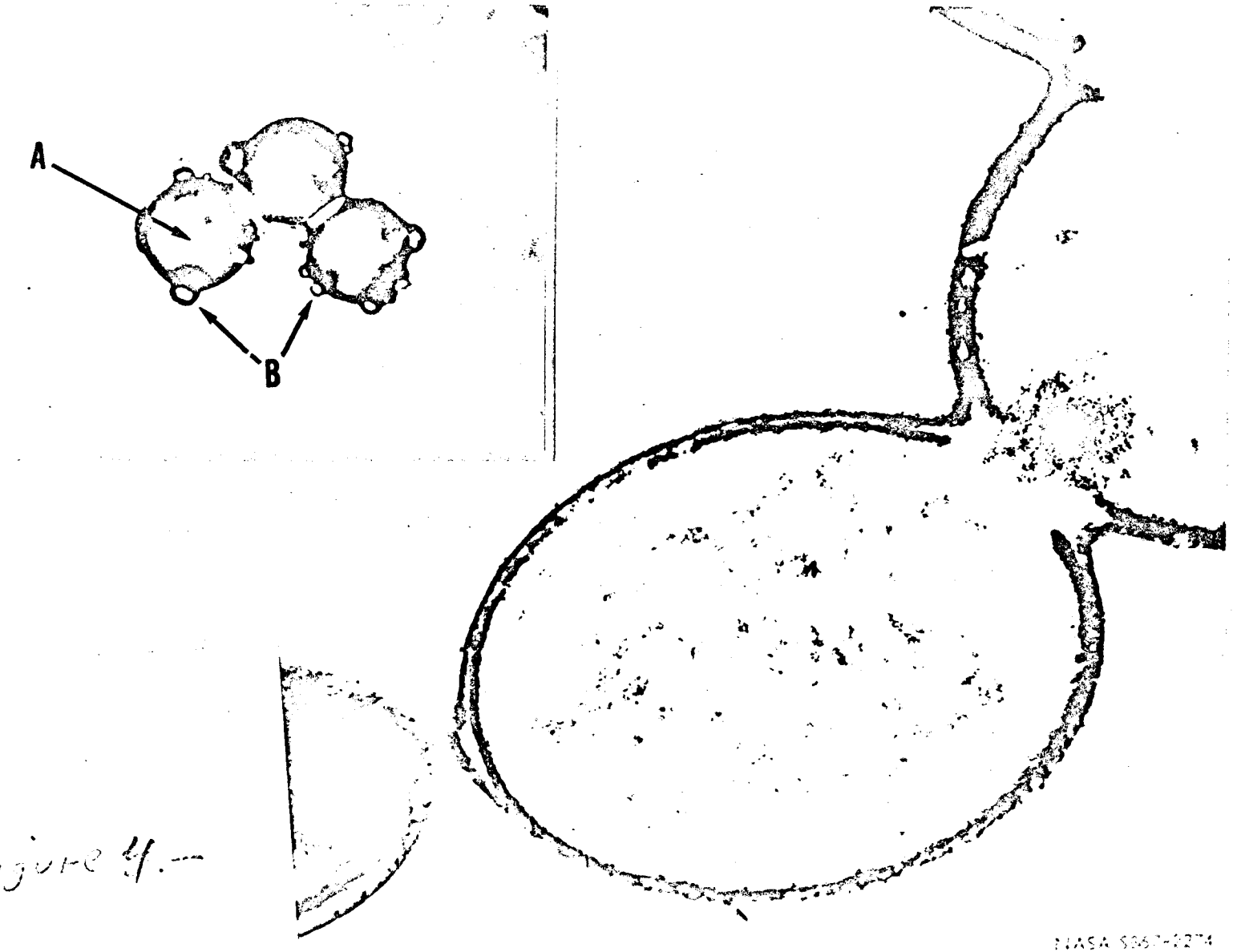

Figure 4.—

NASA S867-2274

microspheres, while the boundary of the spheres remains intact. The thermal model provides a possible mode in the synthesis of the primitive protein and its organization into a structured entity, thus advancing a possible model as to the emergence of the first cell membranes.

In contrast to the above model, the concept of an excitable membrane which preceded the formation of a protocell has been proposed (ref. 32). Investigators have already presented an inorganic proto-life membrane model (ref. 33). The proposed three-dimensional polyphosphate complex with the alkaline earth cations occupying the central atoms approximates a potential gradient such as the one operative in biological membranes.

Many experiments have been performed to ascertain if morphological or other effects can be observed with the addition of salient vital cellular components (such as nucleic acids, lipids, histones) to the thermal proteinoid subsequently incorporated into microspheres. Marked changes in morphology have been observed with lecithin and with histone (refs. 34, 35).

Different characteristics have been found in proteinoid microspheres produced in the presence of enzymically synthesized polycytidylic acid. The microparticles are larger (10-20 μ in diameter). The granularity of the contents is more marked than in proteinoid particles prepared in the absence of polynucleotide.

C. Molecular Biology and Biophysics

Without a strong and a parallel research program in molecular biology and biophysics, studies in abiogenic synthesis become merely an accumulation of simple organic compounds synthesized within the laboratory. Molecular biology and biophysics are the sole avenue for achieving direction and overall understanding with respect to life and its processes. The transition from chemical evolution to biological evolution is a subtle one. Oparin, in his 1924 book on the Origin of Life, stated; "There is no fundamental difference between a living organism and lifeless matter. The complex combination of manifestations and properties so characteristic of life must have arisen in the process of the evolution of matter." Some aspects of this transition from inaminate to animate matter are becoming better understood through studies in molecular biology.

In the area of genetic coding, the most significant achievement is the near solution of the amino acid code. Since 1964, investigators in various laboratories, reviewed by Jukes, ~~et al.~~ (ref. ~~35~~ 36), working for the most part with synthetic polyriboneucleotides, support the conclusion that long sequences of bases

in deoxyribonucleic acid (DNA) spell out instructions which are transcribed into messenger ribonucleic acid (RNA) and are read from left to right in consecutive groups of three bases termed "codons" or "triplets." A picture of RNA molecules is shown in figure 5 taken by a newly developed electron microscope. Messenger RNA consists of long chains of the four ribonucleotides: adenylic acid, guanylic acid, ~~and~~ cytidylic acid, and

(Insert fig. 5)

uridylic acid (A,C,G,andU). There are 64 possible ways in which A,C,G,and U may occur in a consecutive sequence of 3 nucleotides, or a "triplet" joined together by 3', 5'-phosphate ester linkages. Sixty-one of the 64 triplets are codes for the 20 amino acids used in the biological synthesis of proteins.* The various experimental advances in 1965 and 1966 permit representation of the amino acid code as shown in table I.

(Insert table I)

This code enables each of the single-amino-acid replacements to be written in terms of a single-base change in a coding triplet, with one exception, that of glutamic acid/methionine. Forty-five of the 75 possible single-base changes that can lead to single amino-acid changes in the code have been described as mutations occurring in at least one protein at one or more locations. This is summarized in table II, where the underlined examples have been reported to occur in mutations.

(Insert table II)

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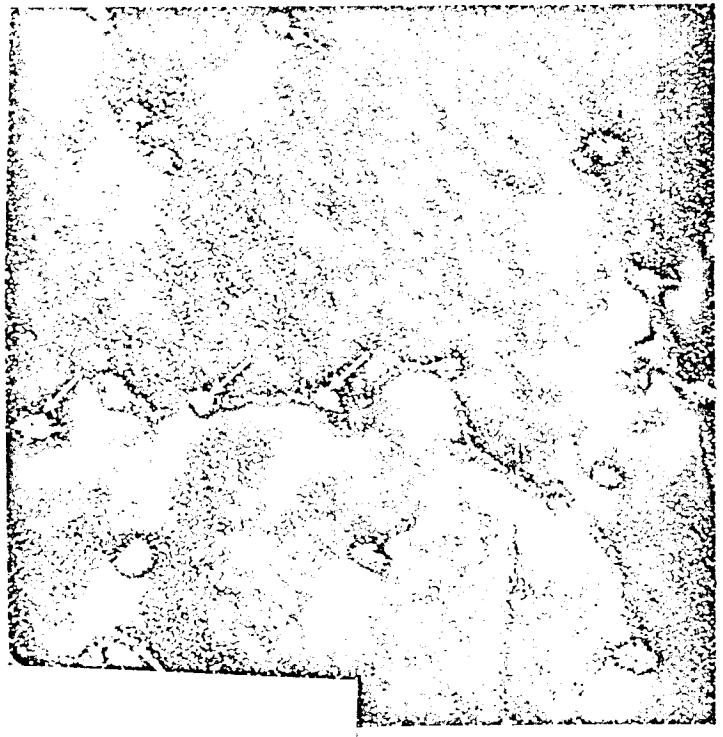
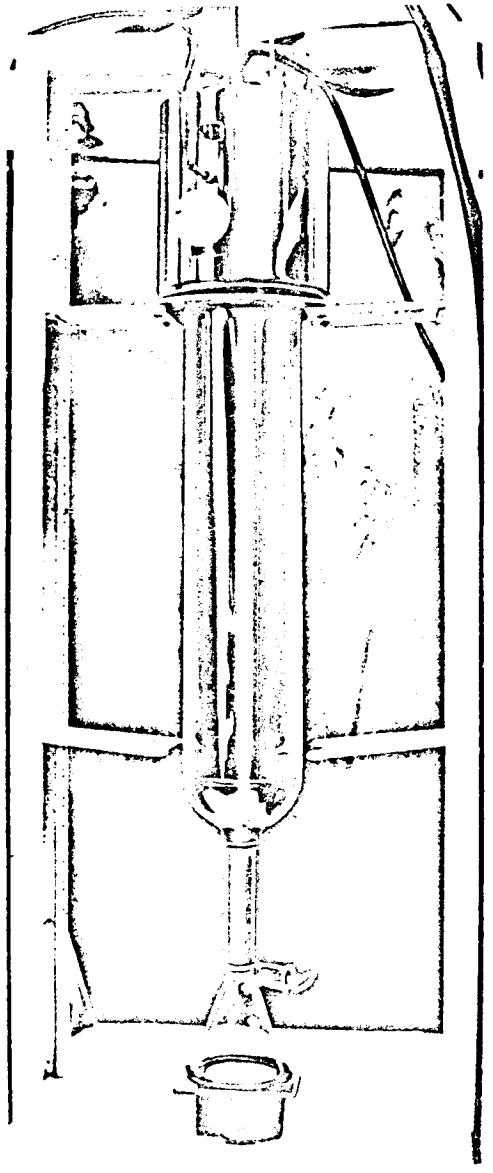
* The abbreviations of amino acids that take part in ptotein synthesis are listed. The same abbreviations are used in tables II, III, and IV.

Alanine	ala	Glutamic acid	glu	Leucine	leu	Serine	ser
Arginine	arg	Glutamine	gIN	Lysine	lys	Threonine	thr
Asparagine	asN	Glycine	gly	Methionine	met	Tryptophan	try
Aspartic acid	asp	Histidine	his	Phenylalanine	phe	Tyrosine	tyr
Cysteine	cys	Isoleucine	ilu	Proline	pro	Valine	val

NEW ELECTRON MICROSCOPE WITH SUPERCONDUCTING LENS



TION SHOWING RNA
OF CHROMOSOMES



SUPERCONDUCTING LENS
WITH LIQUID HELIUM

5.

~~Table 2~~.

Table I.- THE AMINO ACID CODE -Cl

UUb phenylalanine	CUd leucine	AUb isoleucine	GUd valine
		AUA "	
UUe leucine		AUG methionine	
UCd serine	CCd proline	ACd threonine	GCd alanine
UAb tyrosine	CAb histidine	AAb asparagine	GAb aspartic acid
UAe gaps	CAe glutamine	AAe lysine	GAe glutamic acid
UGb cysteine	CGd arginine	AGb serine	GGd glycine
UGA gap			
~~UGe~~ tryp~~o~~tophan		AGe arginine	
UGG			

U=uracil; C=cytosine; A=adenine; G=guanine; b=U, C; d=U, C, A, G; e=A, G

Table II.

AMINO ACID INTERCHANGES THAT CAN OCCUR AS THE RESULT OF SINGLE-BASE CHANGES IN THE CODING TRIPLETS

Amino Acid	Possible Interchanges											
Ala	Asp	glu	gly	pro	ser	thr	val					
Arg	Cys	glN	gly	his	ilu	leu	lys	met	pro	ser	thr	try
AsN	Asp	his	ilu	lys	ser	thr	tyr					
Asp	Glu	gly	his	tyr	val							
Cys	Gly	phe	ser	tyr	try							
GlN	Glu	his	leu	lys	pro							
Glu	Gly	lys	val									
Gly	Ser	try	val									
His	Leu	pro	tyr									
Ilu	Leu	lys	met	phe	ser	thr	val					
Leu	Met	Phe	pro	ser	try	val						
Lys	Thr	met										
Met	Thr	val										
Phe	Ser	tyr	val									
Pro	Ser	thr										
Ser	Thr	try	tyr									

Jukes has postulated the evolutionary changes in the amino acid code that are summarized in table III.

(Insert table III)

Examination of the relationship between the polypaptide chains of myoglobin and alpha, beta, and gamma hemoglobins in terms of the genetic code has led to this conclusion (Cantor, ref. 37): the evolutionary separation of two genes with a common origin is followed by "point mutations," which occur as base changes randomly distributed along strands of DNA. These and other related observations have led to the belief that evolution proceeds by three general types of events: duplication of entire genes or portions of genes, "deletions," and "point mutations." Although it is probable that these events take place on a random basis, the process of selection is nonrandom.

Internal duplications in the molecules of the cytochromes, the hemoglobins, and myoglobins, which have been detected (ref. 37), represent an incident of crossing over and recombination

Table III.—

"Primitive" codes		"Recent" codes	
AAd	asN ~~lys~~	AAb	asN
		AAe	lys
ACd	thr		
AGd	ser ~~arg(?)~~	AGb	ser
		AGe	arg
AUd	ilu	AUb	ilu
		AUA	ilu
		AUG	met
CAd	his	CAb	his
		CAe	gln
CCd	pro		
CGd	arg		
CUd	leu		

"Primitive" codes		"Recent" codes	
GAd	asp ~~(?)~~	GAb	asp
		GAe	glu
GCd	ala		
GGd	gly		
GUd	val		
UAd	tyr ~~gap(?)~~	UAb	tyr
		UAe	gap
UCd	ser		
UGd	cys	UGb	cys
		UGA	gap
		UGG	try
UUd	phe	UUb	phe
		UUe	leu

d = A, C, G, U; b = C, U; e = A, G

within a gene. Following this event, "point mutations" take place and may be so numerous as to obscure the common origin of homologous segments of the protein. This represents a significant advance in protein synthesis. The amino-acid substitutions due to "point mutations" (refs. 38,39) may possibly have been lost as a result of the working effect of evolutionary changes in the amino-acid sequences. Since remnants of the early peptides are still discernible in the forms of partially repetitive sequences, it must follow that protein molecules originated in end-to-end joining of short polypeptide sequences followed by evolutionary changes in the amino-acid sequence of the polymer.

Jukes (ref. 40) presented evidence that the evolutionary divergence of certain transfer RNA molecules from a common archetype took place by genetic duplication followed by functional differentiation; the degree of differentiation was restricted by the need to conserve the secondary and tertiary structure of the molecule. This was the first detailed examination of the primary structures of functionally related nucleic-acid molecules.

Several transfer RNA molecules from yeast have been purified completely, and their base sequences determined. These

were an alanine transfer RNA (tRNA) (ref.41), two serine tRNA's (ref. 42), and tyrosine sRNA (ref. 43). The comparison was made in a manner similar to that used for protein sequences already mentioned. The essential procedure is the insertion of arbitrary gaps which infer that deletions of genetic material have taken place during evolution as shown by alignment of homologous portions of the sequences. The identified portions are presumably preserved for essential functional characteristics, or because the time lapse following duplication is not yet sufficient for randomization for "point mutations" to produce differences in base composition.

Jukes used the "clover-leaf" model of yeast alanyl transfer sRNA proposed by Holley et al. (ref. 41) to provide an evolutionary model for comparing the yeast alanyl, seryl, and tyrosyl sRNA's. The model includes the same four helical regions that are shown in the "clover-leaf" conformation by Holley, with the exception that one of the regions has been shortened by one base pair. It is therefore suggested that one evolutionary deletion is present in each of the three sRNA's. The proposed anticodons are shown in each case in complementary juxtapositions to

messenger coding triplets. ~~(see fig. 8).~~

The three sRNA's are compared for evolutionary homology in their base sequences in which the proposed helical regions, connecting regions, loops, and deletion are all aligned. The most obvious region of correspondence in all three sRNA's is in residues 64 to 72 which includes the sequence common to all yeast sRNA's (ref. ~~43~~ 44). Elsewhere the homology for all three appears only in the secondary structure. ~~(figure 8).~~ This indicates that the common archetype of the cistrons for these two sRNA's probably underwent gene duplication at a time subsequent to the gene duplication which separated the archetype of alanine sRNA's from the common archetype of serine and tyrosine sRNA's.

If sRNA's indeed evolved from a common origin by gene duplications, it would suggest that the genetic code evolved from a small number of anticodons that underwent differentiation as the number of sRNAs became increased by gene duplication (ref. ~~39~~ 40). Each duplication could then give rise to a new anticodon.

A clue to the nature of very early proteins is possibly to be found in the ferredoxins. These are small proteins that transport electrons under extremely electronegative conditions. They are present in certain anaerobic bacteria, such as Clostridia and Chromatium, and also in green plants. The amino acid sequence of spinach ferredoxin shows evolutionary relationships to the clostridial ferredoxins (fig. 6). The latter substances contain a

(insert fig. 6)

preponderance of alanine, cysteine, glycine, valine, isoleucine, aspartic acid, serine, and proline. This may indicate that these eight are the more "primitive" of the amino acids listed in table III.

PERSISTANCE OF A MOLECULE DURING THREE BILLION YEARS OF BIOLOGICAL EVOLUTION

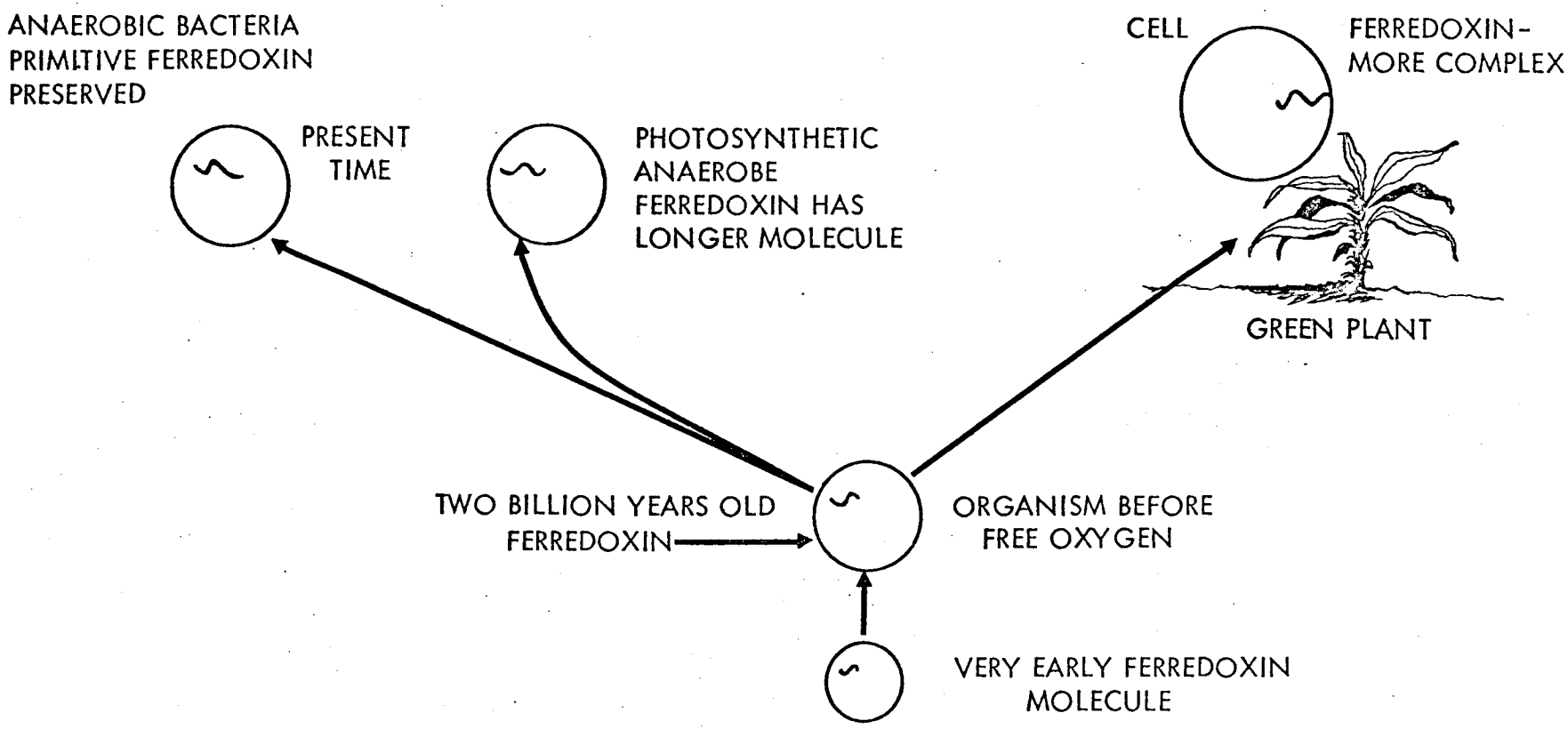


Figure 6.–

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3. LIFE ENVIRONMENT

According to present theories, life could not have originated on the Earth, Moon, Venus, or Mars if their environments were always as they are now. The belief that life exists beyond the Earth is based upon a unified concept that the primordial atmospheres of the Moon and Earth-type planets had a basically similar composition rich in methane, ammonia, and hydrogen, and containing oxygen almost exclusively in chemical combination as water. Up to a point, therefore, it is thought that the chemistry and development of protolife on Earth and its neighbors may have been similar. Each, however, eventually went its own way. Life on Earth has indeed contributed greatly to changing the Earth's atmosphere from a reducing to an oxidizing environment.

In studying chemical evolution it has been tacitly assumed that primitive Earth conditions no longer exist anywhere on Earth and can therefore be only satisfactorily simulated in the laboratory. This assumption is not absolutely correct. There are micro-environments in nature, such as volcanoes, fumaroles, hot springs, and isolated areas in desert lands, mines, and special areas where reducing gases occur locally and conditions ~~which~~ may be quasi-primitive. If some sort of organic synthesis, which can be demonstrated to be abiological, occurs in these specialized environments, we would have further support for the hypothesis of chemical evolution. The chemical study of exotic environments is an integral part of the study of life environments.

Present life on Earth grows and reproduces in a surprisingly wide range of physical and chemical environmental conditions. These extreme environmental parameters are being carefully determined, especially to provide information for making more accurate predictions of whether

indigenous or introduced organisms can grow on other planets. This information will also enable design of more effective remote life-detection instruments.

An interdisciplinary study of life in "exotic" or "extreme" environments on Earth has been initiated by NASA. We are now reaching the point in history where we are beginning to probe for life on other planets — planets whose contemporary environments and evolutionary history are virtually unknown, but surely quite different from the "average" Earth. The philosophy and technology with which we contemplate and approach the problem of study of extraterrestrial life, (and indeed, life on earth), is based on Earth "averages." In fact, many of our life-detection devices are essentially designed to study extraterrestrial organisms on Mars under simulated Earth conditions.

Some of the reasons for this are the lack of understanding of the relationship between life and its environment, the response (both long and short term) of organisms to their environment, and tolerance limits of life in extreme environments. An understanding of the origin and evolution of life, either on Earth or elsewhere in the universe, can come only through consideration of the physics and chemistry of the young planet, that is, molecular evolution, as well as biological evolution.

The ecology and the nutritional and physiological adaptation of microorganisms in non-arable soils is of immediate concern to the life-detection program. Present knowledge indicates that the Martian surface is much more similar to our non-arable, than to our arable, soils, yet practically all of our knowledge of soil microbiology has derived from, and may be largely applicable only to, arable soils. Increase of our general knowledge of the microbiology of non-arable soils is an appropriate objective to

increase our confidence in the selection of the most cogent experiments for life-detection systems.

Study of exotic environments provides sites of the most variable and most unusual habitats on Earth for testing instruments *in situ*. The field ecological studies allow understanding of the distribution, abundance, and interactions of microorganisms with their environment in unusual habitats.

NASA has supported studies of a wide variety of hostile and extreme environments to determine the ranges of the environmental factors. Many species of living organisms were collected and ecological studies ~~were~~ made of their growing conditions. Many organisms have been studied in various laboratories to determine their environmental limitations and relevant physiological mechanisms. This appears to be a most promising area of research.

Expeditions and ecological studies, undertaken in various parts of the world, have increased our understanding of the exact environmental ranges under which Earth life can grow and have provided organisms for laboratory study of the mechanisms of adaptation to extreme factors.

Life on Earth is found in almost every possible environment,ranging from the bottom of the ocean to the highest mountain tops and from cold arctic habitats to the tropical jungles and hot springs. It is found in some volcanic craters, deep wells, salt flats, and mountain snow fields. Earth life has invaded and become adapted to a very wide range of habitats. The physiological and morphological adaptations of life are exceedingly diverse and complex.

Ecological studies of hot dry deserts have been made in the Sonoran Desert of Arizona; Death Valley, Mohave and Colorado Deserts, California; Baja California; Kau Desert, Hawaii; Eastern Sahara Desert, Egypt; and

deserts in Israel and New Zealand (Cameron, refs. 45, 46).

Cold deserts have been studied in the dry valleys of Antarctica (Cameron, ref. 47) including McKelvey Valley and Taylor Valley. The Antarctic polar region — one of the coldest deserts on Earth — has a climate more rigorous and severe than in any other land area on this planet. It is characterized by a very dry atmosphere, the lowest mean temperature on Earth, strong, persistent, dry cold winds, and 5 months of darkness. Bacteria, molds, algae, and other organisms were collected from the ground surface and in permafrost (Fig. 7).

(Insert Fig. 7)

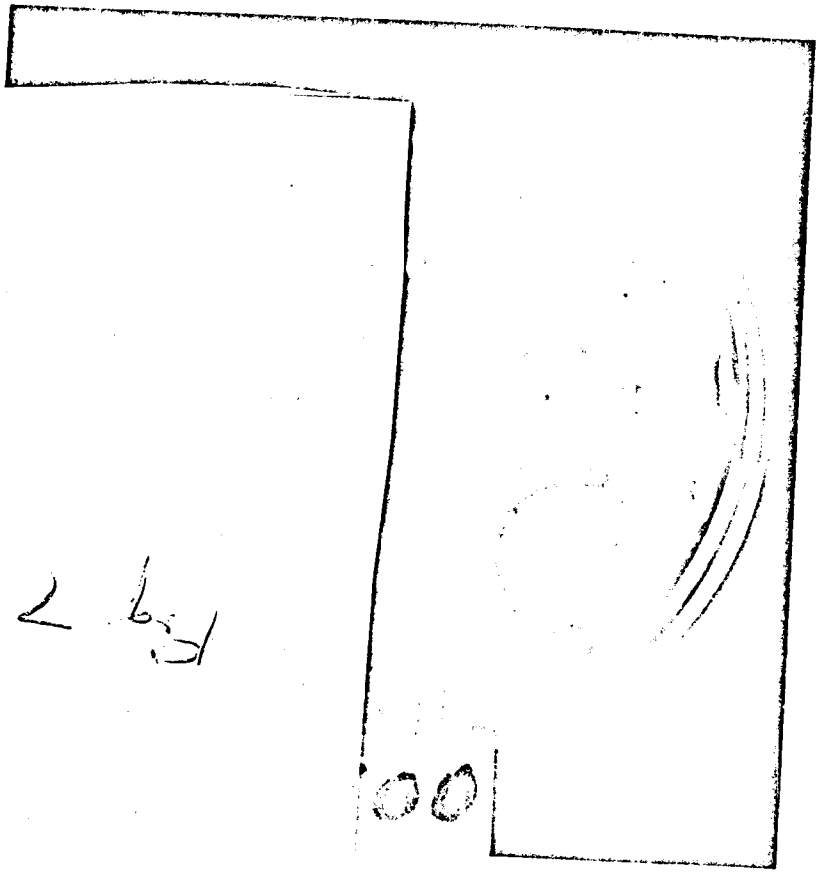
Cold alpine habitats and organisms have been studied in the high Cordillera Mountains of Chile; White Mountains, California (ref. 48); the Rocky Mountains, Colorado (ref. 49), Cascade Mountains, Oregon; ice fields in Juneau, Alaska; the Valley of 10,000 Smokes, Alaska (ref. 50), and Mt. Seymour, British Columbia. Ecological studies have been made of these mountain areas, and red and green snow algae have been studied in detail and new species described (Curl, ref. 51).

Thermophilic organisms have been collected in hot springs in Yellowstone Park, Wyoming; in Death Valley, California; and other hot springs in Western North America. Special studies on thermophilic algae and molds were carried out on organisms in the laboratory.

Volcanoes have been of special interest because of the reducing atmosphere containing hydrogen, ammonia, and methane in the craters. Collections of volcanic material have also been made for studies on synthesis of organic material related to life. The volcanoes studied were Sertsey Volcano, Iceland, and volcanoes in Hawaii and Italy.

LIFE IN ANTARCTIC DRY VALLEYS

BACTERIA AND MOLDS
FROM SURFACE SOIL



BACTERIA FROM PERMAFROST
18 INCHES DEEP

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Highly saline habitats have been studied carefully because of special interest in the water relations of halophilic organisms. Brine pools, salt flats, and bitterns, have been examined, and halophiles ~~were~~ collected and studied in the laboratories. Special studies have been made by Sweeney (ref. 52) at Owens Lake, California.

Organisms for laboratory study have also been collected from deep oil wells and ocean depths; from sulfur mines in the Cordillera Mountains, Chile; and from sulfide-rich environments. Various species of organisms have been collected from special habitats such as an ammonia-rich soil, where an organism which appears to be the same as Kakabeckia umbellata was discovered by Siegel (ref. 53) and which was originally described from ancient rocks over 2 billion years old. Microorganisms have been collected from high-altitude stratosphere by means of special air samplers on balloons at altitudes up to 130,000 feet (ref. 54).

4. SEARCH FOR EXTRATERRESTRIAL LIFE

In viewing the origin and nature of living systems from the most general point of view, the following questions are raised: (1) How did life arise on the planet Earth? (2) Does life exist elsewhere in the Universe? (3) What are the general features of life which would be operative on any planetary surface? (4) Using abiogenic systems, how far can we go toward the synthesis of recognizably living forms? The background of these biological questions lies in the physical nature of the universe, the cosmic abundance of the elements, and the distribution of energy in forms suitable for organic synthesis.

The search for extraterrestrial life is designed to implement the biological investigation of the solar system. While much of the planning has been devoted to the study of Mars it should be recognized that the Moon and other planets of the solar system are also objects of significant biological interest. The study of sites on the Moon which are inaccessible to astronauts, the probing of the upper atmospheric layers of Venus, and the analysis of interplanetary debris in the asteroid belt are programs of biological pertinence. The present emphasis on Mars exploration is not intended to detract from the importance of other objects in the solar system.

The approaches to the observation of complex features of a planetary surface, briefly but inaccurately dubbed "life-detection experiments," can be divided into three types:

(1) Chemical Composition: Search for organic compounds,

identification of organic compounds, identifications of specific molecular architecture, and search for high molecular-weight compounds.

(2) Chemical Activity: Transformation of organic compounds into gases or other easily detectable products, gas exchanges, changes in pH, Eh, conductivity, isotope exchanges, and changes in fluorescence.

(3) Morphology (in its widest sense) : Observation of particle size, observation of particle number, motility, shapes of particles, and images.

Certain results of these observations may then be interpreted as motility, growth, metabolic activity, optical specificity, characteristic shape, and familiar biochemical compounds, in short, what is known as "life."

Among the instruments required to carry out the various types of determinations listed above, certain analytical tools emerge as being particularly versatile. The combination of gas chromatography with mass spectroscopy can serve to identify a variety of products (fig. 8). These gases may be the components of the atmosphere, the pyrolyzed products of soil (Insert fig. 8) components, or they may be the gases produced by metabolic reactions. Optically active compounds can be distinguished by gas chromatography since upon esterification with known optically active alcohols they form molecular species of different physical properties. Hence, the gas chromatography-mass spectroscopy combination can serve for chemical analyses, detect a variety of compounds, identify fragments of organic compounds, and resolve optical isomers. As a

GAS CHROMATOGRAPH
MASS SPECTROMETER FOR MARS SOIL ORGANICS ANALYSIS

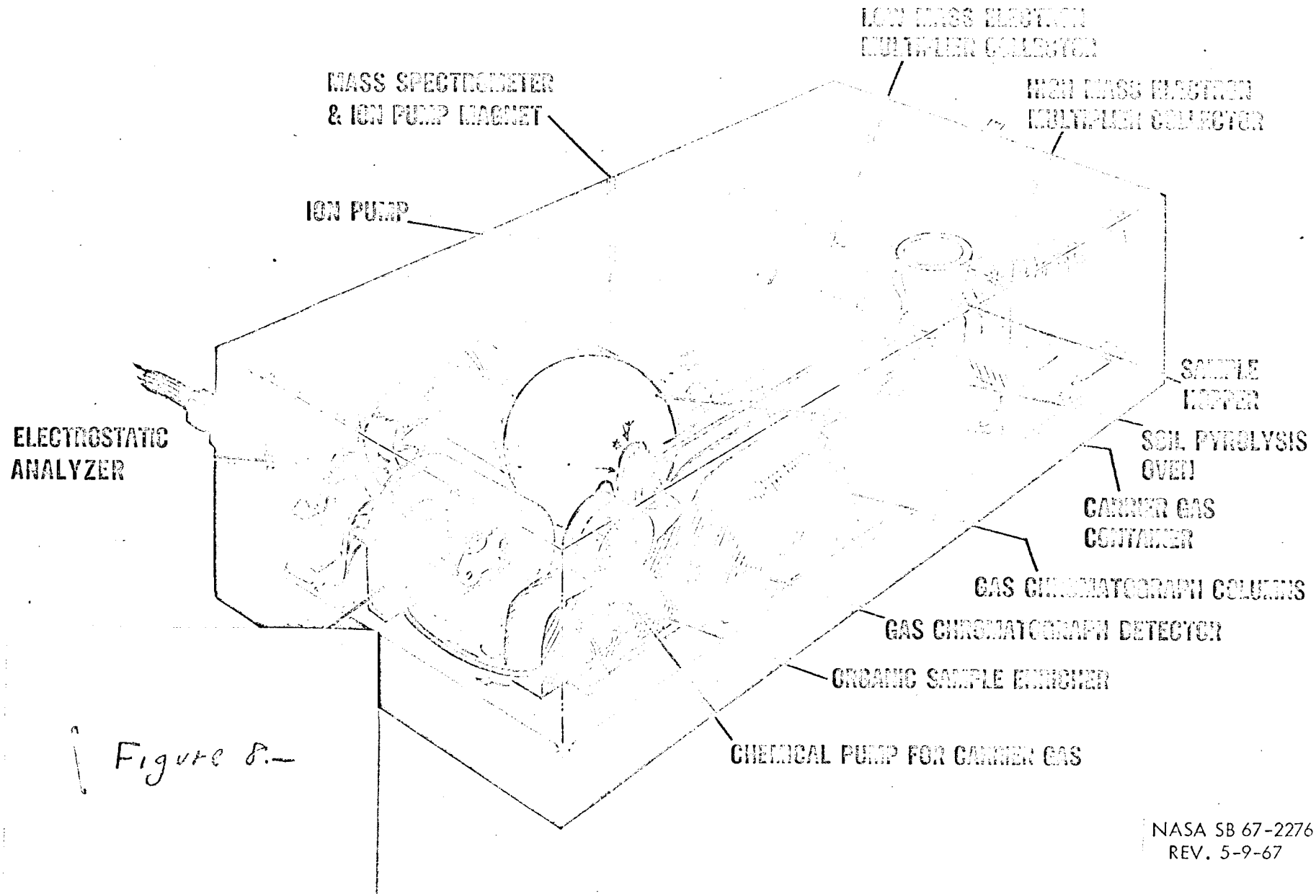

Figure 8.—

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sensitive detection tool for gaseous products it can also follow a variety of activities of living organisms in which gas exchange takes place. A miniaturized flight model has been developed at the Jet Propulsion Laboratory (fig. ~~8~~ 9).

(Insert fig. 9)

There are, on the other hand, a variety of instruments which would carry out significant and revealing measurements, but which are not tied in with a gas chromatography-mass spectroscopy system. These measurements include, but are not limited to, the following examples:

(1) detection of organic compounds by the formation of fluorescent compounds in heated samples;

(2) detection of change in number of particles (growth of microorganisms?) by change in light scattering, and distinction from mineral particles by X-ray scattering;

(3) detection of compounds of high molecular weight by spectroscopic shifts of adsorbed dyes;

(4) detection of motile particles by use of the flying-spot oscilloscope;

(5) microscopic and macroscopic imaging.

There are, in addition, valuable methods which would provide highly informative measurements but which have not as yet gone very far in the development towards flight instruments. The appearance or disappearance of unpaired electrons as a function of light, organic substrates, or other environmental changes could be detected by electron paramagnetic resonance and indicate the nature of the chemical reactions taking place. Chemical or metabolic reactions in the soil can be followed by presently available microcalorimeters which can detect the activity of a few dozen bacteria. The presence of large molecules and complex

MINIATURIZED MASS SPECTROMETER

Figure 9.—

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structures more organized than the environment can be demonstrated by differential thermal analysis regardless of whether the constituent molecules are based on carbon or not.

A. Meteorites

A variety of targets for the search for extraterrestrial life are available. One of these, which has been available to ~~in the hands~~ of scientists for some time, is the carbonaceous chondrite type of meteorite, the most famous example of which is the Orgeuil meteorite, first examined for extraterrestrial life by Louis Pasteur. This and other meteorites are being examined for carbon compounds associated with life (refs. 55-57 ~~13-15~~). The search includes studies of optical activity (Hayatsu, ref. 58) ~~(ref. 16)~~, the detection of sulfur compounds (ref. 59 ~~17~~), and electron-spin resonance studies (Schulz, ref. 60) ~~(ref. 18)~~, all directed towards establishing the presence of indigenous biogenic materials. Studies on other meteorites also sought organic compounds (ref. 61 ~~19~~), the nature of microstructures (ref. 62 ~~20~~), and an isotopic analysis of the sulfur content (ref. 63 ~~21~~). Evidence to date is still equivocal. The chief problem in such investigations is whether or not the biological material found is of terrestrial origin introduced by contamination subsequent to the fall of the meteorite (refs. 64-66 ~~22-24~~). In addition, other than that they are extraterrestrial, the sources of the meteorites remain unknown.

The techniques applied to meteorite analysis are the same as those in detecting evidence of very early terrestrial life in geologic sediments. "Chemical fossils" of biologic origin are being sought in addition to morphological evidence of primeval life on Earth. Work on samples of ancient formations has produced electronmicrographs of fossils whose structure closely resembles that of contemporary blue-green

algae, indicating that life was present on Earth more than three billion years ago (refs. ~~25-30~~ 67-72).

The best analytical method to determine the presence of the various chemical substances in ancient geological formations is the combination of gas chromatography with mass spectroscopy. The instrument of choice is the newly developed, highly miniaturized, double-focusing, high-resolution mass spectrometer (Wilhite, ref. 73) ~~(ref. 31)~~. The method not only reveals the presence of life-related chemicals but indicates that the quantities of these chemicals decrease at a rate consistent with the age of the sedimentary rocks. The technique permits the most precise identification of molecules yet possible. The range of chemicals sought includes hydrocarbons (Oro, et al, ref. 74), ~~(ref. 32),~~ isoprenoid alkanes (ref. 75 ~~33~~), ~~and~~ sterances and pentacyclic triterpanes (ref. 76 ~~34~~), and other types of organic materials (refs. 77 ~~35~~ and 78 ~~36~~).

B. The Moon

Next to meteorites, the Moon offers the most readily available source of evidence of extraterrestrial life. Ranger and, more recently, Surveyor photographs indicate that the Moon, whose entire surface seems composed of in-fall, may contain a wealth of relevant information. It seems likely that, since its formation, the Moon has been sweeping space, actively collecting large quantities of materials, including planetary fragments from a great variety of near and distant sources. An examination of Moon samples, when obtained, may reveal fossils or biochemical remains of once living organisms.

NASA has constructed a major laboratory installation (ref. 79 ~~37~~) at the Manned Spacecraft Center in Houston to quarantine the returning astronauts and lunar samples. Behind the most complete biological barrier ever constructed, a variety of terrestrial organisms will be

exposed to the returned material to see if any infectious diseases result. If no hazards are disclosed, the lunar samples will be distributed to laboratories throughout the world for continued search for evidence of life.

C. Mars

The prime target in this search is Mars, for years the focus of speculation on existence of extraterrestrial life. A recent assessment of all evidence by prominent scientists (Sagan, Feb. 80) ~~(ref. 38)~~ concluded that life most likely existed on that planet.

The pessimism stemming from popular press reports of Mariner IV photographs of Mars was not shared by qualified scientists. The degree of resolution required to detect direct evidence of life on Earth, confirmed (as predicted by the experimenters) the belief that no direct evidence for or against life on Mars had resulted from the flight. Indeed, a planet with the varying topographical features which were revealed for Mars (refs. 81 ~~39~~ and 82 ~~40~~) could provide a wide range of environments, some of which might be suitable for life. Careful study of these photographs established that Mariner IV had given as much encouragement as was reasonable to expect from the design and the purpose of its mission, and the Space Science Board accordingly re-emphasized its recommendations and goal.

Recent analyses of refined infrared spectra, obtained with an improved sensitivity interferometer, strongly indicate the presence of substituted methane in the Martian atmosphere (ref. 83 ~~41~~). It is felt that the presence of these compounds in the presence of the high concentrations of carbon dioxide (ref. 84 ~~42~~) could be explained only by the continuous re-supply of the substituted methane. This dynamic equilibrium could be explained by the participation of living organisms

in the gas exchange. Attempts to repeat the observations, however, have been marginal, indicating that the effect may be seasonal or that the original observation was an instrumental error.

Many investigators have contended that no form of life was possible on Mars because of heavy concentrations of atmospheric oxides of nitrogen, and that these gases were responsible for the advancing wave of darkness; the differences in shading and, possibly, in color of surface markings; the observed clouds; the polar caps; and other phenomena. Within this reporting period, however, a study (ref. 43) of existing infrared spectra of Mars with respect to the oxides of nitrogen concluded that life on Earth was able to exist in atmospheres, particularly in large cities, containing larger concentrations of nitrogen oxides than were apparent on Mars.

If terrestrial life can survive under Martian conditions, this would constitute evidence that life on Mars is not impossible. Accordingly, various candidate Earth microorganisms, seeds, and small macroorganisms have been placed in environmental chambers simulating the Mars environment to the extent that present knowledge permits. A number of Earth organisms survived and some grew under these conditions (ref. 44). A particular item of interest was the finding that vegetative cells exhibited greater survival tendencies than did spores.

In similar studies, not all of which were directed toward Martian conditions, various microorganisms have been grown in atmospheres of 100-percent methane, 100-percent hydrogen, 100-percent carbon dioxide, 100-percent ammonia, 50-percent hydrogen cyanide, and other gases and chemicals frequently considered lethal. In addition, certain plants have been shown to germinate and grow in some of these environments.

For example, onion seeds have germinated in ~~four~~ 4 days in 50-percent ammonia and 50-percent methane atmosphere at 25°C. (Siegel, ref. 87) ~~(ref. 45)~~. However, in all of these studies, attention should be drawn to the fact that a great unknown concerning Martian surface conditions is the moisture content of the "soil." It is possible that this could be lower than the moisture contents prevailing in any of the experimental chambers.

Difficulties in simulating the Martian environment in the absence of more accurate knowledge, has shifted emphasis toward establishing the environmental extremes which hardy forms of terrestrial life can withstand. Then, as planetary information becomes better defined, the biological data will already be at hand to permit a determination of what terrestrial forms, if any, could survive these conditions.

D. Venus

Venus has become of increased interest as a planetary target in the search for extraterrestrial life. Reports of excessively high surface temperatures have not been uniformly accepted. There appears to remain a possibility that the surface temperature of the planet may actually be within the "life zone" range, at least over certain portions of the planet. The clouds, sometimes thought to be ammonia, have now yielded evidence (Strong, ref. 88; Bottema et al., ref. 89) ~~(refs. 46 and 47)~~ that they are composed of water ice and that considerable free water exists in the atmosphere. In a theoretical study of the Venus atmosphere (ref. 90 ~~49~~), a ternary diagram of the elements carbon, hydrogen, and oxygen was generated. If the theory is correct, no equilibrium accumulation of polycyclic aromatics or any other hydrocarbons is possible.

Renewed interest in Venus derives also from a photograph of the

Earth taken from a spacecraft in the vicinity of the Moon. The photograph showed Earth apparently completely shrouded in white clouds, strongly resembling Venus as viewed from Earth, thus suggesting that closer observation of Venus than heretofore possible may show that the clouds are not continuous. If the clouds are not continuous, useful photographs of the planetary surface could be taken from spacecraft, and the sun's light may penetrate to the surface in amounts sufficient for photosynthetic activity, further buoying the prospects for life.

E. Jupiter

Only recently has Jupiter become of interest to biologists. Early considerations of the massive planet, its tremendous gravitational constant, and its awesome depths of atmosphere were thought to preclude the presence of life. Recently, however, the high gravitational constant was viewed as a possible biological asset in that all of the gases originally present on the planet should still be there. This indicates the strong probability that the presumed original reducing atmosphere of the planet prevails. Inasmuch as this condition is believed to have been responsible for the early evolution of organic compounds on Earth, speculation exists that the pre-biological and, perhaps, life-generating evolutionary processes still operate on Jupiter.

In laboratory experiments simulating the assumed Jovian atmospheric composition, the production of a number of significant organic molecules have been reported. The compounds include adenine, hydrocyanic acid, aminonitriles, and amino acids. Further speculation on Jupiter would suggest that an almost unlimited number of ecological niches might exist through the great depth of the atmosphere. Thus,

it is conceivable, that "life zones" are available to carry forth the evolutionary process continuously fed by the production of abiogenic organic compounds.

On the possibility that the atmosphere of Jupiter might contain large quantities of ammonia, growth studies of selected terrestrial micro-organisms in 100-percent ammonia atmosphere have been conducted ([illegible]) with the result that growth and survival occurred.

F. Methods and Experiments for Extraterrestrial Life Detection

In the final analysis, theoretical considerations of extraterrestrial life must be reduced to practical experiments if evidence is to be obtained. Six types of experiments are being developed under NASA support. They are: physical and chemical assays for simple organic compounds of biological interest, morphological evidence of living forms, detection of intermediate or complex biochemicals, evidence of metabolism, evidence of growth, and evidence of reproduction. These constitute attributes of life and are listed in approximately increasing order of significance. Experiments seeking physical and chemical determinations of relatively simple compounds would be those most likely to yield results. However, the evidence thus obtained could not definitely establish the presence of life. Organic compounds, up to a moderate degree of complexity, are known to be generated abiogenically. On the other hand, while those experiments seeking evidence of reproduction would yield the most conclusive results, they are the least likely to be successful. This is because such experiments must be based on assumptions concerning the general nature and function of the living systems sought.

Morphological experiments under development include a near-scan, high-resolution television system and a vidicon microscope. Should the extraterrestrial life encountered resemble Earth forms,

such experiments might be highly successful. However, it is quite possible that the alien morphologies may be ~~xxxx~~ completely unrecognizable. Furthermore, it is frequently difficult to distinguish terrestrial organisms, particularly unicellular ones or parts thereof, from inorganic surroundings.

The detection of biochemicals of medium to advanced complexity, particularly the macromolecules associated with terrestrial life, would be of great significance. Although, in themselves, they do not offer proof of life, nevertheless, their presence would establish, as a minimum, that abiogenic evolution had proceeded to a relatively advanced state. This would support the growing theory of prebiological evolution.

The unambiguous detection of metabolism, growth, or reproduction falls into another category, and could firmly establish the presence of life. Furthermore, metabolic rates, metabolic pathways, the manner of growth, and the generation period might be determined. Through such experiments, ultimately, the nature of the life encountered could be compared with terrestrial life for the paramount determination of whether the two forms of life are similar.

Inasmuch as an earlier publication (ref. 91) describes many of the life-detection experiments, the present report will deal only with some of the significant developments in this area within the past two years.

Physical and Chemical Assays for Simple Compounds. - While not producing direct evidence for life, determination of the chemical nature of the environment will be valuable in designing life-detection experiments for subsequent landers. The analytical methods being developed are discussed elsewhere in this report.

Biochemical Assays. - Perhaps the greatest advance in detecting and identifying chemicals of biological origin or interest has been in the field of gas chromatography and mass spectrometry. In addition, thin-layer (Balck, ref. 92) ~~(ref. 51)~~ chromatographic techniques are being developed to assay for chemicals of biological interest in soil samples taken by planetary landers.

In the case of the gas chromatograph, the surface material is pyrolyzed and the vapor fragments of large organic molecules are determined. In a similar type of experiment, the vaporized fragments are assayed by mass spectrometer. When both instruments are coupled, ~~together~~, a particularly powerful analytical tool results. Following pyrolysis of the sample, the gas chromatograph separates the vapor fragments into specific fractions. The fractions are fed into the mass spectrometer for accurate analysis. A frequency distribution analysis of these fragments can then be made with the aid

of a computer in an attempt to reconstruct the chemical composition of the compounds originally present in the sample (ref, 93). Reference 94 (Whitten) offers a description of a gas chromatograph-mass spectrometer specifically designed for space-exploration experiments. This general type of instrument has been used to analyze for paraffinic hydrocarbons in animal products (ref. 95).

Several researchers have been working on a particularly sophisticated application of the instrument, relating to the determination of stereoisomerism in biochemicals. For some time, stereoisomerism has been considered an excellent clue to the biogenic origin of any compound. Whenever the structures of biochemicals permit stereoisomerism, all known terrestrial living systems select for one form only. Until recently, attempts to detect this structural specificity in biochemicals have been confined to looking for the rotation of polarized light passing through a solution of the compound in question (ref. 96), the Optical Rotary Dispersion Experiment (fig. 10). More recently, however, the gas chromatograph (refs. 97, 98), the mass spectrometer (refs. 99-102), and the gas

(Insert fig. 10)

chromatograph-mass spectrometer combination (refs. 103, 104) have been used to detect stereoisomerism. The underlying concept is similar in the experiments using the gas chromatograph, the mass spectrometer, and the two in combination. The sample, possibly →

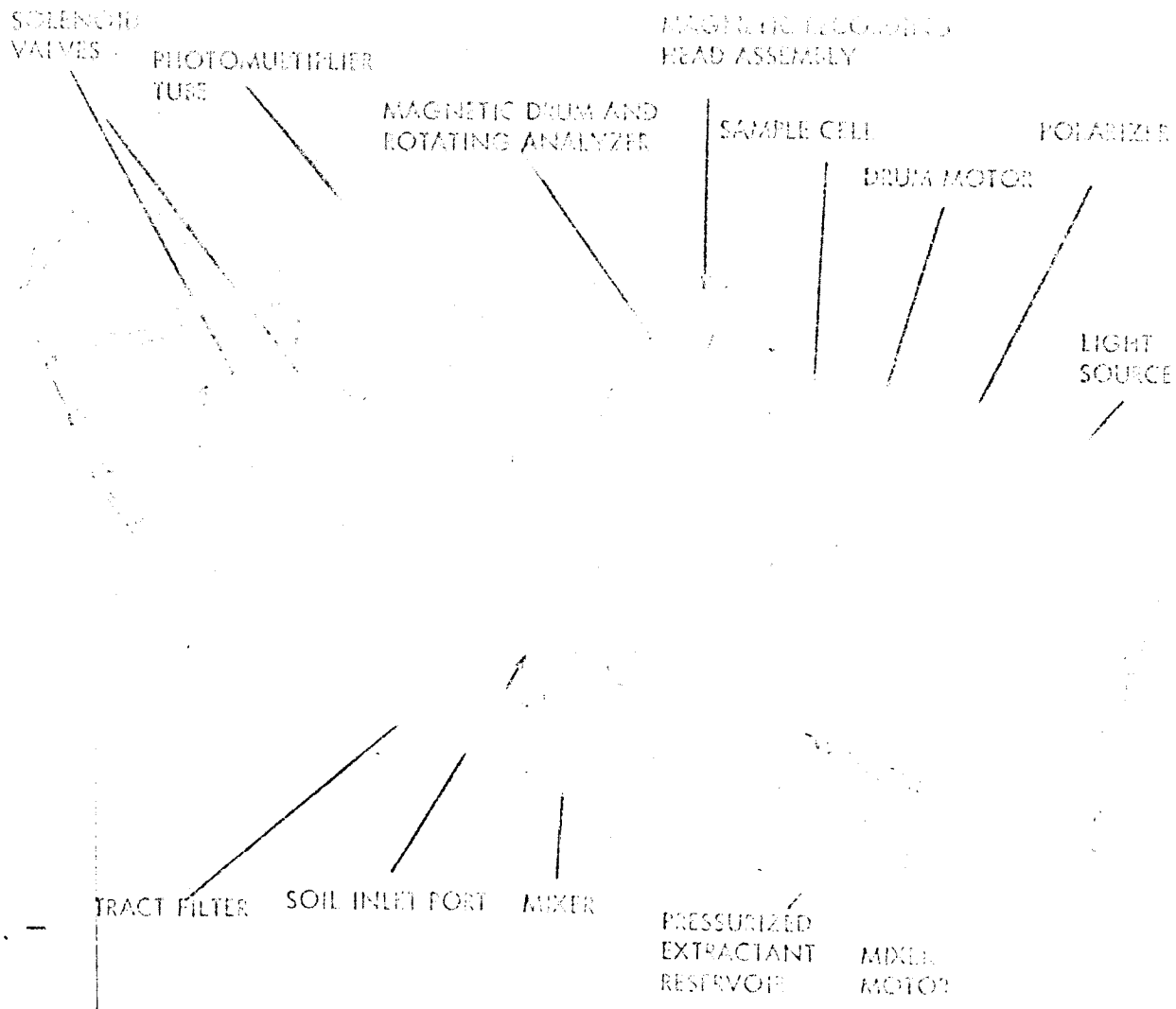

Figure 10.—

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Figure 10.—

containing protein or peptides, is degraded through pyrolysis or hydrolysis to the constituent amino acids. The amino acids are then esterified in a manner producing esters of enantiomers of the respective amino acids which then migrate through the gas chromatograph at different rates. That is, the esters made from a racemic mixture of the stereoisomers of the respective amino acids will have sufficient structural differences so that migration of the esters occurs at different rates. After separation in this manner, the esters are broken, releasing the original amino acids. The amino acids, now separated into stereospecific fractions, are then identified by the instrumentation as discussed earlier. The presence and the ratios of the stereoisomers are thus determined.

Another approach to the detection of biochemicals is through the observation of fluorescence. Generally, macromolecules associated with life fluoresce or can be made to fluoresce at characteristic frequencies. This fact underlies several life-detection tests under development for a number of years, such as the J-band and the ultraviolet spectrophotometer experiments. A better understanding of the mechanism of a fluorescence-producing complexing reaction which occurs upon the introduction of a cationic dye was recently achieved (Bean et al, ref. 105) ~~(ref. 64)~~. The fluorescent-assay technique is also being developed to detect enzymatic activity, as in the case

of the Multivator life-detection test. New discussions of this technique have been published (refs. 65 and 66).

Adenosinetriphosphate (ATP) is essential to energy transformation at the cellular level in all known forms of life. This fact is one of the important unifying concepts of biological evolution. Several years ago, ATP was abiogenically synthesized in the classic Miller-Urey type experiment. Thus, from a biological as well as a pre-biological standpoint, the detection of ATP on another planet would be highly exciting. Known as Diogenes, the firefly bioluminescent assay is under development (refs. 67-69) as a highly sensitive means for detecting ATP. The reactants, luciferase, luciferin, and magnesium are recovered from firefly lanterns. When this mixture is placed in aerobic solution and ATP is added, light is emitted. The amplitude is a measure of the amount of ATP. Advances in the Diogenes experiment have reported the detection of as little as 10^{-18} moles of ATP. Tests have detected the ATP from as few as 200 E. coli, one alga, or one protozoa within an elapsed time of two minutes.

Based on this concept, an instrument, Firefly, has recently been developed which can automatically execute four ATP assays in rocket-borne flight within a two minute period. Initially conceived to be used in determining the microbial profile of the Earth's atmosphere, this experiment might be performed during entry aboard a hard lander approaching Mars.

Metabolism, Growth and Reproduction. - As stated, evidence of metabolism, growth and/or reproduction would yield the least ambiguous data for concluding that extraterrestrial life has been discovered. Instrumental concepts to obtain one or more of these types of data have been reported in previous years. Perhaps the most advanced of these are the Wolf Trap, Gulliver, ~~the~~ Multivator, and Diogenes. An excellent summary of these and other life-detection instruments is given in Appendix I in reference 2.

A complete account of the current status of the Wolf Trap is provided in a recent final report (Buckendahl et al, ref. 112) ~~(ref. 70)~~ on that instrument. The instrument aspirates a surface sample into five separate growth chambers containing different media. Evidence for life is an increasing optical density in the culture, preferably in the form of an exponential growth curve. Continuous pH measurement of each culture is made to confirm the biological nature of the increasing turbidity. The latest model of this instrument can detect 1,000 cells of typical soil bacteria. It was recently field-tested successfully in mountain and desert environments in California.

Recent advances in Gulliver (fig. 11 ~~4~~) have also been reported (refs. 113 ~~71~~ and 114 ~~72~~). (Insert fig. 11) A new version of this instrument operating in situ has

IN SITU "GULLIVER" (OPERATING POSITION)

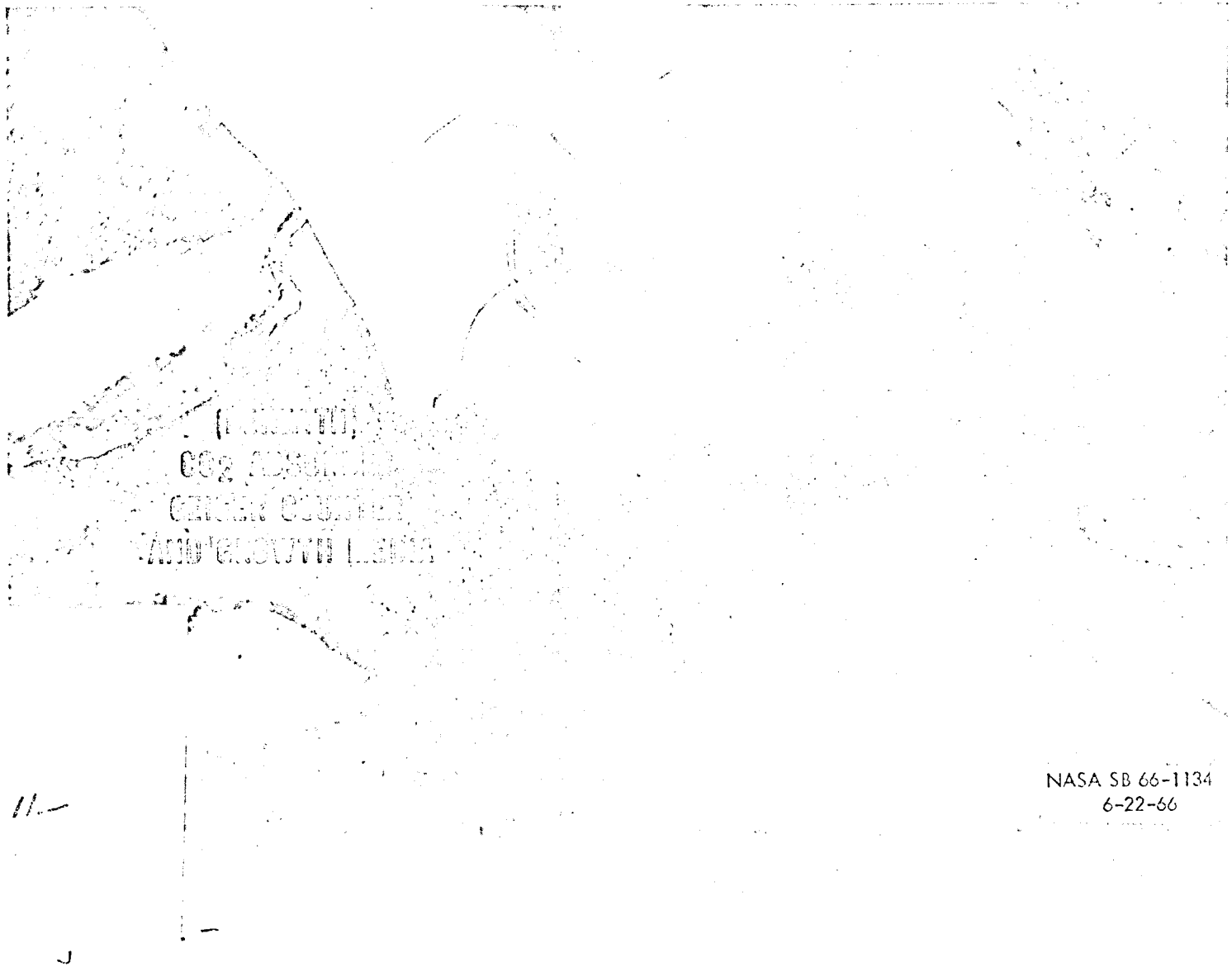

Figure 11-

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been demonstrated. Radioisotopes are applied directly to the surface of the planet as substrates. The instrument then detects metabolic assimilation of these substrates through the production of expired, labeled gases. Conclusive evidence of life has been obtained within a matter of minutes by this technique when performed manually on the soil. The instrument produced a positive test in approximately one hour.

The inclusion of the "delta time" concept into the Diogenes experiment converts it into a test for metabolism, growth, and reproduction. The detection of an increase of ATP with time in a culture of planetary surface material would be excellent evidence for life. Laboratory experiments have verified this approach over a broad spectrum of unicellular organisms.

Most biologists believe that any extraterrestrial life is likely to be carbon based. However, life detection experiments seeking the metabolic involvement of other important elements can improve the prospects for detecting carbonaceous life as well as non-carbon based life. Inorganic phosphate is essential to all known life. It is believed to have been evolutionarily selected because of its unique energy-storing capacity in the trimer form. Accordingly, a phosphate-uptake experiment (Levin, Ref. III) is being developed in which the disappearance of dissolved orthophosphate

from the culture medium is monitored as a function of time. A poisoned culture, or one in which phosphorylation is uncoupled, serves as a control. As few as 200 cells of E. coli per ml ~~milliliter~~ can be detected in this manner within 3 ~~three~~ hours. A similar experiment (ref. 111) ~~(73)~~, based on the same philosophy, is being developed to detect the uptake of sulfur, as sulfate (which, in an alien biochemistry, might substitute for phosphate) or otherwise.

An interesting "new entry" into the field of life-detection experiment candidates was recently described (Kok and Varner, ref. 115). ~~(ref. 74)~~ It seeks to establish oxyanion exchange between water and ions of phosphate, nitrate, and sulfate. This exchange would be attributed to metabolism. The system is applicable to carbon and non-carbon based biological systems equally. Only water, atypical in its stable isotopic composition, is added to the sample. Aliquots of the water are then examined at time intervals to detect the production of labeled ions of the oxyanions mentioned. The mass spectrometer is used as the analytical instrument.

G. Voyager Biological Laboratory

A major development in the preparations to conduct the search for extraterrestrial life has occurred over the past two years. Although NASA support in this area has largely been directed towards the support of individual life-detection experiments, such as those reported above, a growing realization has come to the biological community that the expense and importance of the planetary exploration program require the integration of individual experiments into a single instrument package generally designated as an automated laboratory (refs. 116-119) ~~75-78~~.

An intensive conceptual study (ref. ~~80~~ 120) of the Automated Biological Laboratory has been supported by NASA. Now designated the Voyager Biological Laboratory (VBL), this laboratory (actually a series of laboratories) would constitute the biology payloads for the Voyager Program. A concept model of the VBL is shown in figures ~~5~~ 12 and ~~6~~ 13. This is based on a study incorporating 38 different instruments (fig. ~~7~~ 14).

(Insert figs. ~~5,6,7~~ 12,13,14)

The VBL concept is based upon the plan of looking for as many characteristics of life as possible at one time. The VBL must therefore have the capability to reprogram its analyses, to replicate its experiments, and to perform the necessary computations on the data it obtains. It must also be capable of selecting and reducing appropriate data, and transmitting the information to Earth.

Ideally, the VBL would be capable of detecting existing life at the cellular level, and at higher evolutionary levels by means of more ambitious visual, acoustical, electronic, and mechanical means.

Characterization of life, if present, is a scientific goal of the biological exploration of a planet, and requires a computer-managed, reprogrammable, automated biological laboratory. Such a laboratory should provide reprogrammable computer controls with a set of laboratory functions and operations which would permit a large set of possible variations of the experiment and experimental conditions. The choice of the optimum variation will no doubt originate from Earth after the initial data are received. This approach is to be distinguished from the automation of a prewired, discrete set of experiments, even though integrated, the sequencing of which is

AUTOMATED
BIOLOGICAL
LABORATORY

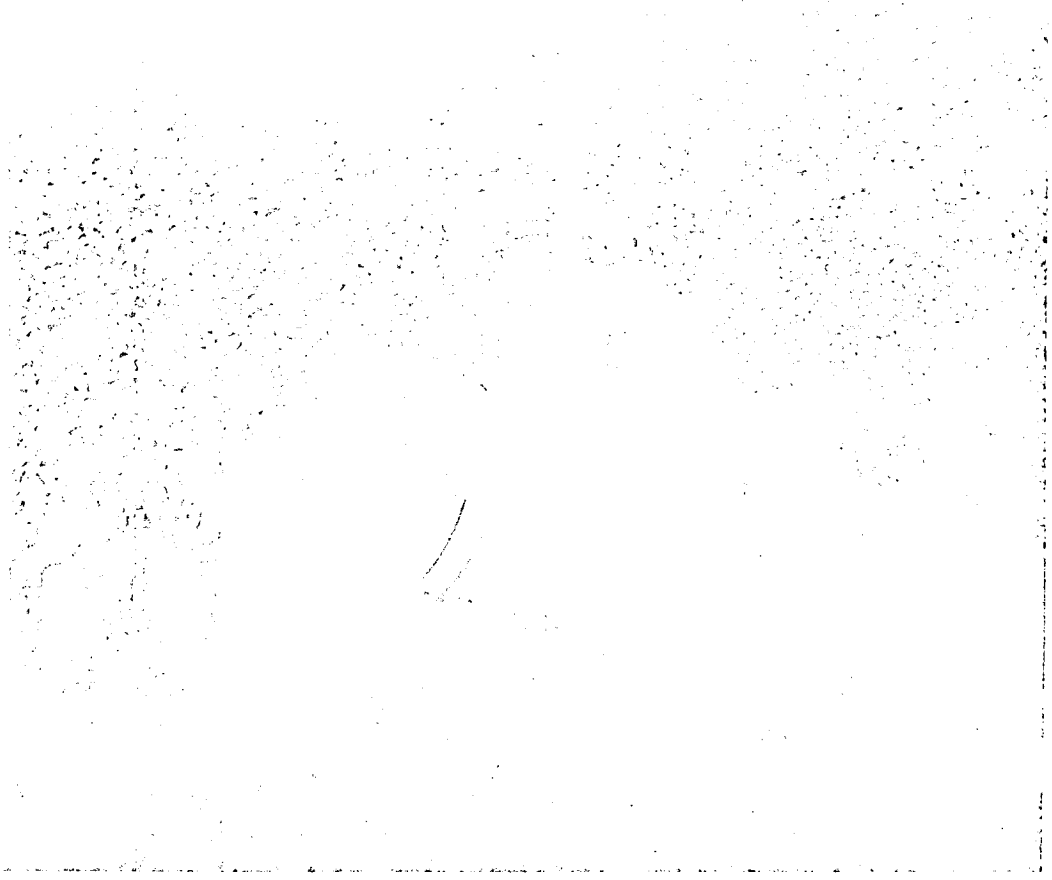
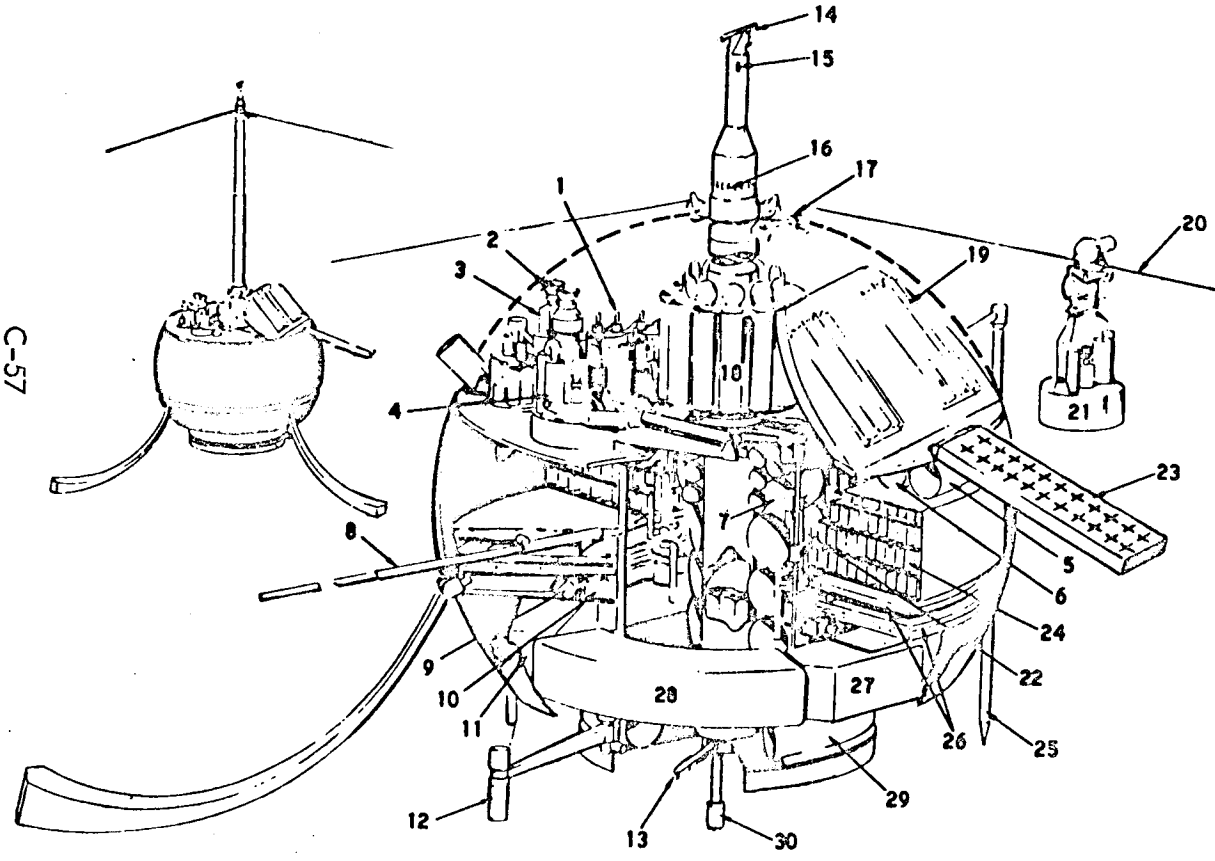


Figure 12.—

AUTOMATED BIOLOGICAL LABORATORY



1 ATMOSPHERIC GAS CHROMATOGRAPH
2 SOIL PULVERIZER
3 SEPARATOR SCALE
4 SURFACE SAMPLER
5 WATER STORAGE
6 CLEANING SOLVENT STORAGE
7 BULK STORAGE FOR GASES
8 PROBE FOR WIND TRANSPORTED PARTICLES
9 LEG SUPPORT TRACK
10 BATTERY
11 IR SPECTROPHOTOMETER
12 IN SITU CHAMBER
13 SURFACE SAMPLER
14 ATMOSPHERIC PROPERTY DETECTORS
15 MACROIMAGING PORT
16 MOTION DETECTOR
17 REMOTE SAMPLER HOIST
18 SOIL PRESERVATION CAPSULES
19 RTG
20 STAY WIRE
21 REMOTE SAMPLER
22 CULTURE-REACTION CHAMBER
23 DIRECTIONAL ANTENNA
24 REAGENT STORAGE (AMPULE)
25 SUBSURFACE PROBE
26 CHAMBER ATTACHMENT STORAGE
27 SPECTRAL ANALYZER
28 DATA AUTOMATION SYSTEM
29 WASTE STORAGE
30 CORE DRILL

Figure 13. -

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ABL EXPERIMENT COMPLEMENT

	INSTRUMENTS AND SENSORS	THERMOMETER	ATMOSPHERIC PARAMETERS SENSOR	GOLD FILM/Al_2O_3 H_2O VAPOR DETECTOR	MICROPHONE WITH RESONATOR	OMNIDIRECTIONAL MICROPHONE	2π BETA AND GAMMA COUNTER	CORE HOLE SONDE	SOIL MECHANICS APPARATUS	OPTICAL MOTION DETECTOR	OPTICAL DENSITY COMPARATOR	MACROIMAGING SYSTEM	INFRARED RADIOMETER	SOLAR INSOLATION RADIOMETER	pH METER	$Ba(OH)_2$ CONDUCTIVITY CELL	BETA IONIZATION COUNTER	ALPHA SCATTERING ANALYZER	GAS CHROMATOGRAPH NO. 1	GAS CHROMATOGRAPH NO. 2	GAS CHROMATOGRAPH NO. 3	GAS CHROMATOGRAPH NO. 4	GAS CHROMATOGRAPH NO. 5	UV AND VISIBLE SPECTROPHOTOMETER	IR SPECTROPHOTOMETER	FLUORIMETER	POLARIMETER	MASS SPECTROMETER
1	ATMOSPHERIC PRESSURE, TEMPERATURE, AND WIND																											
2	ATMOSPHERIC HUMIDITY																											
3	WIND TRANSPORTED PARTICULATE MATTER																											
4	ACOUSTICAL MONITOR																											
5	ULTRAVIOLET AND VISIBLE INSOLATION																											
6	BETA AND GAMMA RADIATION BACKGROUND																											
7	ATMOSPHERIC CONSTITUENTS																											
8	SOIL TEMPERATURE AND WATER VERSUS DEPTH																											
9	SOIL ELECTRICAL CONDUCTIVITY																											
10	SOIL DENSITY BY GAMMA SONDE																											
11	SOIL MECHANICS																											
12	SOIL SAMPLE ENCAPSULATION AND PRESERVATION																											
13	SOIL ELEMENTAL ANALYSIS																											
14	SOIL GAS ANALYSIS																											
15	SOIL SOLUBLE INORGANIC IONS AND pH																											
16	SOIL ORGANIC MATERIAL																											
17	SOIL GAS EXCHANGE																											
18	AMINO ACID ANALYSIS																											
19	AMINO ACIDS AND OPTICAL ACTIVITY																											
20	PORPHYRINS																											
21	FLAVINS																											
22	NONSAPONIFIABLE LIPIDS																											
23	SAPONIFIABLE LIPIDS																											
24	MACROMOLECULES BY VISIBLE ABSORPTION																											
25	MACROMOLECULES BY ULTRAVIOLET ABSORPTION																											
26	OPTICAL ACTIVITY OF WATER SOLUBLE MACROMOLECULES																											
27	WATER SOLUBLE MACROMOLECULES BY PYROLYSIS GAS CHROMATOGRAPHY																											
28	FUNCTIONAL GROUP ANALYSIS																											
29	LIGHT-STIMULATED $C^{14}O_2$ FIXATION AND DARK $C^{14}O_2$ FIXATION VERSUS TEMPERATURE																											
30	CO_2 EVOLUTION BY NORMAL METABOLISM																											
31	$C^{14}O_2$ EVOLUTION FROM LABELED SUBSTRATE																											
32	$C^{14}O_2$ UPTAKE IN LIGHT AND DARK AND SUBSEQUENT EVOLUTION BY METABOLISM																											
33	CULTURE EVALUATION AND GROWTH DETECTION																											
34	MOTION DETECTION																											
35	MACROIMAGING AND INFRARED SCANNING																											

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Figure 14.-

perhaps controlled by local computation of data or from Earth. Besides its impact on the particular experiment under discussion, such a laboratory would have other interesting unique characteristics. Participating scientists would be competing not for payload weight or engineering support, but for pro-rated time on the system. Computer logic would direct the execution of the main subroutines of, say, sampling and biochemical analysis, but would be supervised and reprogrammable by command from Earth to allow full advantage to be taken of stepwise or scientifically competitive programs. Such a concept allows for the broadest possible scientific participation. The scientist in his own laboratory could direct and evaluate the results of an experiment carried out on Mars. He could initiate an experiment any time during the lifetime of the laboratory without the need of having had to design space-qualified hardware years earlier.

Such a laboratory should be coupled with facilities for visual observations on scales ranging from microscopic to macroscopic. The singular value of visual observations is based on their non-specificity and generality. This advantage can only be preserved by the transmission of enormous amounts of data, at least initially without the limitations imposed, for bandwidth reduction purposes, of a set of possible a priori interpretive algorithms. It is the interaction of these data with the collective store of scientific intelligence represented by observers viewing the images on Earth that gives power to the experiments that can be carried out by the reprogrammable biological laboratory.

5. SPACECRAFT STERILIZATION AND PLANETARY QUARANTINE

A. Background of the Sterilization Requirements.

The United States effort in controlling terrestrial contamination of the Moon and planets has greatly expanded within the past three to four years. NASA created the Office of Planetary Quarantine in 1963 with responsibility for preservation of the planets from Earth microorganisms. The search for extraterrestrial life must not be compromised by contaminants on spacecraft launched from Earth. Contamination of scientific apparatus would prevent determination of whether positive results were due to Earth contaminants or organisms native to the explored planet. In addition, Earth contaminants may alter or destroy the planet's flora or fauna.

Responsibility for implementation of the program is in the Bioscience Programs Division of the Office of Space Science and Applications. The program consists of developing and publicizing techniques of spacecraft sterilization, estimating probabilities of contamination from both sterile landing capsules and non-sterile spacecraft, monitoring throughout spacecraft assembly, and certifying microbial levels of completed spacecraft or the sterility of the capsule where sterility is required (ref. [21]).

The NASA space policy has embodied various recommendations of the Space Science Board (SSB) of the National Academy of Sciences-National Research Council (NAS-NRC). The NAS-NRC was one of the

first scientific groups to recommend that the biological entity of the planets be preserved. Of similar influence, but on the international level, is the Committee on Space Research (COSPAR) of the International Council of Scientific Unions (ICSU). COSPAR's charter (ref. 122 2) aims to

> "provide the world scientific community with the means whereby it may exploit the possibilities of satellites and space probes of all kinds for scientific purposes and exchange the resulting data on a cooperative basis."

The Fourth International Space Science Symposium (COSPAR, Warsaw, 1963) made recommendations which were to become a part of NASA's Planetary Quarantine Program. These recommendations were: (1) Venus and Mars landers should be sterilized before launch; (2) Venus and Mars fly-bys should have a trajectory such that the probes themselves and all ejects miss the planets; and (3) decontamination of lunar landers would be acceptable in lieu of sterilization inasmuch as the lunar environment does not appear to offer a suitable habitat for multiplication of terrestrial microorganisms.

The basis of the NASA sterility requirement lies in Resolution 26, and as subsequently modified, submitted to COSPAR by the Consultative Group on Potentially Harmful Effects of Space Experiments. This resolution was adopted by the General Assembly at the Fifth International Space Science Symposium held in Florence, Italy, in May 1964. The highlights of Resolution 26 are as follows (ref. 122 3):

> 26.5 - The level of sterilization of a planetary lander be such that the probability of a single viable

microorganism aboard the spacecraft be less than 1×10^{-4}; the probability of planetary impact by an unsterilized vehicle be less than 3×10^{-5}.

26.6 - Sterilization requirements for lunar landers will be less severe than those for planetary landers.

26.7 - Nations are urged to forego planetary launchings until sterilization requirements can be met.

26.9 - The Consultative Group is urged to convene an International Sterilization Conference whenever it deems necessary, providing sufficient participation by both of the leading space launching nations is assured.

These recommendations were tentatively accepted by COSPAR as interim objectives for sterilization parameters. Nations involved in space activities were urged to report changes in objectives, directions, or technology whenever the results of new studies so indicated.

NASA has concluded that the probability of contamination of Mars can be analyzed by any number of mathematical models, each resulting in somewhat divergent values. Therefore, an attempt will be made to meet these probabilities even though absolute proof of having met the requirement is not possible (ref. 123).

At the Florence Symposium, Hall and Bruch (ref. 124), discussed the procedures deemed necessary to prevent planetary contamination. They rejected the concept that deep space environment will sterilize spacecraft since low temperature and vacuum are used to preserve bacteria for long periods. Anaerobic sporeformers, in particular, are believed capable of withstanding the space and Martian environ-

ments and recent studies suggest that some vegetative cells might adapt to Martian conditions and multiply. Of the available sterilization methods, only dry heat and radiation achieve internal and external sterilization. Strict standards of cleanliness through use of clean rooms, filters, protective clothing, and personnel training programs in preventive measures should result in a spacecraft with no more than 10^5 micro-organisms. Further engineering developments should produce spacecraft components capable of withstanding sterilization temperatures; heat labile components should be kept to a minimum and techniques of sterile insertion developed. ~~(ref. 5).~~

The Army Biological Laboratories (Ft. Detrick) originally reported that a microbial accumulation of 2×10^8 organisms could be expected on a spacecraft with a surface of $10m^2$. It was later realized that this figure did not account for the microbial die-off and, therefore, the count could be expected to be several orders of magnitude below the original estimate.

In its 1964 report to COSPAR (ref. 125 ~~6~~) the SSB recommended dry heat for the sterilization of spacecraft and their components, but noted that the time/temperature relationship was in need of additional refinement (fig. 15). The SSB reported that survival rates had been obtained for bacterial spores and indigenous soil flora (insert fig. 15) but emphasized that the survival curves were not linear, since some organisms were heat resistant for long periods. It was also noted that culture media varied in ability to promote growth of heat-damaged organisms.

CONCEPTUAL DEVELOPMENT OF STERILIZATION TIME-TEMPERATURE RELATIONSHIP

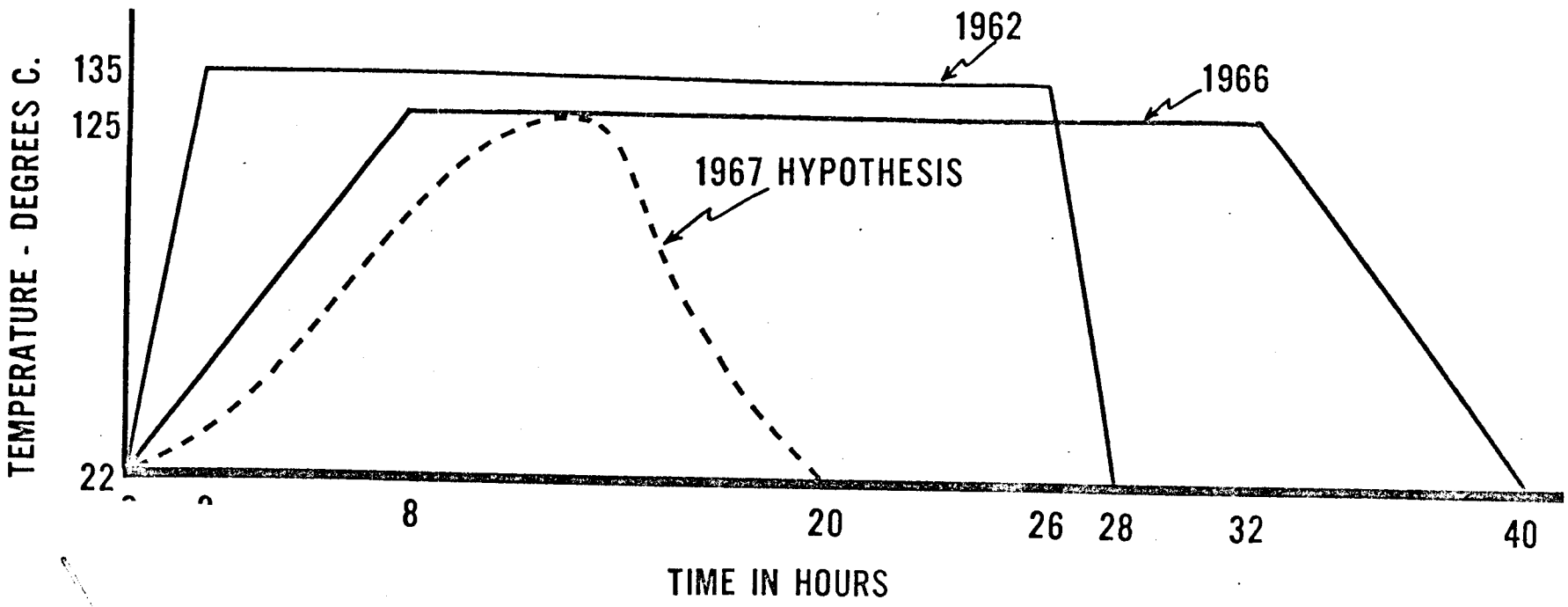

Figure 15.-

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Standards for sterilization of spacecraft were cited by a study group on the grounds that contamination could alter a planet's ecology by destruction of indigenous biota. It would also negate any opportunity to investigate the pre-biological chemistry of that planet. The probability of contamination could be reduced by controlling the spacecraft trajectory, continuing spacecraft sterilization programs, and introducing programmed self-destruct systems (ref. 126).

The SSB recommended clean-room assembly as an essential step in the production of a sterile lander (fig. 16). ~~(ref. 8).~~ This was based on studies (Insert fig. 16) of the Army Biological Laboratories wherein microbial fallout in uncontrolled areas was found to be ten times greater than in controlled areas (ref. 127). Fallout contamination on walls and ceilings was reported to be one-tenth that on upward-facing horizontal surfaces. The importance of humidity in the effectiveness of ethylene oxide as a decontaminating agent was noted as well as the fact that Bacillus subtilis spores (1×10^7) could be sterilized in 48-72 hours at 105° C.

At the request of NASA, the American Institute of Biological Sciences established the Spacecraft Sterilization Advisory Committee in 1965. The Committee reviews the broad aspects of the spacecraft sterilization program, examines the research data on sterilization upon which policy decisions are based, and prepares recommendations for changes in sterilization policy. The Committee membership consists of nine scientists.

The Lunar and Planetary Programs Office has the function of spacecraft-sterilization engineering. Problems facing the engineer in the

VERTICAL LAMINAR FLOW CLEAN ROOM

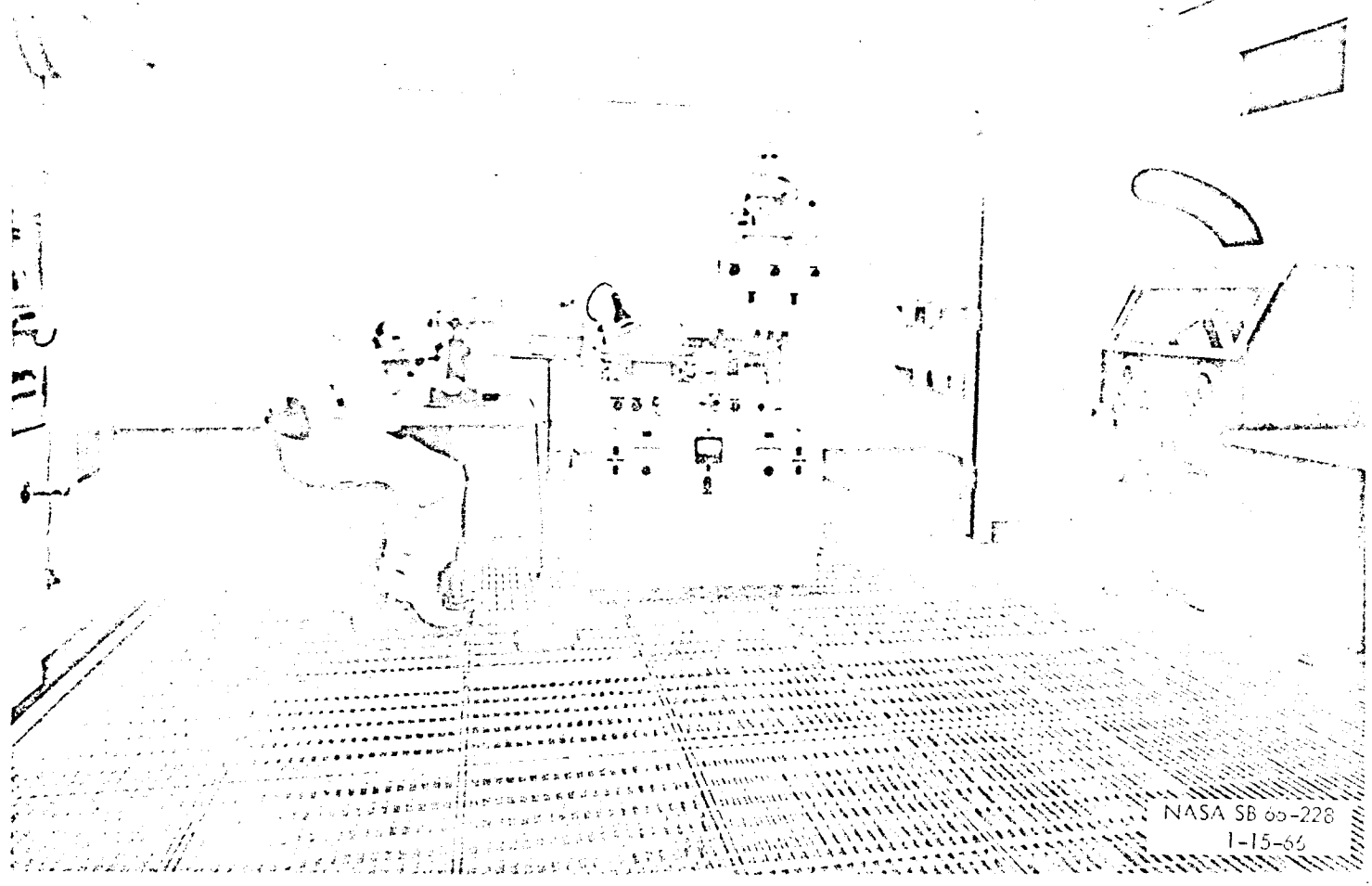

Figure 16.—

production of suitable hardware for the space program were stated by Nicks and Miles (ref. 128). The program is essentially a three-step procedure comprised of clean manufacture and assembly in a controlled environment, terminal sterilization, and the prevention of recontamination. In addition, spacecraft components must withstand 24 hours exposure to 12-percent ethylene oxide — 88-percent Freon 12, undergo three heat cycles at 145° C. for 36 hours, survive a variety of environmental tests, and still have an operational reliability of 10,000 hours. It was found that temperatures below 135° C. can be applied to components for periods longer than 24 hours, which reveals that degradation of hardware may be more a function of temperature than of time. The obvious disadvantages of heat sterilization are component degradation and the prevention of repair inside the sterile barrier. Some progress has been reported in the use of ionizing radiation for sterilizing purposes, although its application has not been approved by NASA, since some degradation in polymeric plastics as well as electronic components has been noted. Development of fracture-resistant components would eliminate the need for the internal sterility requirements in many instances. Biggest design problems revolve about impact limiters, batteries, electronic piece parts, soldered connections, and polymers. Components nearest the outside of the spacecraft must be more heat-resistant that those closer to the center because of the heat-up time, a problem of significance to the design engineer.

During the Buenos Aires COSPAR meeting in 1965 the Consultative Group on Potentially Harmful Effects of Space Experiments established

a Panel on Standards for Space Probe Sterilization.

No ¶ The objectives of spacecraft sterilization were reviewed by Davies and Horowitz (ref. 129). Prevention of confusion of exobiological experiments and contamination of the planetary ecology was believed to be of primary importance; although some microorganisms probably would survive the harsh environment, multiplication of terrestrial organisms appeared unlikely. Dry heat alone would not solve the problem of contamination because: (1) some spacecraft components do not survive the heat treatment and (2) certain scientific experiments would also suffer the effects of high temperature. A lowering of the sterilization temperature by 10°-15° C, while increasing time of exposure, would ease engineering problems considerably. Concerning release of entrapped microorganisms in solids and electronic gear, it was asserted that electronic components are bacteriologically clean and that there is no diffusion of spores within solids. On the basis of some experiments revealing only slight erosion of Lucite it was concluded that release of spores from solids would be unlikely. Recommendations included investigation of statistical studies to determine distribution of microorganisms within components, development of sterile insertion techniques, and further studies to determine the heat sensititivity or heat resistance of various microorganisms. The opinion that some internal contamination was permissive was contrary to the opinion of the Space Science Board.

In its 1966 report to the COSPAR Executive Council, (ref. 130), the Consultative Group recognized that the 1964 recommendations were too detailed and recommended revision by suggesting 1×10^{-3} as the basic

probability that a planet will be contaminated during the period of biological exploration, and that this figure be accepted as the criterion for the exploration of the several planets of biological interest. In applying this criterion the Group recommended that:

(1) Every available means should be utilized by members to insure that all factors which might contribute to the total planetary contamination probability are considered.

(2) Probabilities which are assigned to each individual factor should be determined by experiment, testing, or experience whenever possible and practical.

(3) If probabilities for certain factors cannot be determined by experiment, testing, or experience, conservative methods should be used for statistical calculation of these probabilities.

(4) The mathematical model used for final combination of probabilities for individual factors should be conservative and based on standard statistical procedures.

(5) The computations should assign a probability of not less than 10^{-3} that sterilization-damaged organisms landed on the surface of a planet will grow and spread (changed from an inferred 10^{-2} in the COSPAR model on the basis of the probability of random species of terrestrial sterilization-damaged organisms infecting random media). The similar value for organisms that have not been sterilization-damaged should not be less than one.

(6) Members of COSPAR should make available to it, within three months after launch, the sterilization procedures and computations used for each flight to assure prevention of contamination of the planet consistent with the probability of 10^{-3} for the period of biological exploration.

(7) The values and rationale upon which these computations may be based should be reviewed by COSPAR each year and updated in the light of newly acquired knowledge.

(8) Constraints in terms of probabilities should be replaced by specific procedures as soon as such procedures can be developed in details that can be applied to any mission.

Light, et al. (ref. 131), in reviewing COSPAR premises relative to sterilization requirements, believed that newer estimates of the period of unmanned exploration of Mars made a change in these requirements necessary. The probability of the release of microorganisms during exploration of the planet is assumed to be 1×10^{-3} and prompts two constraints: (1) the probability of impaction by a non-sterile vehicle shall not exceed 3×10^{-5}, and (2) the probability of the release of a viable organism on the Martian surface or in the atmosphere on any one mission shall not exceed 1×10^{-3}. Five possible contaminating sources cited were: (1) failure of the sterilization procedures, (2) recontamination of a sterile capsule, (3) accidental impact of the launch vehicle, (4) accidental impact of a non-sterile spacecraft, (5) impact of ejecta.

Through the use of clean-room technology, the biological load of a spacecraft can be held to 1×10^{7} organisms before terminal sterilization (fig. 11). The time required to reduce the microbial population 90 percent (D value) at 125° C. is 4.4 hours for soil microorganisms (insert fig. 17) and 3.5 hours for organisms entrapped in Lucite. For a margin of safety, 3 D values must be added to the time for the microbiological load on the spacecraft to be reduced to zero to meet the constraint of 1×10^{-3}.

The authors concluded with the recommendation that current sterilization standards are in need of revision because they fail to take into account all potential sources of contamination. The line of action recommended was that:

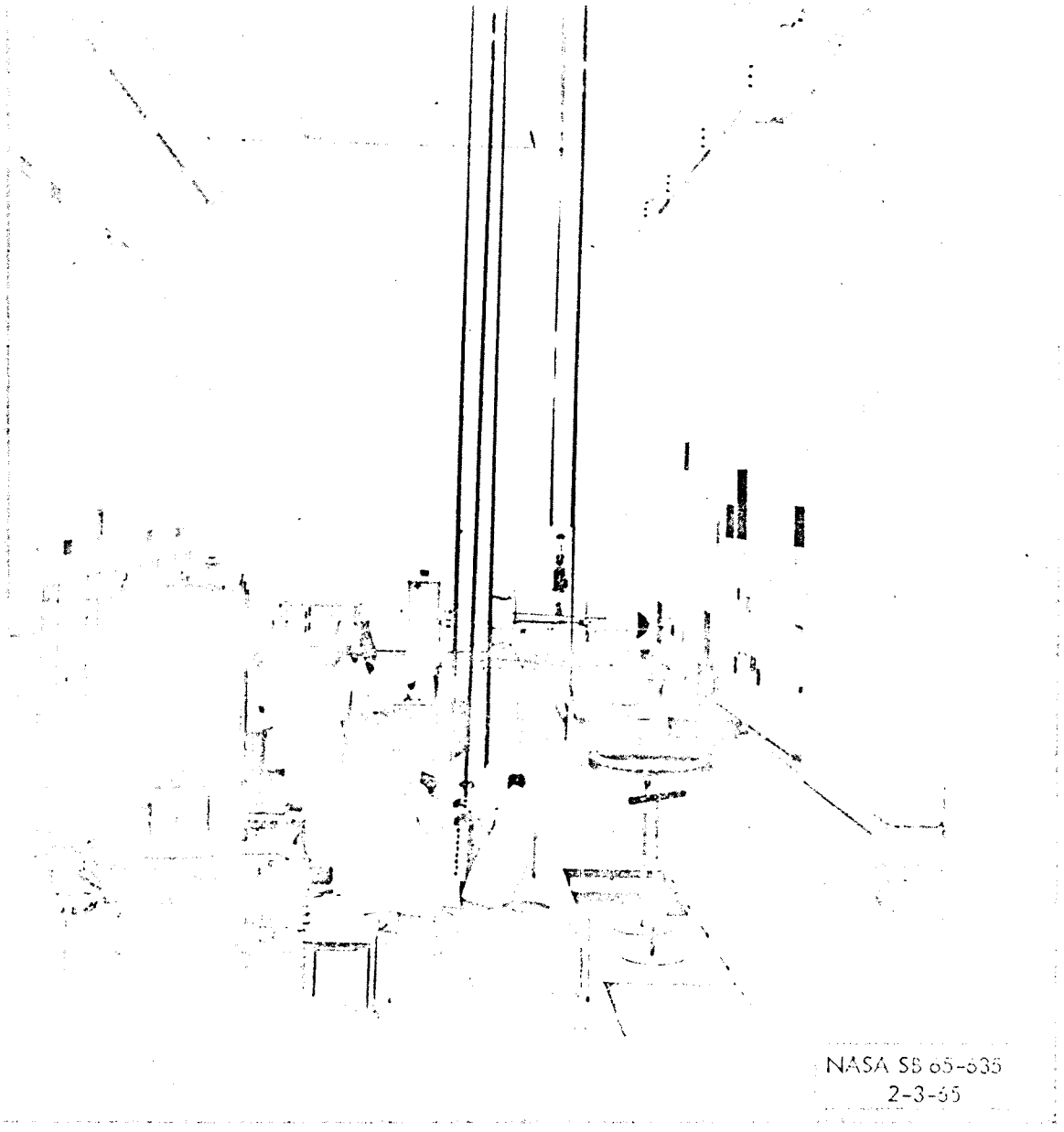

Figure 17.—

"The planetary quarantine policy shall require 99.9% confidence that the unmanned exploration of Mars will not contaminate the planet with terrestrial organisms."

In its 1966 report to COSPAR (ref. 132) the Space Science Board announced findings made available through the Experimental Assembly and Sterilization Laboratory (EASL) of the Jet Propulsion Laboratory. A contamination level of approximately 200 microorganisms per square meter of spacecraft was obtained through use of this facility. The amount of control exercised over persons working on spacecraft components and the use of skin-contact barriers were found to materially lessen contamination. A technique for a high level of microbial recovery from Lucite was mentioned as well as the feasibility of sterile insertion techniques for the post-sterilization replacement of inoperable spacecraft components.

At the conclusion of the Seventh International Space Science Symposium, the Organizing Committee for a Symposium on Techniques for Space Probe Sterilization made the following recommendations to the COSPAR Executive Council (ref. 133):

> that a symposium entitled "Sterilization Techniques for Instruments and Materials" will be held in London during the summer of 1967 and that topics include sterilization endpoints, combined sterilization techniques and sampling and verification procedures.

Examination of Sterilization Requirements

The ability of terrestrial microorganisms to survive simulated Martian environments has been studied by several groups (ref. 134 ~~15~~). In order to determine the probability of contaminating a planet by impact of a non-sterile spacecraft, by ejecta, or by a lander which has undergone a sterilization process, mathematical models relating to the planetary quarantine requirements have been proposed.

Sagan and Coleman (Ref. 135 ~~16~~) analyzed the problem of Martian contamination and proposed that the probability of such contamination be kept to 0.001 during 1000 experiments and that the number of viable microorganisms deposited on the Martian surface by each vehicle be kept to 2×10^{-4}. The probability of accidental impact should be kept to less than 4×10^{-5} for this same contamination probability. At a 1965 meeting of the NASA Bioscience Subcommittee of the Space Science Steering Committee, Schalkowsky (ref. 136 ~~17~~), pointed out the need for a re-examination of the Sagan-Coleman analysis with a concomitant change in sterilization requirements and simplification of procedures. In comparing COSPAR values to those of Sagan-Coleman it was made known that the former dealt with the probability of a single viable organism being aboard a vehicle while the latter dealt with the probability of release of an organism on the Martian surface. There is little numerical difference between the two values:

	COSPAR	SAGAN-COLEMAN
Probability of Viability	σ 1×10^{-4}	0.75×10^{-4}
Probability of Impact	Pi 3.5×10^{-5}	2×10^{-5}

Using the parameters of Sagan-Coleman, Schalkowsky calculated 740 lander missions over a 10-year period. This figure was inconsistent with the 30 fly-bys tentatively scheduled for the same period. This report led to the formulation of a more complex model which would consider the many sources of contamination and the available launch opportunities during the 1966-2000 period.

A working group (ref. 137 ~~18~~) developed a standard nomenclature for mathematical terms in accordance with COSPAR recommendations. Symbols were defined to describe certain events with recommendations for their formulation in planetary quarantine standards. Figure 18 ~~4~~ illustrates the 1966 COSPAR Model (Insert fig. 18) which defines symbols for certain probabilities. A comparison of planetary quarantine mathematical models (1965) is shown in table 4. A re-examination of the 1964 COSPAR resolution concluded that the probability of 1×10^{-3} that a planet would be contaminated was an acceptable premise; it also assigned a probability (not less than 10^{-3}) for the growth and spread of organisms that survived the sterilization treatment and unity for non-sterilized organisms. (Insert Table 4)

The rationale of modeling has been succinctly stated by the Mathematical Models Subcommittee of the Spacecraft Sterilization Advisory Committee (ref. 138 ~~19~~):

"The purposes of modeling are to provide:

1. The basis for a clean means of communication.

   a. Precise definitions
   b. Simplified vocabulary

2. A framework for analysis

(1) $P_c = n_L \cdot P + n_U \cdot P'$

- P' — probability of contamination due to a non-lander
- n_U — number of non-lander vehicles
- P — probability of contamination due to a lander
- n_L — number of landers
- P_c — probability that planet will be contaminated

(2) $P = P_N \cdot P_R \cdot P_G$

- P_G — probability of growth and spreading
- P_R — probability of release
- P_N — probability of one viable organism

(3) $P' = \sum^{i} (P'_T)_i \, (P'_R)_i \, (P'_G)_i$

- $(P'_G)_i$ — probability of growth and spreading
- $(P'_R)_i$ — probability of release
- $(P'_T)_i$ — probability of transferring viable organisms to planet

i = distinct, independent sources of contamination

(4) $P_c = n_L \cdot P_N \cdot P_R \cdot P_G + n_U \cdot \sum^{i} (P'_T)_i \cdot (P'_R)_i \cdot (P'_G)_i$

Figure 18.- 1966 COSPAR Model

Table IV.- Comparison of Planetary Quarantine Mathematical Models

ITEM	Description	Planetary Contamination Parameters			Biological Judgment Parameters		Allocation Parameters		Implementation Parameters			
		P_c	n_L	n_U	p_G	p'_G	$n_L P$	$n_U P'$	p_R	p_N	p'_R	p'_T
1	Sagan and Coleman	10^{-3}	60* #667(1)	30	10^{-2}	1	5×10^{-4}	5×10^{-4}	1	2×10^{-4}	1	4×10^{-5}
2	Corrected values of Sagan and Coleman (2)	10^{-3}	# 667(1)	30	10^{-2}	1	5×10^{-4}	5×10^{-4}	1	7.5×10^{-5}	1	2×10^{-5}
3	COSPAR	10^{-3}	667#	17	10^{-2}	1	5×10^{-4}	5×10^{-4}	1	10^{-4}	1	3×10^{-5}
4	Present Model case (a)	10^{-3}	70	30	10^{-3} (3)	1	5×10^{-4}	5×10^{-4}	1	7×10^{-3}	1	1.7×10^{-5}
5	Present Model case (b)	10^{-3}	70	30	10^{-3} (3)	1	7×10^{-5}	9.3×10^{-4}	1	10^{-3}	1	3.1×10^{-5}

*Stated
#Inferred

(1) This value of n_L is not explicitly given in the Sagan-Coleman analysis. It has been calculated using data provided on the desired number of successful experiments and the various success probabilities, and by adding a probability of 0.9 that the capsules launched will successfully land on Mars.

(2) Differences between values in this item and item 1 stem largely from the erroneous use by Sagan and Coleman of logarithms to the base 10 rather than base e as called for in their formulation.

(3) Reduced from previous models on the basis of the probability of a random terrestrial species of heat-damaged organism infecting a random media.

3. The interrelationships of parameters and alternatives

   a. Sensitivity studies
   b. Uncovering new relationships previously unrecognized

4. A quantitation of factors

5. A predictive decision tool

   a. Management tool
   b. Operates within a time-cost constraint matrix in order to optimize resource utilization

6. A guide for research: priority ordering."

The role of mathematical models in determining the planetary quarantine constraints has been summarized by Cornell (ref. ~~20~~ 139):

"The role of mathematical models in the planetary quarantine program is to provide a basis for communication and decision, for quantifying and inter-relating factors, for prediction and analysis, for directing research toward factors about which information is needed and for explicitly representing variation and uncertainty in terms of probability distribution."

B. Environmental Microbiology

The requirements for the sterilization of capsules can be divided into four steps: (1) design and development of sterilizable flight hardware, (2) reduction of microbial load, (3) terminal sterilization, and (4) prevention of recontamination. The first phase is an engineering rather than a biological problem and will not be discussed here. The major focus at this time is on the development of sterilizable solid propellants, polymers, batteries, impact limiters, certain electronic components, and soldered joints. The reduction of microbial load of a capsule and its components can be achieved through clean assembly practices (use of clean rooms, protective clothing, proper training, and correct techniques.)

A tank to measure the microbial shedding from humans (microbiotank) was devised at the National Communicable Disease Center, Atlanta, Ga. (ref. 140). The tank is large enough for one adult and can be steam-sterilized before use. After a period of exposure to human contamination, the tank is sprayed with culture media to recover the microorganisms present, and after collection in a large wash bottle, is centrifugated, diluted, and put in the appropriate culture media in order to recover and identify any organisms present. Studies with this equipment demonstrated that (1) shedding of organisms of human origin could be reduced through the use of sterile scrub suits, clean room garments, and masks, and (2) special washing and delinting of "bunny suits" are more effective in reducing fallout than are sterile

surgical scrub suits (ref. 141).

Evaluations of the effect of surface materials on viability of various species of microorganisms demonstrated that the nature of the surface does not appear to be a factor in viability loss. Surface types included in the study were glass, vinyl, stainless steel, and ceramic tile (ref. 141). As might be expected, the die-off rate of sporeforming organisms was much less than that of non-sporeformers.

Many factors affect the level of contamination in clean rooms: number of personnel, degree of activity in which they are engaged, and type of clothing worn. Studies have shown contamination rates to be highest from personnel in street clothes and lowest from relatively inactive persons in protective clothing and wearing masks (ref. 142). The value of environmental controls was shown by comparing microbial fallout over a 1-year period in a clean room and a factory area. In both areas the surface contamination remained fairly constant but the count in the factory area was over 19,000 aerobes per square ft. while the clean room count was 510. Contamination levels in clean rooms can be minimized by requiring proper garmenting, personnel and equipment decontaminating procedures, and training of personnel.

Further evidence of the greatest source of viable contaminants has been reported by the Phoenix Field Station of the U.S. Public Health Service (ref. 143). When the environment and personnel are rigidly controlled, organisms associated with humans (Staphylococci and Corynebacteria) are predominant; when controls are relaxed, organisms are mostly associated with soil and dust. It is interesting that the

microbial fallout in hospital operating rooms is approximately one order of magnitude higher than in clean rooms and often equal to the contamination level of some factories. The predominating organisms isolated in operating rooms are sporeformers, molds, and Actinomycetes.

In correlative studies (ref. 144), 75 percent of clean room isolates were commonly associated with humans and only 10 percent were sporeformers. Transfer of contaminants from humans to surfaces through handling varied from one individual to the next. The mean microbial die-off rate was progressive for organisms deposited on stainless steel, aluminum, and teflon test strips. After storage, there were reductions of 99 percent in viable organisms on stainless steel, and 90 percent for those on aluminum and solder strips. Lowest die-off rates were recorded for organisms deposited on epoxy, Lucite, and copper strips, the latter revealing the highest mean number of survivors. An analysis of heat susceptibility of these organisms indicated a 1-hour exposure at 135° C. was sufficient to sterilize a strip having a mean contamination level of 76.5 microorganisms per strip. Effectiveness of control measures was established through the combined use of a hexachlorophene soap and gloves, which reduced the mean contamination level to less than one organism per assembled unit. Sporeforms isolated were reported destroyed at temperatures below those required by NASA operation plans.

In assaying microbial contamination on surfaces, ultrasonic energy has been found superior to mechanical agitation in that it is more efficient for removal of organisms from rough surfaces (refs. 145, 146).

The difference in efficiency between mechanical agitation and ultrasonication has sometimes been more apparent with articles contaminated through handling than those contaminated through natural fallout. A cold medium yields greater microorganism recovery, since low temperature retards spore germination; by withstanding heat shock, spore formers can be separated from vegetative forms and thus improve sensitivity of the test. A wetting agent has been found to increase the percentage recovery of heat-damaged organisms and also to aid in surface dispersion of microbial cells.

Survival of microorganisms on surfaces depends on chemical composition , relative humidity, care exercised in processing and manufacture of components, length and temperature of storage, and number and types of organisms (ref. 146). Organisms which have been naturally deposited on stainless steel strips and electronic components exhibit varying rates of die-off after several weeks storage. Aerobic mesophiles (moderate heat in oxygen) have been found to decrease by 50 percent while non-sporeformers decreased by 80 percent. Species unaffected by storage are aerobic sporeformers, molds, and Actinomycetes. With cultures of Bacillus and Clostridium (deposited on stainless steel), die-away rates were found greater at relative humidities of 47 and 96 percent than at 12 percent. The die-off of spores deposited on Lucite discs has been observed to be greater at 0 than at 46-percent relative humidity.

Many studies concerned with the recovery of microorganisms from plastic surfaces and stainless steel have made reference to the

"plateau phenomenon." This steady state of microbial level is apparently caused by the fact that the number of organisms dying is equal to the number being deposited on a given surface.

Studies of microbial load of various electronic piece parts are in progress at the Jet Propulsion Laboratory (ref. 147). Upon receipt in the laboratory, parts are quarantined for 7 days and thereafter handled only by sterile forceps. Microbiological studies revealed that vegetative bacterial cells are the most prevalent organisms isolated--actual numbers range from zero to 3×10^5, while aerobic isolates outnumber anerobic 26:1. The results of these assays (type of organism and level of contamination) are fed into computers where the information on each part is stored for retrieval as occasion demands. The present schedule averages 100 piece parts per week.

The Sandia Corporation of Albuquerque, New Mexico, has been a leader in joint industrial-governmental development of particulate contamination-control guidelines. Federal Standard 209 (rev. 209a)--a result of such cooperation (ref. 148)--seeks

> "to prescribe air cleanliness classes and other air environmental conditions required for achieving and maintaining the levels of cleanliness specified in the product specification."

HEPA (high efficiency particulate, air) filters reduce air contamination 99.97 percent. Improved design of cleanroom ventilating systems has resulted in easier and lower cost maintenance. Development of new instruments for detection of contaminants and filter leaks has improved cleanroom operation. Through the proper use of air showers,

protective garments, and enforcement of rules, a reduction in contaminants has been possible. Newer techniques of packaging can reduce the number of contaminants contributed by handling, and protect sensitive components from damaging humidity and dust.

Areas of concern in environmentally controlled rooms are air, surfaces, personnel, and spacecraft hardware. General monitoring methods have been described by McDade et al. (ref. 149). Dust and fibers constitute non-viable contaminants while microbial aerosols constitute viable contaminants. Air-sampling methods include sedimentation, filtration, centrifugation, electrostatic precipitation, liquid impingement, and impaction on solids. Slit samplers are commonly used to determine the number of viable particles per cubic foot of air. The agar settling plate is also frequently used in laminar down-flow rooms. The contamination monitoring of spacecraft and hardware is a multi-staged operation beginning with the interior and exterior examination of piece parts and proceeding through the several stages of subsystems to the completed capsule.

The influence of the number of personnel in a cleanroom area upon the total viable count is shown in figure 19 (ref. 149). With (insert fig. 19) each decrease in personnel there is an immediate and marked decrease in the viable particle count. Air sampling studies conducted in a hospital operating room (figure 20) reveal similar findings. Laminar air flow has been found to be an extremely efficient method of cleaning up contaminated areas and preventing contamination of spacecraft hardware.

(insert fig. 20)

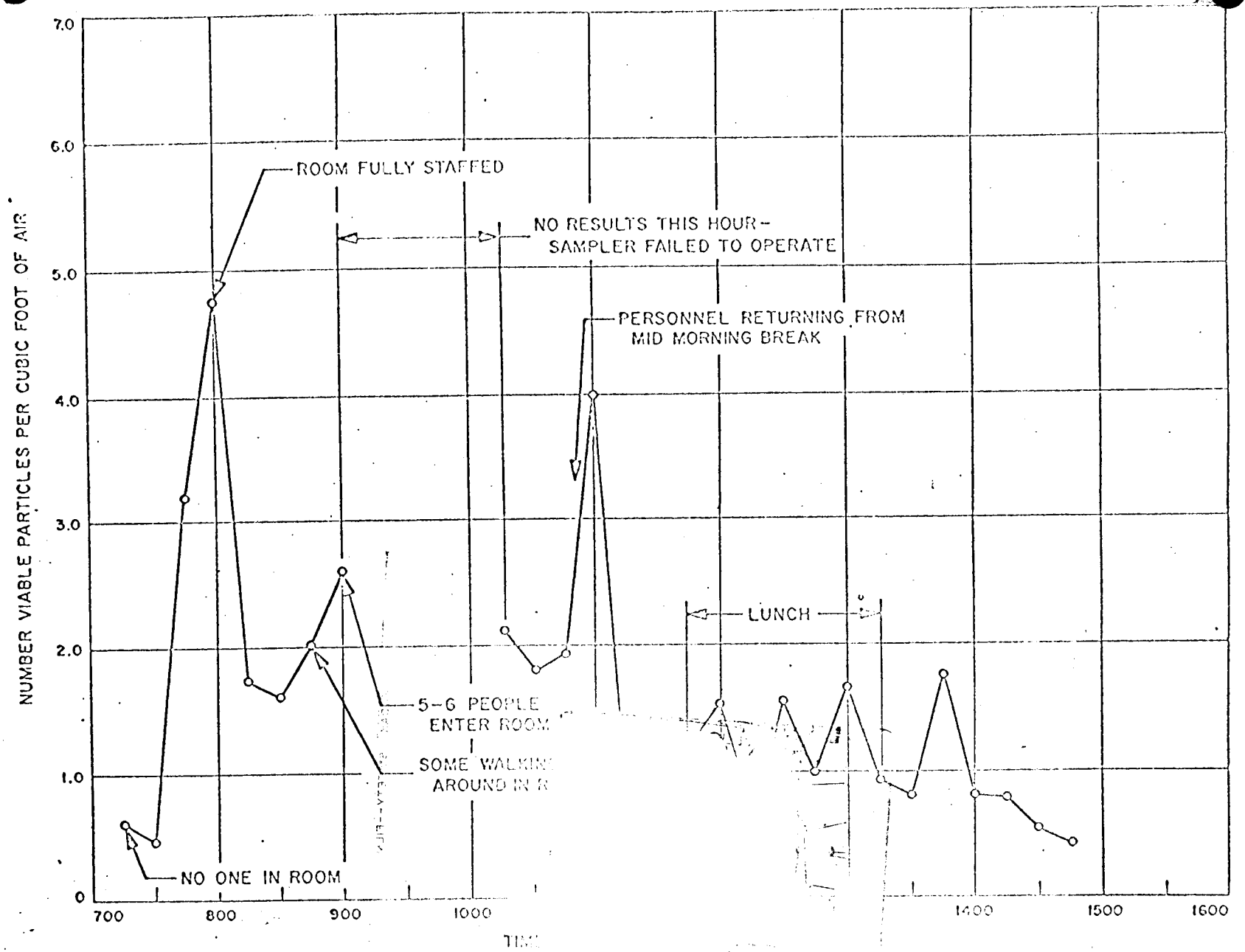

Figure 2. - Air Sampling Studies Conducted In A Conventional Clean Room

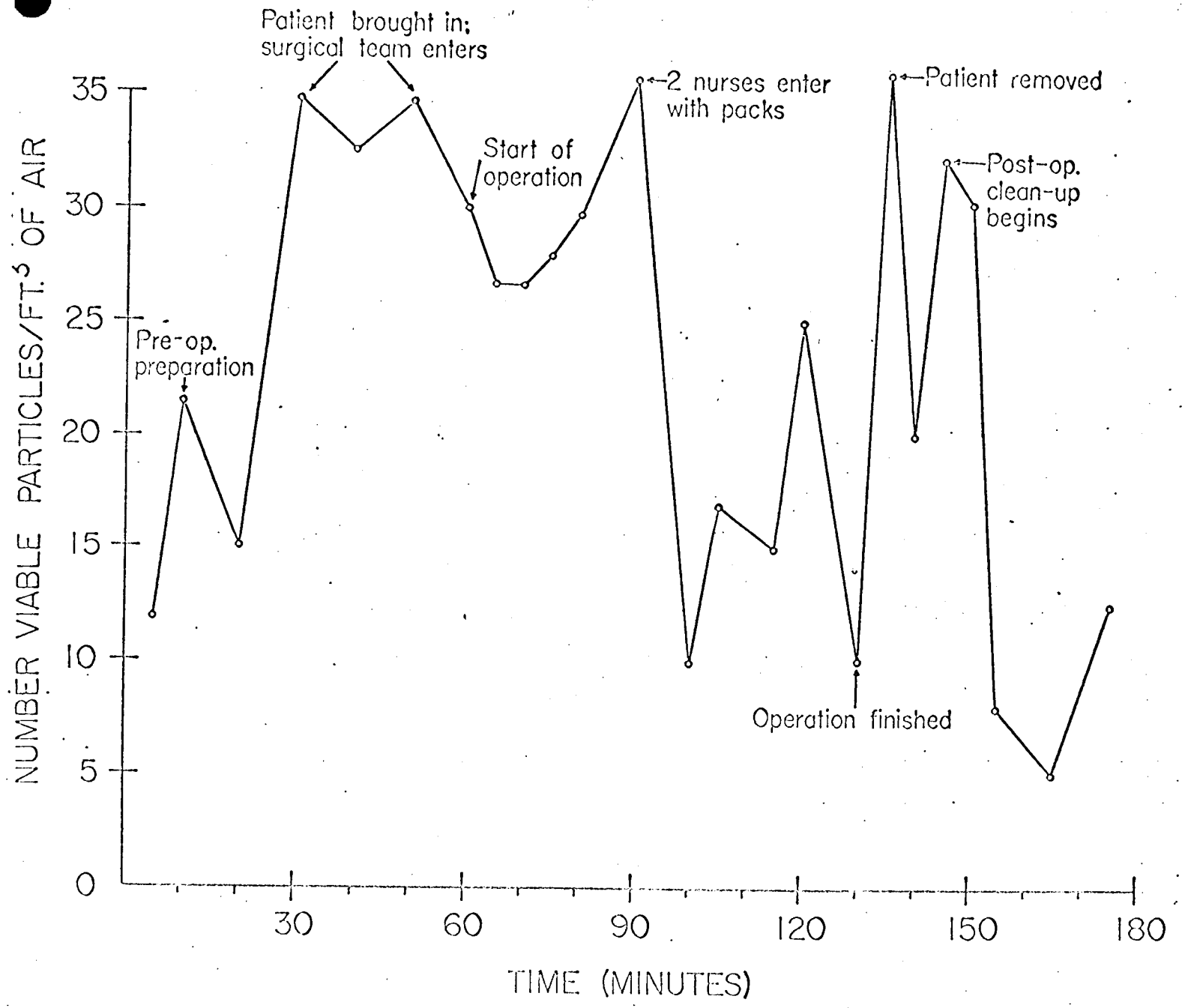

Figure 20. - Air Sampling Studies Conducted In A Conventional Operating Room

Recognizing the need for a more sensitive instrument capable of a higher rate of sampling in a laminar airflow system, Sandia Laboratory developed an improved system capable of sampling for small numbers of particles in laminar air-flow clean rooms (ref. 150 ~~35~~). Most counters are not reliable (without extensive testing) below 1000 particles per cu~~bic~~ ft. foot. With the development of an improved optical system, a prototype model was designed, capable of attaining a sampling rate of 1 - 5 cu~~bic~~ ft. ~~feet~~ of air per minute. Used to monitor clean rooms and check the efficiency of HEPA filters, the instrument is ideally suited for use in clean room environments.

Other investigators at this laboratory reported (ref. 151 ~~36~~) on a surface-sampling device, called a vacuum probe, designed to sample large surface areas having minimal particulate contamination. Reported to remove more than 90 percent of viable contaminants from a smooth surface, the instrument also tends to break up large clumps of micro-organisms in the process. It appears possible to incorporate a membrane filter or some existing slit-air samplers, with the probe, thereby further refining it as a useful clean room tool.

To carry clean room control to a more sophisticated level, studies are underway at the Jet Propulsion Laboratory on automated contamination monitoring (ref. 152 ~~37~~). Early phases of the work involve analysis and evaluation of equipment in use by various organizations and a determination of the validity of such instruments if applied to clean room monitoring.

In June 1966 a NASA task force was established to develop a manual of standard procedures for microbiological examination of space hardware (ref. 153 ~~38~~). Providing guidance in assessment of microbial contamination in the clean room environment and on space hardware (both natural and that deposited by handling), the manual specifies: (1) surface sampling of accumulated contamination through the use of stainless steel strips (gauge 16, 1 x 2 inch) which simulate the surfaces of interest, (2) flat environmental surface sampling by means of Rodac plates or, if irregularly shaped surfaces are sampled, by the swab-rinse technique, (3) volumetric studies of microbial contamination of the environmental air through the use of slit samplers, (4) agar settling plates for gravimetric sampling of air, (5) exterior surfaces of piece-parts sampling through the use of ultrasonication techniques, and (6) assaying interiors of piece parts following pulverization. All ~~of~~ the sampling methods with the exception of (6) are of value in monitoring spacecraft contamination in that no damage accrues to surfaces during these sampling procedures. These methods constitute the NASA-approved procedures and deviation is permitted only with the approval of the NASA Director of Bioscience Programs.

A promising method for sterile repair or replacement of spacecraft components involves the adaptation of germfree animal techniques to space-age requirements (ref. 154 ~~39~~). The method demonstrates that sterile parts can be passed through a contaminated zone with a minimal chance of becoming contaminated in the process. Sterile lock insertions

were made in an atmosphere of 12-percent ethylene oxide-88 percent Freon 12 and a relative humidity of approximately 30 percent. Of 23 insertions challenged with a microbial suspension of 10^6/cubic feet, 20 were sterile. The 3 contaminated insertions were determined to have come from accountable sources. Contamination leaks did not occur as long as positive pressure was maintained in the flexible film isolator through which passages were made. Further studies in this application of gnotobiology are contemplated by NASA at the various field installations concerned with the problem of repair of spacecraft or insertion of scientific packages.

Pilot-plant studies relating to contamination control have been undertaken at the Jet Propulsion Laboratory through the construction of the Experimental Assembly and Sterilization Laboratory (EASL) and the Sterilization Assembly Development Laboratory (SADL). The purpose of these clean room facilities is to gain experience in the assembly of space-flight hardware, evaluate engineering techniques, and sterilize an assembly as a system while meeting the planetary quarantine requirements. The developmental phases include construction of a capsule training model, initiation of a training program, evaluation of assembly techniques, and sterilization of the test capsule (ref. 155).

Evaluation of microbial load entails examination of solids for viable microorganisms. This problem, peculiar to the requirements of the space age, revolves upon the possible release of organisms from the interior of solids in the event of a space probe's accidental impact on a planet's surface. Many techniques (sawing, grinding, filing,

pulverizing, and temperature-induced disintegration) have been employed to reduce solid materials to a state in which they may be cultured.

In early work on contamination detection inside components, (ref. 156 ~~41~~), the following percentages of parts examined were found to have internal contamination: 4 out of 28 and 6 out of 8 capacitors; 1 out of 24 and 1 out of 5 resistors; 1 out of 4 transistors; and 4 out of 5 solar panel pieces.

In additional studies of 218 components (ref. 157 ~~42~~), all were found to be internally sterile; prior to this lot, 7 percent of all components tested were found contaminated. Control cultures, incubated in the presence of some components, were not inhibited in ability to grow.

Although ethylene oxide was known to exert no deleterious effect on the functional ability of some electronic components, it occasionally has proven inadequate as a sterilant. On exposed surfaces, however, a 6-hour exposure to ethylene oxide has been adequate to achieve sterility--when operations are within the prescribed limits of to all known parameters.

Resins used to pot electronic components have been studied for sporicidal activity. Among five ~~5~~ types studied, little such activity was noted, although one ~~1~~ resin exhibited some sporicidal effect over a 5-week period. If contamination were minimal, it would appear that this resin could inhibit bacterial growth (ref. 158 ~~43~~).

A considerable body of data on recovery of organisms from solids at the Phoenix Field Station (ref. 159 ~~44~~) indicates that the

condition of the spore suspension (inoculum) greatly influences rate of recovery. Recovery rates for untreated spore suspensions average 16-18 percent, while suspensions treated with lysozyme show a recovery rate of 87 percent. Ethylenediaminetetracetic acid (EDTA) as a solvent for plaster of Paris pellets has been very successful and has served as a control for evaluating pulverization methods. Other solvents (acetone, methanol, 2-propanol, and ethanol) have been used in conjunction with plastics to determine a model system for recovery of microorganisms from solids. Little loss in microbial viability resulted from use of these solvents.

The method of pulverization also exerts an influence on the recovery rate: 70.7 percent using mortar and pestle, and 44.5 percent using a blendor-mill. These recoveries were from dental-inlay material stored for a period of 50 days. The use of the blendor-mill is acceptable in that it produces uniformity of particle size and reduces variables introduced by the individual performing the pulverization. Proper evaluation of all recovery tests is dependent upon the use of valid controls and a clean spore suspension (achieved through chemical treatment or centrifugation).

Spores have been incorporated into polystyrene by lamination with carbon tetrachloride and then recovered through dissolution of the plastic and subsequent passage through a membrane filter. During polymerization of methyl methacrylate, a 90-percent reduction in viable organisms was noted. After polymerization, no further die-off was noted if the material was stored at -4° C. Compared to the

control (release of organisms through dissolution), use of a blendor-mill recovered approximately 99 percent fewer viable spores. A 5-minute period of grinding was found sufficient to release all viable spores in the plastic (no increase was observed following dissolution), but a 1-minute grinding was deemed insufficient.

Studies in which Bacillus subtilis spores were incorporated in epoxy resin indicate that D values may be twice as high as those for Lucite (ref. 160 45, 161 46). The D value for the former (135° C.) was determined to be 2.5 hours and that of the latter, 1.35 hours. The D_{125}° C. values for Lucite-entrapped spores have been reported as 3.08 and 3.44 hours, while the D value for spores in epoxy was 5.3 hours. Water activity (W_A) is suspected of being one of the major factors in the resistance of organisms to dry heat.

Studies on the interior contamination of solids included these materials: paraffin, paraplast, epoxy, ice, parlodion, silicon rubber, and Lucite (re 162 47). Organisms were recovered using suitable solvents, mortar and pestle, sanding discs, and blendor-mill. A novel and promising recovery method employed cryogenic grinding which renders the material friable. It was concluded that pulverization exerted little lethal effect on spores and that the blendor-mill was the most efficient method of reducing materials (71 percent of the particles were less than 10 μ ~~microns~~ in diameter.) The die-off rate of spores embedded in paraffin during a 3-month storage period at room temperature was found to be 66 percent.

The Jet Propulsion Laboratory (ref. 163 ~~48~~) is developing a parts list which will ascertain if conditions of manufacture will sterilize components. If possible, a redirection of manufacturing processes would be encouraged in order to produce sterile parts.

To determine the heat regimen necessary for the sterilization of capsules, study of heat-resistance of typical bacteria was necessary. Bruch et al. (ref. 164 ~~49~~) screened strains of Bacillus and Clostridium at 120° and 125° C. Several dry heat-resistant strains of Bacillus were isolated; however, isolates from soil samples proved several-fold more resistant than spore suspensions. It was noted that vacuum decreased, while spore entrapment in solids increased, sterilization time.

A review presented at the 1963 COSPAR Meeting (ref. 165 ~~50~~) cited several time/temperature cycles sufficient to sterilize clean materials:

Time (hrs)	Temperature
0.5 - 1.0	170°C.
1.5 - 2.0	160
4.0	150

These cycles were deemed adequate and safe, although surface sterilization temperatures would be inadequate for spores entrapped in solids. From exposure cycles derived from soil studies, it was recommended that space-probe time/temperature cycles be based on heat resistance of organisms collected by air samplers in spacecraft assembly areas.

Original parameters for capsule sterilization temperatures centered around 135°C. for 22 hours; recommendations for lower temperatures and longer times were based on a desire to preserve electronic components from the deleterious effects of high temperatures.

Present plans call for use of physical (thermocouples) rather than biological indicators to determine the effectiveness of a given heat cycle.

To assess the effectiveness of temperatures of 105° C. in the sterilization of microorganisms (ref. 166 ~~51~~), spores of Bacillus subtilis var. niger were exposed to dry heat at 105° C. on glass surfaces (embedded and not embedded in plastic) and sterilized after 48 hours exposure. On non-embedded slides, the requisite sterilization time increased with the concentration of spores; the level of spore contamination did not exert as marked an effect in determining the sterilization time for plastic embedded slides. For spores not embedded, sterility was achieved in 24 hours at 105°C., while 48 hours was required for plastic embedded spores.

The effectiveness of dry heat on spore contamination of mated surfaces was studied to determine whether longer exposure periods were needed (ref. 167 ~~52~~). Dry heat was equally effective against spore contamination, whether on mated or exterior surfaces and, therefore, extended exposure periods probably will not be required to sterilize mated surfaces.

Diatomaceous earth as a test material for entrapped spores has been suggested (ref. 168 ~~53~~), since it is relatively easy to handle, allows for the release of entrapped organisms, and gives quantitative and reproducible results. The thermal death rate for spores at 105° C. or 125°C. is slower when they are embedded in diatomaceous earth than when exposed on a glass surface.

The dry-heat resistance of spores on various surface materials is believed related to water activity (ref. 169). Spore-inoculated Lucite powders stored for 2 weeks at relative humidities of 20, 40, 60, and 80 percent formed into rods, and exposed to 135° C. have been found to have D values of 73.5, 88.7, 67.4 and 36.0 minutes respectively. Spores on mated surfaces torqued at 150 in-lb are more heat resistant than those on surfaces mated at 12 in-lb. In addition, spores encapsulated in epoxy resins or Lucite show a greater resistance to heat than spores on mated surfaces. Survival would appear to be related to the porosity of the surface material, the surface area, and reduced water loss. The physiological state of the spore, the purity of the suspension, and water activity are important factors in survival.

In summary, the sterilization of capsules is a four stage procedure: (1) production of sterilizable components, (2) assembly of the spacecraft under untra-clean conditions, (3) terminal sterilization by dry heat, and (4) protection of the capsule from recontamination before it reaches its destination. At the time of the terminal sterilization, the capsule (that part of the spacecraft that will land on a planet) may be sealed within a canister, which serves to prevent recontamination following sterilization. The canister would remain in place until it reaches deep space. An alternative method would be to heat the capsule outside of its canister and then encapsulate it by means of tunnel suits and glove ports in the wall

of the sterilization chamber. Once the vehicle is free of the Earth's atmosphere, the canister would open and free the capsule on its trajectory toward a designated planet.

CONCLUSIONS

In less than a decade the first unmanned lander is scheduled for a Martian landing. This program, designated VOYAGER, will seek the chemical, physical, and biological examination of the planet's surface. The sterilization technology developed in recent years will be applied to this automated spacecraft series. The requirements for stresses applied to the capsule for sterilization have been reduced appreciably through directed research. To assure engineering reliability, subassemblies may be exposed to heat and ethylene oxide cycles exceeding the terminal sterilization cycle. Thereafter, contamination control will be exercised through the use of bio-clean facilities and microbiological barriers until the time of terminal sterilization.

Continuing needs of the Planetary Quarantine Program include technology utilization, improved monitoring procedures, newer cultural methodology, sterilization techniques for heat-labile materials, analysis of the release of organisms from solids, and on-going program review and definition.

The effort of program managers, scientists, and engineers throughout the country have already overcome many seemingly insurmountable problems. Program requirements will be altered and procedures refined as new technology is developed. Indeed, as work progresses in this relatively new field of space endeavour, benefits accrue to consumers, medicine, and industry. As

frequently happens in technological development, answers to specific problems often find application to other, and often unrelated, areas.

6. ENVIRONMENTAL BIOLOGY

Introduction

Living organisms, from microbial forms to man, are extremely responsive to their environment. The history of science tells us that living systems have adapted successfully their life forms and life cycles to an inconceivable variety of biologic environments. The space environment provides still another variety of unique environmental factors to which living Earth systems are being exposed.

Studies of the effects of the space environment on living organisms are necessary in the pursuit of three prime NASA goals. First, the fundamental characteristics of life can be investigated in the space environment in relative isolation from interfering influences. Such studies can contribute toward the development of a general theory of biology. Secondly, before men can be launched into extended space flights, the hazards of lengthy exposure to the space environment must be understood to permit the necessary protection to be built into the spacecraft. Finally, better understanding of the effects of the space environment on biological and pre-biological material will be of value in planning and evaluating experiments designed to carry out the prime bioscience mission of NASA — the search for life beyond our planet.

This chapter deals with those research projects seeking to determine the effects of various aspects of the space environment on subjects

ranging from the amoeba to man. Whether of an applied or basic nature, the studies have made progress toward the three goals mentioned. However, in these early days of the space program, a logical emphasis has been placed on problems confronting man's sustained flight through space. Most of the environmental biology studies, whether they be on frog-egg development in an orbiting Gemini or biosatellite capsule, or on chickens revolving in a centrifuge, reflect this concern.

The projects described in this chapter are only one portion of the research in progress in the multi-phased environmental biology program. Other aspects appear in the chapters entitled "Chemical Evolution and Molecular Biology," "Life Support," and "Flight Programs."

The rigors of space flight in toto as well as individual physical factors (acceleration, vibration, radiation, and weightlessness) impose a stress which the organism must withstand by conditioning and adaptive processes. It may be that the organism will not encounter any difficulties in adapting to weightlessness. However, the readaptation to the 1-g environment following the stresses of space flight and prolonged weightlessness may pose serious problems of short and long duration.

A. Space Flight Factors

1. Acceleration

There is little systematic information available concerning the effects of mechanical forces on complete biologic systems, especially the way they respond to mechanical forces. Gravity has not been a matter of great concern among biologists, due perhaps to its constancy and consistency. With the development of space bioscience, however, this information becomes critical inasmuch as man and other Earth organisms will be exposed to a wide range of dynamic space-flight factors and environments.

The hen and its egg appear to be good models for studying the response of biologic systems to accelerative forces. Chickens are used since they, like man, are bipeds. The hen's egg provides us with a simple system for studying changes in density gradients that may be of functional importance in responding to accelerative forces.

Fertile avian eggs from the domestic fowl showed a measurable loss of weight and a reduction in hatchability when exposed to varying intensities of centrifugal force for 10 minutes (Beach et al., ref. 170). A more detailed report followed of the morphologic changes in the avian egg (ref. 171). The reduced hatchability is the result of yolk-particle displacement which erodes the blastoderm cells. There is no apparent effect of the accelerative force on the vitelline membrane which surrounds the yolk. Concerning the orientation of the yolk (ref. 172), chicken embryos assume a characteristic position within the egg prior to hatching. Under certain circumstances these embryos may assume a "malposition" resulting in a decrease in hatchability. Important implications in weightlessness are ascribed by these results.

An important aspect of body composition is size — usually indicated in terms of weight, or one of its derivatives. Under natural conditions, increasing weight represents increased mass — resulting from development in immature animals or fattening in mature animals. However, weight also may be changed artificially through alterations in the ambient accelerative force, which is equivalent, physiologically, to a change

in gravity. After prolonged exposure to a different accelerative force, changes in growth and body composition may be anticipated as a result of changes in work requirements for tonus, locomotion, etc., as well as of more specific effects on individual organs.

Indeed some of these changes were observed in chickens exposed to chronic acceleration of low intensity (1.5 to 3.0 g) for periods of several weeks to several months(ref. 173). These changes were designated a syndrome and referred to as "chronic acceleration sickness." Changes occurred in the cardiovascular system, skeleton, and body size (fig. 21). Permanent organic debility was not involved as evidenced by the ready reversibility of the sickness upon return to normal gravity.

(Insert fig. 21)

Changes in ambient accelerative force lead to changes in chicken muscle size — much like the effects of exercise. This is to be expected, since the direct effect of the accelerative force is an increase in the specific locomotion, maintenance of tonus, etc. In this regard, the differential effect on extensors and flexors is interesting. The work occasioned by increased weight is borne by the extensor — the "anti-gravity" muscle — and this muscle increases in size systematically with the ambient accelerative force. The basis for the acceleration-induced reduction in flexor size is less obvious. Perhaps the increased weight reduces its functional requirements — or perhaps the increased work by the extensor leads to some reciprocal inhibition to the flexor. The cause of hypertrophy and atrophy of skeletal muscle is not well understood. It is not possible to determine from the available information if the growth response (or its absence) of skeletal muscle is caused by the contractile activity <u>per se</u> or by nervous factors

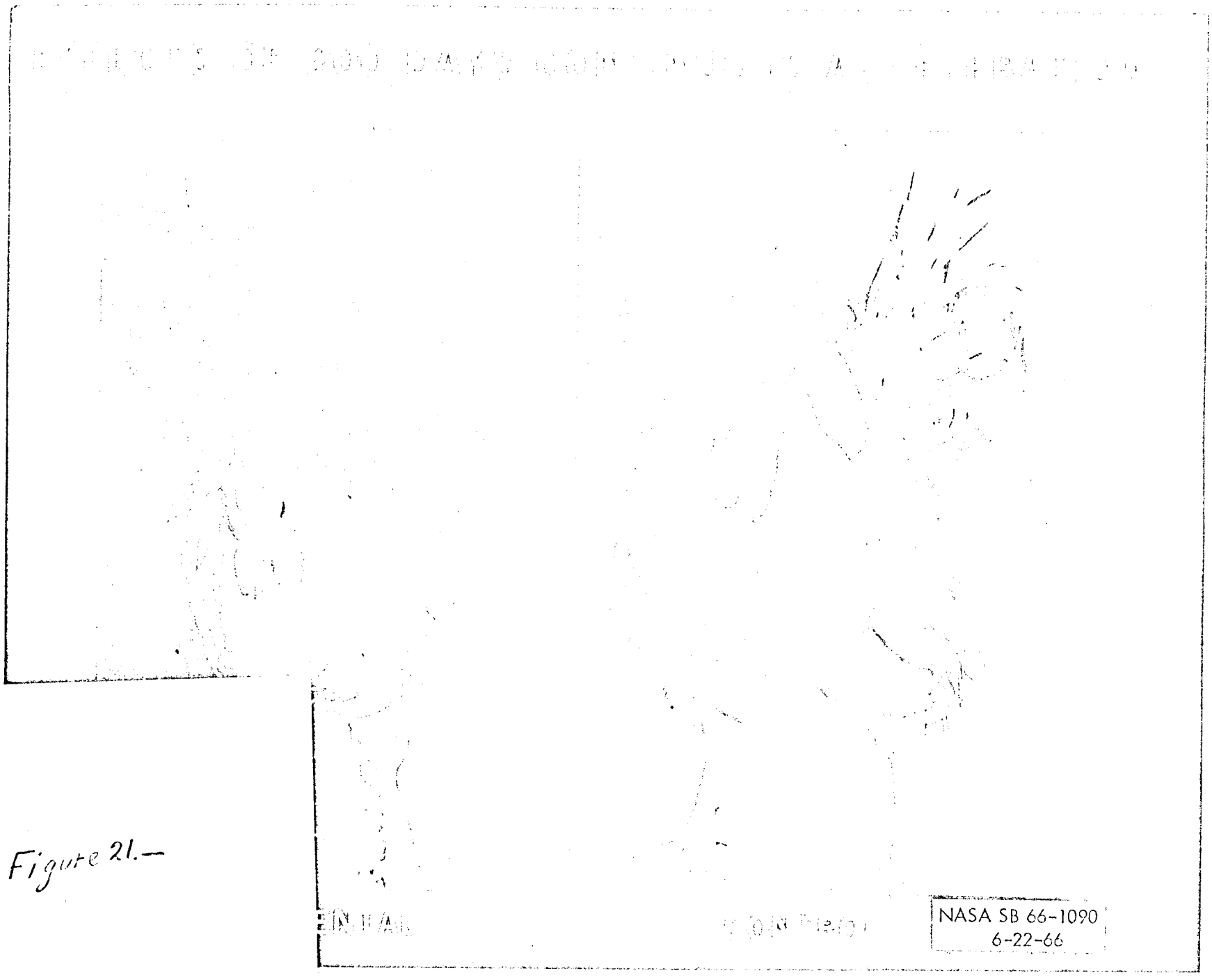

Figure 21.—

unrelated to contractile stimulation.

The anticipated reduction of muscle size in weightlessness also may be akin to a "disuse atrophy". However, the analysis of the data indicates that such reduction would have limits -- and not proceed indefinitely. Thus, the reduction in muscle size in hypodynamic environments would represent a physiologic adjustment-- rather than a complete pathologic atrophy. Mathematical predictions of weightlessness effects are made and they should be tested in actual weightlessness (ref. 174) ~~(5)~~. The potential importance of these phenomena to prolonged weightlessness has been stressed in considerations of space exploration.

Apparently fat is a major source of energy for these skeletal muscles in man and birds. Recently, it has been suggested that intracellular muscle lipids are not as important as extracellular lipids as an energy source for muscular activity in man. At increased acceleration fields more energy is required for equivalent activity with less fat being available in the muscle. Other data indicate an increased fat turnover occurs in hyperdynamic environments, perhaps a result of this greater energy need. It is possible that energy reserves may be a limiting factor regarding muscular activity in hyperdynamic environments, and that a reduction in physical movement would enhance survival.

The fat concentrations of these individual muscles are more gravity-sensitive than is total muscle fat. The dynamic environmental effects concerning the fat content may vary among certain muscles, with the extensor ("anti-gravity") muscle being more depleted. Consequently, there is an inverse relationship among individual muscles between change in fat content and degree of hypertrophy, which is further indication

of the weight-dependent nature of these phenomena. This relationship also indicates that the changes are at least partly dependent upon local phenomena -- rather than only the result of "whole body" phenomena.

Since the effect of increased acceleration upon the fat of skeletal muscles is significant, an increase in fat concentration in the weightless state may be anticipated (cf. 174).

2. VIBRATION:

Considerable concern has been generated about the effects of vibration on living systems. Reports of Russian space-flight experiments have indicated abnormally high NUMBERS members of chromosome breaks and rearrangements, reportedly due to vibration and acceleration. Consequently, Knepton and Beischer initiated A pilot series of experiments that would reveal a feasible approach to discover whether or not the vibrations present aboard spacecraft are harmful to living organisms, and in particular, to man's chromosomes (Knepton, ref. 175). Conidia of Neurospora crassa (L-prolineless mutant), N. crassa (N.R.C. No. 865 A) Tradescantia paludosa, and Drosophila melanogaster were selected and vibrated at 40 and 70 cps. At each frequency they were exposed to two displacements which gave g forces of 10 and 20. These vibratory frequencies and g forces did not alter the genetic material of N. crassa (mutant), did not produce chromosomal rearrangements in T. paludosa microspores, nor did they result in chromosomal aberrations or mutants in D. melanogaster.

In a subsequent study (ref. 178) the investigators exposed T. paludosa microspores to low frequencies (40 - 100 Hz) of vibration at various levels of acceleration (5 - 40 g). Slight differences ($P < 0.05$) were found in the number of spherical chromosomal fragments observed in the experimental and control groups of microspores, the greater number being among the experimentals (Figure 22). It was also observed that specimens vibrated for short periods (3 minutes) exhibited larger effects than those vibrated for longer periods ~~of time~~ (15-60 minutes). No significant impairments of the mitotic mechanism or growth disturbances were observed.

Based on USSR Reports (ref. 177) that chromosomal aberrations have been traced to dynamic factors accompanying launch and recovery (I meant Fig. 22), and not to weightlessness, Grosch undertook the elucidation of the combined effects of radiation and space flight factors (acceleration and vibration) on stresses in cell structure (ref. 178).

Three separate experiments, each with two replications, were performed in which wasps (Habrobracon) were centrifuged for 24 hours in a ^{60}Co built gamma field. During this period a total dose of 750 r was delivered. This radiation dose was adequate to give a pronounced dip in egg production by the sixth day following exposure.

The pattern of response was so consistent that all replications from all experiments could be pooled for summarization here. Fecundity and fertility were followed for a 15-day period. Centrifugation alone

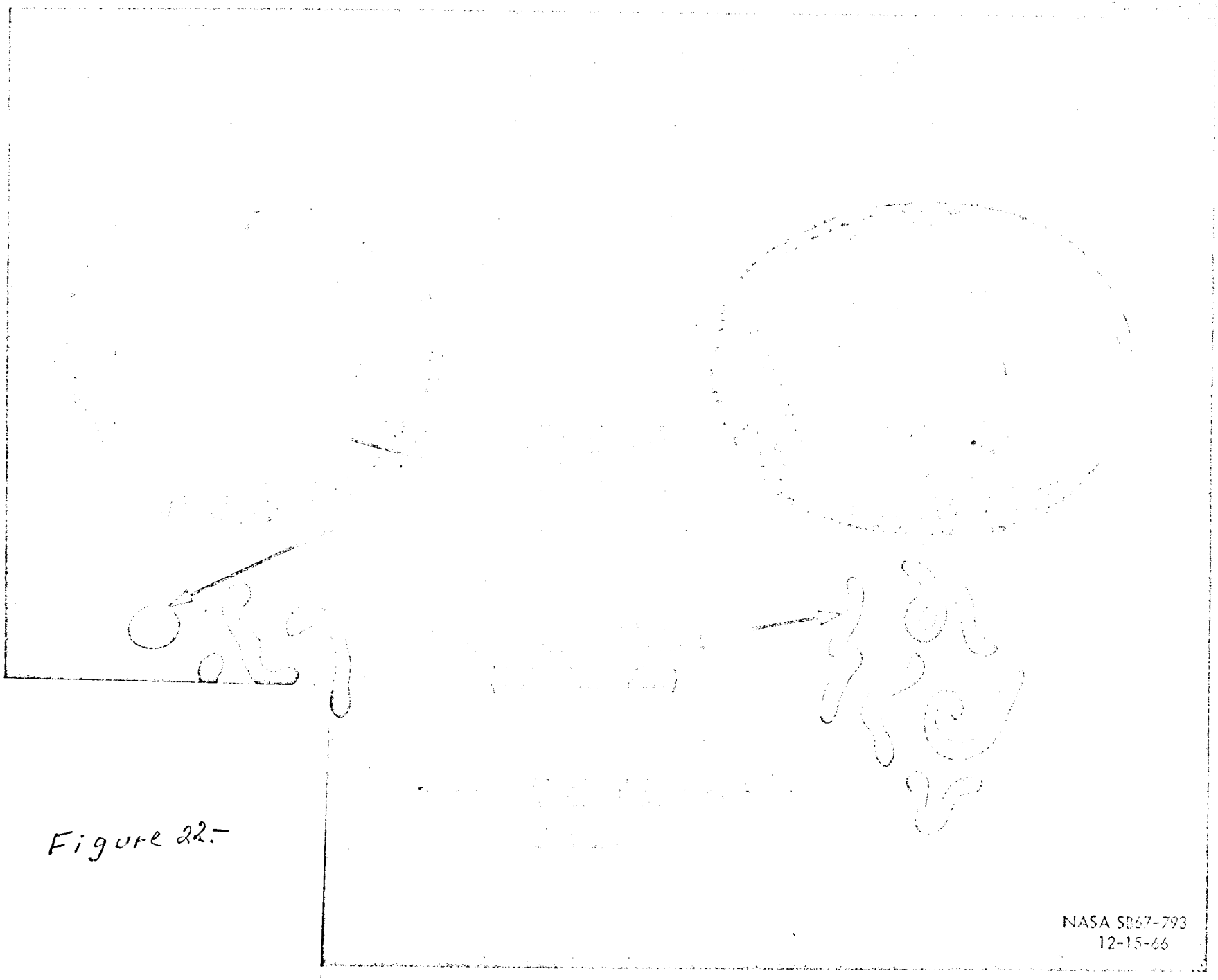

Figure 22.–

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resulted in egg-production curves slightly lower and parallel to the curves representing control values. The combination of treatments resulted in curves intermediate between those obtained from centrifugation and irradiation alone. Tabulation of egg production provides a summary which can emphasize cumulative effects. As shown in Table V, the average total egg production per female for the combination of treatments is intermediate between that obtained from centrifugation and irradiation alone. This pattern of response was also revealed for each period of oviposition corresponding to a different type of cell at the time of exposure.

omit underscoring

Table V.—

Egg production and life span of braconid wasps centrifuged in a ^{60}Co field

TREATMENT	DAYS AFTER EXPOSURE			TOTAL EGGS	MEAN LIFE SPAN (DAYS)
	1-5	6-10	11-15		
Control	89.9	91.5	80.6	262.0	21.9
Centrifuged	72.9	77.6	62.9	213.4	22.5
Irradiated	56.0	30.5	48.1	134.6	22.8
Both	60.6	50.7	59.0	170.3	21.6
Cytological stage when treated	Oocytes	In transition	Oogonia		Somatic Tissue

Thirty female wasps were exposed to gamma rays from ^{137}Cs (Cesium) at a rate of 1000r per minute for two (2) minutes. Immediately half of them were exposed to vibration (120 cps) which continued for ~~four~~ 4 hours, a period earlier determined to include the major part of the operation of recovery mechanisms. During the same period the remaining half was

held in an identical container. Also a suitable group of controls was prepared as well as a group of wasps subjected only to vibration.

In this experiment egg production was followed for 20 days, ~~which~~ includes oviposition records for senile females. These data are summarized in Table ~~III~~ VI. In total egg production the sequential combination of radiation and vibration resulted in more eggs than deposited after radiation alone. However, the pattern differs from that of the centrifuge experiments. A deficit occurring during the first few days was compensated by the higher fecundity subsequent to the fifth day. ~~Mean life span included on Table III more closely approaches the control value for wasps receiving combined treatment than in the case of either vibration or radiation exposures.~~

TABLE ~~II~~ VI.—

The hatchability of the Table I eggs. Percentage values

TREATMENT	DAYS AFTER EXPOSURE		
	1-5	6-10	11-15
Control	90.2	85.3	76.8
Centrifuged	88.0	83.3	71.1
Irradiated	76.7	68.9	60.5
Both	76.5	71.4	62.0

The results presented clearly indicate a lack of synergism, and, instead, a consistent trend toward antagonism, however slight.

3. MAGNETISM

The fact that all living matter exists in a magnetic field of 0.5 ± 0.1 Oersted generally has been disregarded. Knowledge is lacking ~~in~~ of how variations in this field can affect biologic material. Until recently, research has been concerned with the effects of fields greater than ambient on living systems. Inasmuch as application of strong or high magnetic fields has been under consideration for protection of organisms, including man, against cosmic radiation, in ion propulsion, and other purposes, man could be exposed to such intense fields for long periods. ~~of time.~~

In preparation for such an eventuality, Beischer and Knepton (Beischer, ref. 179) conducted a systematic search for possible physiologic effects of high magnetic fields in squirrel monkeys (Saimiri sciureus). Electrocardiograms of four monkeys were recorded during exposure of the animals to magnetic fields of 20,000 to 70,000 oersted. Neither breathing rate nor R-wave amplitude of the EKG was affected. A decrease in the heart rate, an increase in the degree of sinus arrhythmia, and a considerable augmentation of the amplitude of the T wave are newly observed biomagnetic effects. The effect of a magnetic field on the myocardium appears to be a typical example for events in which the magnetic properties of matter are not of first importance. The close coupling of electrical and magnetic fields requires constant consideration of the influence of an external magnetic field on all electrical properties of living tissue, including cerebral electrical processes.

Conversely, Greene and Halpern (ref. 180) observed selected life processes in low magnetic fields. Tissue cultures of HeLa, Chinese hamster, and

chick embryo were exposed to magnetic fields of 0.0005 oersted, 0.5 oersted (ambient), 350, 400, 500, 550 and 600 oersted for ~~four~~ 4 days. Cell counts of the experimental culture after exposure to the magnetic fields did not show any significant quantitative growth differences compared to controls.

A consistent difference, however, was observed by Halpern (ref. 181) ~~(12)~~ in the growth of shoots and roots of wheat seedlings subjected to 50-gamma. The seedlings grown in the low field were larger and more robust than their controls. A reproducible pattern of increased growth in very low natural magnetic fields (less than ~~one~~ 1 mG milligauss) was observed, thereby indicating a growth enhancement.

As a preliminary postulate, the removal of the Earth's magnetic field may result in the release of some governing force which normally controls the rate of cellular growth and proliferation. In a null field environment, the growth process seems to be accelerated as evidenced by marked cellular proliferation.

4. RADIATION: Clc

Chronic acceleration and Whole-Body Irradiation of Rats

Rats survived and physiologically adapted to accelerative forces up to 3 g without apparent effects other than a suppression of body mass and a decrease in body fat as compared to control rats (ref. 182) ~~(13)~~. Exposure to acute whole-body irradiation followed by acceleration increased radiation mortality, but the detrimental effects of acceleration were operative only during the first few days following irradiation. Stressing effects of initial exposure to acceleration were clearly evident by the loss of mass during the first ~~three~~ 3 days. By the second

week, adaptation had occurred and the lost body substance had been regained. In some cases the rate of increase in mass exceeded that of non-accelerated rats.

Results following 700 rads irradiation of rats showed that prior adaptation to acceleration did not increase the ability to withstand irradiation, change the radiosensitivity of the tissues, or affect the repair of radiation damage. Two endogenous agents are known to influence radiation mortality: (1) feeding desiccated thyroid to mice from ~~one~~ 1 to ~~eight~~ 8 days following irradiation increases mortality; (2) although supplemental treatment with pituitary thyrotropic hormone increases acceleration tolerance in chickens, the reverse effects are not observed by inhibiting the thyroid with thiouracil. These factors may have some effect on the observed increase in mortality.

Although stress imposed by exhaustive exercise does not duplicate continual exposure to increased accelerative forces, the finding that exercise following irradiation increases mortality may bear some relation to the finding of increased mortality following acceleration. If thyroid and adrenal functions were determined for animals in each situation, similar changes in function would indicate that common mechanisms are involved.

Acceleration following irradiation has a detectable effect on body mass during the first ~~seven~~ 7 to ~~ten~~ 10 days following radiation exposure. However, the absence of any continued or subsequent effect on mass for periods up to ~~seven~~ 7 months after irradiation indicates that only during a short critical period are acceleration and irradiation effects synergistic. Once having survived the critical period, body

mass of irradiated rats is comparable to that of accelerated controls although the effects of irradiation on body mass are detectable in normal gravity rats. The lack of body fat in accelerated rats is the only detectable difference, both grossly and histologically, between accelerated and non-accelerated groups. The lack of body fat in accelerated rats may be related, in some degree, to the relatively lower alpha-2 globulin found in long-term accelerated-irradiated rats. Since alpha globulins are intimately related to lipid transport and metabolism, these findings suggest that chronic acceleration produces alterations in lipid metabolism.

The absence of pathological findings is highly significant. No histological changes are found in rats sacrificed after 30 days to 10 months of acceleration. This reflects the ability of rats to adapt physiologically to the different environment.

Musacchia and Westhoff (Ref. 183), in studying the natural protection of hibernation against radiation, used intestinal absorption of D-glucose as a criterion. Male and female ground squirrels, Citellus tridecemlineatus, received a single, total body exposure of 2000 r of x-irradiation (250 kv). The dose and time of test were programmed so as to insure a heavy dose of radiation and a measure of the effects at an optimal time for desquamification, generation, and regeneration of the intestinal epithelium. The irradiated hibernating animals showed enhanced active absorption in all intestinal areas. Tentatively, it is suggested that radiation-accelerated absorptive processes in hibernators and hibernation affords protection against radiation damage. These results were

substantiated in another experiment under the same conditions except for which ^{60}Cobalt provided 1000 r of gamma - radiation (Ref. 184).

Montgomery, Reynolds and Cook (Ref. 185) have shown recently that nucleolar changes are induced by ultraviolet microbeam irradiation (0.18×10^{-17} ergs/sec/cm^2) of the nucleolus and nucleoplasm. Individual Chang liver cells were continuously exposed from 4 to 24 hours to a very low intensity heterochromatic ultraviolet microbeam directed to the nucleolus. The cells irradiated for more than 5 hours exhibited distinctive ultrastructural alterations of their nucleoli. The nucleoli of these irradiated cells showed collapse of the nucleolonema, an apparent decrease in nucleolar size, and a reorganization of the nucleolus resulting from the segregation of the various types of granular components of the nucleolus. Nucleolar plaques and light nucleolar "caps" were formed. The significance of this reorganization is not known; however, this same type of reorganization occurs in cells treated with carcinogens and known chemical inhibitors of nucleic acid synthesis, resulting in an injury at the DNA level of cellular metabolism.

It has been previously shown that high-energy protons and alpha particles cause a relatively greater intestinal injury than hematopoietic injury as reflected by the mode of radiation-induced lethality. It has been determined that a single dose of heavy-particle radiation alters the radiation sensitivity of the gut to a second dose of irradiation (Ref. 186). Preliminary studies show two radiosensitive periods; one at the time of the first irradiation, and the

other, 8 hours later. The effect is no doubt related to the pattern of recovery and cellular proliferation in the intestinal lining. In an effort to understand better the role of infection in the intestinal syndrome, some pilot radiation experiments were carried out with germfree mice. These animals are about twice as resistant to radiation than unprotected ones. The relative effectiveness of 730 MeV protons for producing dominant lethality in mice is about 10 percent higher than 250 kv X-rays, and that of 900 MeV alpha particles, 20 percent higher. These results were based on the number of abnormal embryos in relation to the total implantation. Mouse lethality was also studied in a proton beam of approximately 200 MeV.

A relatively uniform whole-body dose of 200r was delivered to female beagles, utilizing the 730 MeV proton beam of the 184" Cyclotron, degraded to a residual energy of 270 MeV. The animals were then immediately subjected to a simulated altutude of 18,000 ft. for a period of almost 2 months. Initial test on combined application of anoxia and radiation indicates that about 6 days following radiation exposure, the animals are unable to compensate for partial anoxia. Studies have been completed on this group of animals , relating changes in blood chemistry, blood counts, iron kinetics, bone-marrow differential, and total body volumes. Based on this pilot experiment, one may tentatively say that the results indicate a certain degree of synergism between hypoxia and irradiation (ref. 186).

[illegible]. Since some Russian space-flight experiments have demonstrated abnormally high numbers of chromosome breaks in space flight, (ref. 177) ~~(19)~~, ~~we have conducted~~ ~~some~~ experiments have been conducted to test if these effects could be due to synergism of radiation effects and vibration. Working with mammalian cells in culture, no synergism was found for cell survival at 100 or 1000 cps ~~cycles per second~~ even at quite high sound intensities. On the other hand, at the ultrasonic frequency of about 10^6 cycles, a very weak ultrasound produced marked synergistic effects with radiation, both on cell survival and on chromosome breaks. The ultrasound by itself, at the intensity used, had no demonstrable effects ~~(19)~~ (ref. 186).

Tested on an organism of larger dimensions, Tribolium confusum, sound was found to have manifold effects. When the organisms were allowed to be suspended in flour, their natural living habitat, part of the sound energy was transformed to heat. When the organisms were surrounded by soft protecting support, the synergistic effects of sound with radiation were reduced to negligible value.

5. SIMULATED WEIGHTLESSNESS

Physiological Effects of Bedrest

Because prolonged bedrest simulates the confinement and many of the conditions of weightlessness associated with orbital space flights, bedrest studies have become important in investigating the effects of weightlessness on humans.

A marked increase in heart rate, decrease in blood pressure, and, in some cases, syncope are manifestations of the cardiovascular deconditioning that occurs in returning a person to the upright position after prolonged bedrest. Indeed, bedrest studies had predicted

these and other manifestations of cardiovascular deconditioning associated with space flight (20) (Vogt, p. 187).

As a result of these studies, a mechanism explaining cardiovascular deconditioning during recumbency or weightlessness has been postulated. During physical activity on Earth, changes in the vascular pressure resulting from the interaction of gravitational and muscular forces in the dependent parts of the body may be partially or totally responsible for the normal maintenance of compensatory reflexes that regulate the cardiovascular system and the volume and distribution of fluids in the different compartments of the body. The absence of gravitational force vectors in recumbency results in a redistribution of the hydrostatic pressure along the cardiovascular axis. This may be the initial cause of the chain of events that leads to the orthostatic changes in blood pressure and heart rate as experienced by astronauts following space flight.

The so-called Flack maneuver is known to produce marked pressure changes in both venous and arterial systems. Therefore, it was thought that such activity might offer a possible means of preventing the development of cardiovascular deconditioning. Accordingly, during a 3-day bedrest period, the subjects performed Flack maneuvers for 30 seconds at 30-minute intervals from 6 A.M. to midnight. However, such repeated Flack maneuvers during bedrest did not prevent the manifestation of cardiac deconditioning, including convulsive syncope.

The deterioriation of the subjects' ability to tolerate passive tilt following bedrest is evidenced by negative slopes of the regression

line of blood pressure vs time and the positive slope heart rate vs time. (~~20a~~)(ref. 187a). In another study, comparing the effects of ~~three~~ 3-day and 14-day bedrest periods, it was demonstrated that cardiac deconditioning became progressively more pronounced with increased bedrest. Blood pressure increased throughout the period of confinement. Circadian rhythms in the cardiac dynamics were detected. Isometric exercise during bedrest improved the subjects' tolerance to passive tilt, but did not reverse the negative slope of the blood pressure. However, yet another study of ~~three~~ 3-day and 14-day bedrest periods did not fully support these results. There were no obvious signs of circulatory deconditioning after ~~three~~ 3 days other than for circadian ~~fluctions~~ fluctuations in the cardiac cycle. Blood pressure did rise during the 14-day confinement and the systolic and isotonic ratios of the pulse-wave velocity indicated stress. In this study, the introduction of isometric exercise during bedrest increased the degree of stress shown by the subjects.

The effects of 14-days' bedrest on the tolerance to physical work has been tested with the bicycle ergometer (ref. ~~20b~~ 187b). Oxygen consumption, carbon dioxide production, pulmonary ventilation, breaths per minute, and frequency of the heart were measured at different work loads. The recovery time of the heart rate after cessation of exercise was also determined. Changes in respiratory gas exchange, ventilation, and mechanical efficiency were not significant. Heart rate at rest and during exercise was significantly higher after bedrest. Isometric exercise carried out during bedrest did not prevent changes in heart rate.

The effect of bedrest on blood volume, on urinary volume, and urinary electrolyte excretion was also examined, (ref. 187e). During the 14-day bedrest period, the subjects were found to exhibit diuresis and naturesis with resulting increase in urine volume. Urinary sodium and potassium excretion were both increased during bedrest. This suggests that a shift of fluids and diffusible electrolytes from the extravascular compartments may occur. It has been reported that increased excretion of potassium accompanies an increased excretion of sodium under many conditions. A decrease in the urinary sodium-potassium ratio was observed as bedrest progressed, suggesting the possibility of a secondary aldosterone effect. However, this decreased ratio could be accounted for by a downward trend in sodium excretion alone. When a bedrest period included isometric exercise, there was a significant decrease in blood volume compared to bedrest without exercise.

Bedrest appears to have little, if any, effect on plasma levels of 17-hydroxycorticosteroids (ref. 187). Plasma levels at 8 A.M., noon, 4 P.M., 8 P.M., and midnight were determined on healthy subjects who were submitted to bedrest for 3 days. During bedrest, the peak level, occurring at 8 A.M., seemed a little lower than normal, but the circadian rhythm of 17-hydroxycorticosteroids was not modified. During bedrest and isometric exercise, the rhythm and the levels of 17-hydroxycorticosteroids were normal. Relatively short periods of physical inactivity seem to have no effect on the circadian rhythm of 17-hydroxycorticosteroids.

Effect on Bedrest on Bone Demineralization

No 9 One of the early factors of concern noted in manned space flight was bone demineralization. The loss of bone calcium during orbital flight was reported in American and Soviet space crews. Because the loss seemed related in a positive manner to the duration of flight, research has been undertaken to assess the importance of this factor in manned space flights of long duration.

Since it was known that long periods of muscular inactivity resulted in bone demineralization, experiments have been directed at determining what degree of the observed bone losses occurring in the astronauts and cosmonauts might have been caused by their confinement during space flight. Were this to be the sole, or principle, cause of bone demineralization during flight, preventive physical exercises might be devised. However, if the phenomenon is associated with weightlessness, or some other factor in the space environment, its potential limiting effect on the duration of manned space flights will require careful scrutiny.

Studies have been undertaken to determine the effect of confinement, with and without exercise, on bone demineralization in the normal Earth environment (Mack, refs. 188, 189). ~~(21, 22)~~. In these studies, a series of 14-day experiments were conducted with human males under carefully controlled calcium-intake regimens. The subjects served as their own controls during pre-bedrest and post-bedrest periods. Their calcium balances during these periods were compared with those of 14-day bedrest periods with and

without exercise. During the pre-bedrest period, diets were carefully administered and the nutritional status of the subjects was monitored by a variety of biochemical tests. By the end of the pre-bedrest period, all subjects were within the ranges accepted for optimum state of nutrition. During separate 14-day bedrest experiments, daily calcium intake by the subjects was controlled at: (a) 2 grams; (b) 0.5 grams; (c) 0.3 grams; and (d) 0.8 grams. The daily calcium regimens for the corresponding pre- and post-bedrest periods were: (a) 2.0 grams; (b) 1.5 grams; (c) 2.0 grams; and (d) 0.8 grams, respectively. During the 0.8 gram experiment, the effects of inactive bedrest were compared with those incorporating isometric and isotonic exercises.

The calcium balance of the subjects was monitored by daily fecal and/or urinary calcium analysis. Daily radiographic bone densitometry of the hand and/or foot and weekly X-rays of the lateral view of the patella were performed.

During pre-bedrest, positive calcium balances were always observed. In each study, the onset of the bedrest period was marked by a shift to negative calcium balance with the demonstration of concomitant loss in bone mass. In most instances, the rate of calcium loss during the bedrest periods was higher for the first several days of bedrest than for the remaining days of the 14-day bedrest period. However, the negative calcium balance prevailed throughout the bedrest period.

The pattern of calcium balance of individuals during the post-bedrest periods varied somewhat. In the 2-gram-calcium-per-day-bedrest

test, a positive calcium balance was re-established during the post-bedrest period. When the calcium intake was reduced to 0.5 gram daily during the bedrest period, individual calcium balances during the post-bedrest period varied somewhat, but a mean positive balance was established. Positive calcium balances in all subjects were re-established in the experiments in which calcium intake was limited to 0.3 grams and 0.8 grams per day during bedrest.

The general pattern established was that of a positive calcium balance for subjects in optimum nutrition states during ambulatory periods. The onset of bedrest, at all levels of calcium-intake studies, was accompanied by a relatively sharp inflection to a negative calcium balance for which the slope was greater during the early days of the bedrest period. A negative slope was maintained throughout the bedrest period. The post-bedrest period was one in which the calcium balance became positive and approached, but did not quite attain, that of the pre-bedrest period.

Bone losses during the bedrest phases of the experiments varied from ~~three~~ 3 percent to as much as ~~fifteen~~ 15 percent. These losses compared with losses of bone mass in Astronauts Cooper and Conrad during ~~eight~~ 8 days of flight of 15.1 and 8.~~2~~9 percent, respectively (c.f. Table ~~III-VII~~. ~~(Figure 1)~~)

The effect of exercise, either isometric or isotonic (the latter conducted with the same exercise equipment and activities as with the astronauts in Gemini VII) demonstrated that exercise somewhat offset the

loss of calcium during bedrest, but did not preclude the development of a negative calcium balance in each case. Thus, bedrest throughout the range of calcium intakes administered during these studies, accompanied or unaccompanied by exercise, resulted in a negative calcium balance which correlated with loss of bone mass.

Throughout these studies, phosphorus balance was also monitored. Phosphorus intake was always maintained as close to the desired calcium-phosphorus ratio as the calcium intake permitted. No consistent, significant veriations in phosphorus balance were observed.

An interesting observation was made during the pre-bedrest phases of all studies reported in this series. At the start of the pre-bedrest periods, almost all subjects were normal in the biochemical assays conducted to determine nutritive state with the marked exception of the ascorbic acid level. This was almost without exception quite low. Daily administration of orange juice brought the ascorbic-acid level to within the normal range prior to the onset of the bedrest period.

Measurement of Bone Mineral Content:—

No ¶ As a corollary to the bone-demineralization studies, extensive efforts have been made to develop methods for the *in vivo* determination of bone density. These methods include X-ray densitometry; measurement of mono-energetic, ionizing photons transmitted through the sample; the use of soft X-rays in narrow-beam geometry; the use of isotopic radiography; the use of beta-X-ray sources; and the use of sonic measurements. These techniques have been applied to a variety of human and animal subjects and bone parts. The ultimate objectives are to determine,

with reproducible accuracy of ~~two~~ 2 precent or less, bone-mass changes at specific sites or areas and to determine whole-body, bone-mass changes. The techniques and instruments developed will have considerable "spin-off" application in normal, clinical medicine.

The principal difficulties encountered are changes in fat and other non-bone tissue between observation times, and the necessity for accurate repositioning of the organ to be measured so that subsequent determinations are comparable. Most of the attention is focusing on X-ray or isotopic radiation methods. In the latter area, a promising method, using tin-shielded ^{125}Iodine as a source of essentially monoenergetic photons, is in an advanced state of development (ref. 190). Using a scintillation detector to count transmitted photons, the system provides a quantitative measure of absorption which is reported to provide reproducibility to ~~two~~ 2 percent. By contrast, normal X-ray film procedures are qualitative only, rarely detecting bone mineral changes of less than 30-60 percent.

Because of the importance of accurately measuring bone demineralization, a conference on "Progress in Development of Methods in Bone Densitometry" was sponsored in Washington, D. C. by NASA, March 25-27, 1965. The proceedings, NASA SP-64, 1966, issued under that title, provide a comprehensive review of the state of the art (ref. 191).

Immobilization:

N.G. In studies investigating the effect of immobilization on bone-tissue metabolism, male guinea pigs were maintained on ~~three~~ these levels of dietary ascorbic acid in Purina rabbit chow: ~~The levels were~~ 0, 400, and 2,000 mg, of ascorbate per kg, of diet. Seven days following

start of the dietary study, the right rear limb of each animal was immobilized with plaster of paris casts. After 14 days, each animal was given ^{45}Calcium intra-peritoneally. Twenty-one days after initiation of dietary treatment, the animals were sacrificed and metabolic studies conducted with bone tissue taken from the free and immobilized hind limbs. (ref. 192) ~~(25)~~

Reporting a normal increase in oxygen uptake and lactic acid in the immobilized state, the studies find these to be dependent on the presence of ascorbic acid. However, both free and immobilized tissue from the scorbutic animals exhibited values for these factors much higher than tissue from ascorbate-supplemental individuals. Skeletal ^{45}Calcium deposition, its active and inactive release during <u>in vitro</u> incubation were all altered significantly by the dietary deficiency. Immobilization appeared to have little effect on these parameters (as opposed to results on human studies reported herein) with the exception of a highly significant increase in the release of ^{45}Calcium by heat-inactivated tissue in both ascorbate-supplemental groups. Alkaline phosphatase and citric-acid levels of bone tissue were not affected by immobilization.

From the results of the study it could be surmised that vitamin-C deficiency resulted in the deposition of a bone salt with a higher degree of lability. From this it seems possible that maturation of the bone crystal could have been inhibited. Immobilization caused no additive influence to the ascorbate deficiency, but in completely-fed individuals there was evidence of increased bone-salt lability, in the immobilized limb, suggesting that the similar responses elicited by bone to the two

separate stress conditions were mediated via different routes. Since cellular metabolism appeared to be elevated by each stress (deficiency or disuse) it may be that such activity reflects a general compensatory response which is energy-dependent. Another consideration is the possibility that biological turnover of calcium in immobilized tissue may be influenced by the blood ascorbate level. The immobilized-to-free ratio for ^{45}Ca calcium concentration was strongly related to blood vitamin-C levels. This relationship suggests that, with disuse, the biological turnover of bone salts is inhibited, but may be stimulated first by normal levels of ascorbate and further pushed by excess amounts. (8)

Studies were also undertaken to determine whether bone loss resulting from immobilization is due to an increased catabolism or an inhibition of anabolism. In vitro assessments of oxygen uptake and lactate production showed that, 48 hours after the initiation of immobilization, they were both elevated. ^{45}Calcium disappeared from bone at comparable rate for both cancellous and compact tissue. There was no significant difference between free and immobilized tissue.

6. GRAVITY NULLIFICATION Clc

Most organisms detect the direction and strength of the gravitational acceleration to which they are exposed, ~~the~~ responding to the gravity field in the course of their development and in their behavior. In many instances, the mechanisms by which the organism senses acceleration is incompletely known. This is especially true in the case of the higher plants. Morphologic responses (especially in plants) in growth patterns, establishment of polarity and symmetry, and the time course of development ~~as~~ are well-recognized responses to the

gravitational vector in many cases (~~(26)~~ ref. 193).

One way of studying these mechanisms by which gravity influences plant development is to "eliminate" or nullify the effects of gravity with a clinostat (FIG. ~~26~~ 23). After ~~its~~ placement in a clinostat in a horizontal position and rotation, the corrective growth response (Insert fig. 23) in the axis of a plant is known to be mediated by an imbalance in auxin, induced by gravity. Our present understanding of growth curvatures produced by negative geotropism is based on the concept of excess auxin or indole acetic acid (IAA) in the lower side. Immature plants from a clone of Coleus blumer Benth were used to trace the movement of [^{14}C-2] indoleacetic acid during development of geotropic curvatures in branches and the main axis. Unaltered IAA was found to be distributed in a ratio of approximately 40:60 in the upper and lower halves of the curvatures. This evidence supports the concept of downward transport of unaltered IAA as the basis for more rapid growth of the lower tissues in a geotropic curvature of a branch or main axis (Lyon, ref. 194). ~~(27)~~ Subsequently, studies were undertaken of axial growth rates and auxin transport. Radioassays of the distribution of extractable, radioactive IAA within a curved stem showed that a large excess of this auxin had been transported from a terminal supply into the convex half of the curvature. These curvatures of stems and the imbalances in auxin distribution did not appear, however, when the plants stood erect to gravity. The evidence from these isotope studies supports the interpretation of the regulatory action of gravity on auxin transport and upright growth as being due to the mechanism of negative geotropism (~~(28)~~ ref. 195).

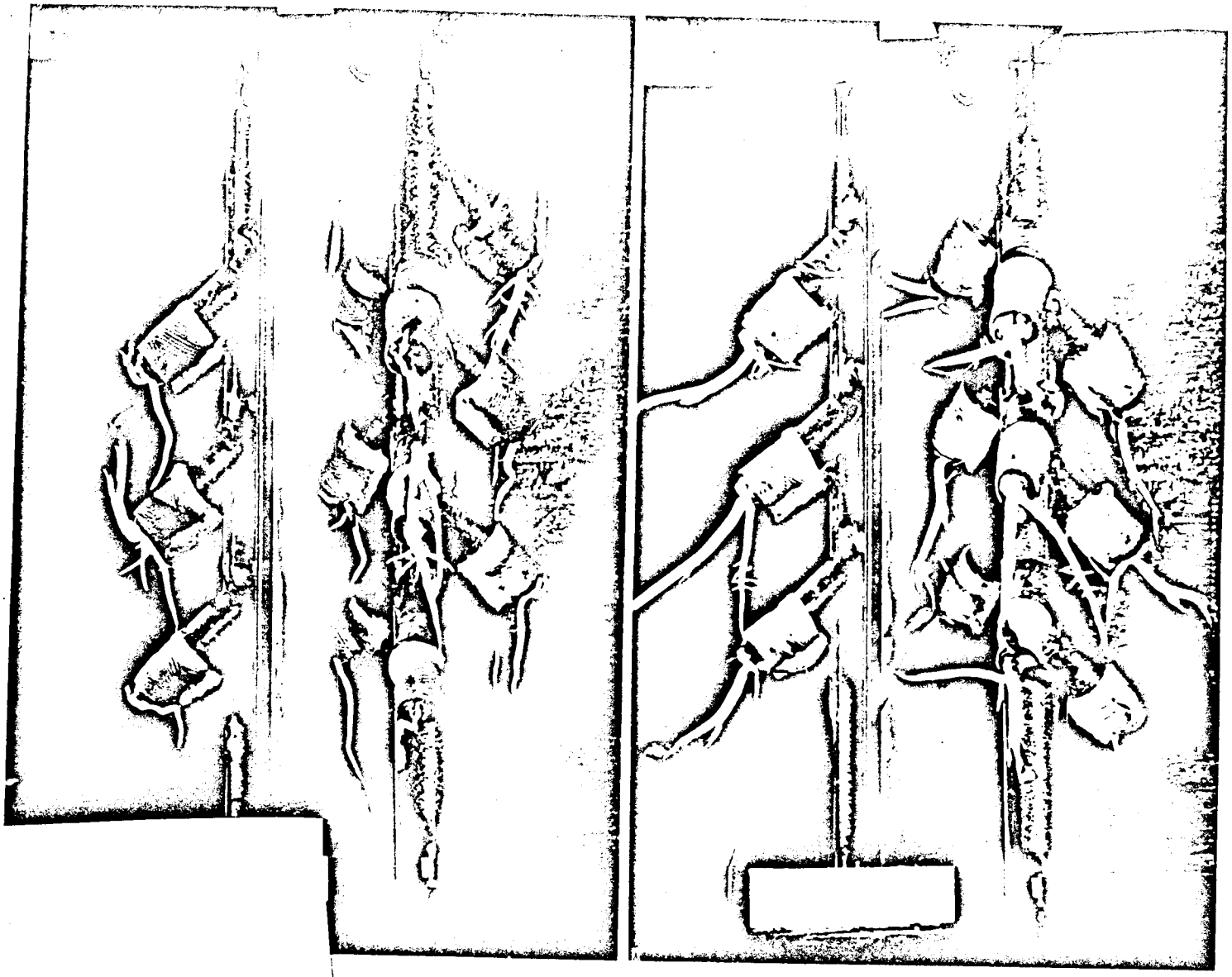

Figure 23.- CLINOSTAT EFFECTS ON PEA SEEDLINGS. (Left) PEA SEEDS, ERECT TO GRAVITY, GROW NORMALLY, i. e., ROOTS DOWN AND SHOOTS UP. (RIGHT) SEVEN DAYS OF CLINOSTAT ROTATION RESULTS IN DISORIENTATION i. e., ROOTS TEND TO GROW IN HORIZONTAL PLANE AND SHOOTS GROW DOWNWARD

Experimenters have sought to relate causally the phenomena known as geoelectric effect to a lateral redistribution of the growth hormone, auxin, and thence to geotropic curvature. The geoelectric effect is a difference in potential between the upper and lower sides of the shoot and root tips developed in many plant species after they are reoriented from a vertical to a horizontal position. Experiments ~~(29)~~ (ref. 196). have demonstrated a lack of either a causal or a direct relation between the geotropic and geoelectric effects. Auxin is required for both responses to gravity.

Additional support for this conclusion was obtained in experiments showing that gravity-induced asymmetry of transport of indoleacetic acid in horizontal segments is greater under anaerobic than under aerobic conditions. As geoelectric potentials do not occur under anaerobic conditions, it is concluded that geoelectric potentials are not a mandatory consequence of asymmetric auxin distribution (~~(30)~~ Dedolph, ref. 197).

Other experiments indicate that enhanced plant responses to horizontal clinostat rotation, where rates of rotation are sufficient to nullify the directional component of the gravity-force vector, are caused primarily by increases in metabolism and not by a modification of auxin availability (~~(31)~~ ref. 198). Auxin uptake by the tissues, a metabolically dependent component of the transport system, is consistently increased by horizontal rotation. It is suggested that metabolism may be accelerated by horizontal clinostat rotation and the concomitant nullification of the directional component of the gravity-force vector. The increased curvature response to geostimulation of horizontally rotated coleoptiles may result from enhanced metabolism and not a transport-induced auxin asymmetry.

Implicit in clinostat experiments is the concept that rotation about a horizontal axis cancels biologically the directional component of the gravity-force vector. Studies (Ref. 199) show that geotropic response is dependent upon the calculated degree of nullification of the directional component of the gravity-force vector. This relationship increases the probability that clinostat rotation at appropriate rates produces conditions simulating low gravity. The geotropic curvature response increases with the force imposed. Forces of 0.06 and 0.08 x g were sufficient for maximum response of oat roots and coleoptiles, respectively.

The 2π Compensator (~~Figure 4~~ 24) represents another experimental approach bearing on the question of the thresholds of graviperception. In operation, each radial spoke turns about its horizontal longitudinal axis at 2 rpm as a clinostat; simultaneously, it moves about the central vertical axis, in a horizontal plane, so that an outward force is applied in the manner of a centrifuge. Compensation, in two coordinates, is thus achieved for the directional component of the gravitational vector (Ref. 200). ← (Insert fig. 24) → Oat ~~Avena~~ seeds were germinated and grown in the dark in this apparatus under continuous compensation. Differences for any organ at forces less than 10^{-3} x g did not vary significantly (~~Figure 5~~ Fig. 25). One might consider, then, the region between the forces of 10^{-2} and 10^{-3} x g as the "threshold range" of perception under these experimental conditions. The experiments indicate that accelerations yielding continuous or pulsating forces with vectors no greater than 10^{-3} x g appear permissible for in-orbit bioscience experimentation. 10^{-4} x g would be conservative by one order of magnitude.

(Insert fig. 25)

GRAVITY COMPENSATION IN TWO COORDINATES (2π)

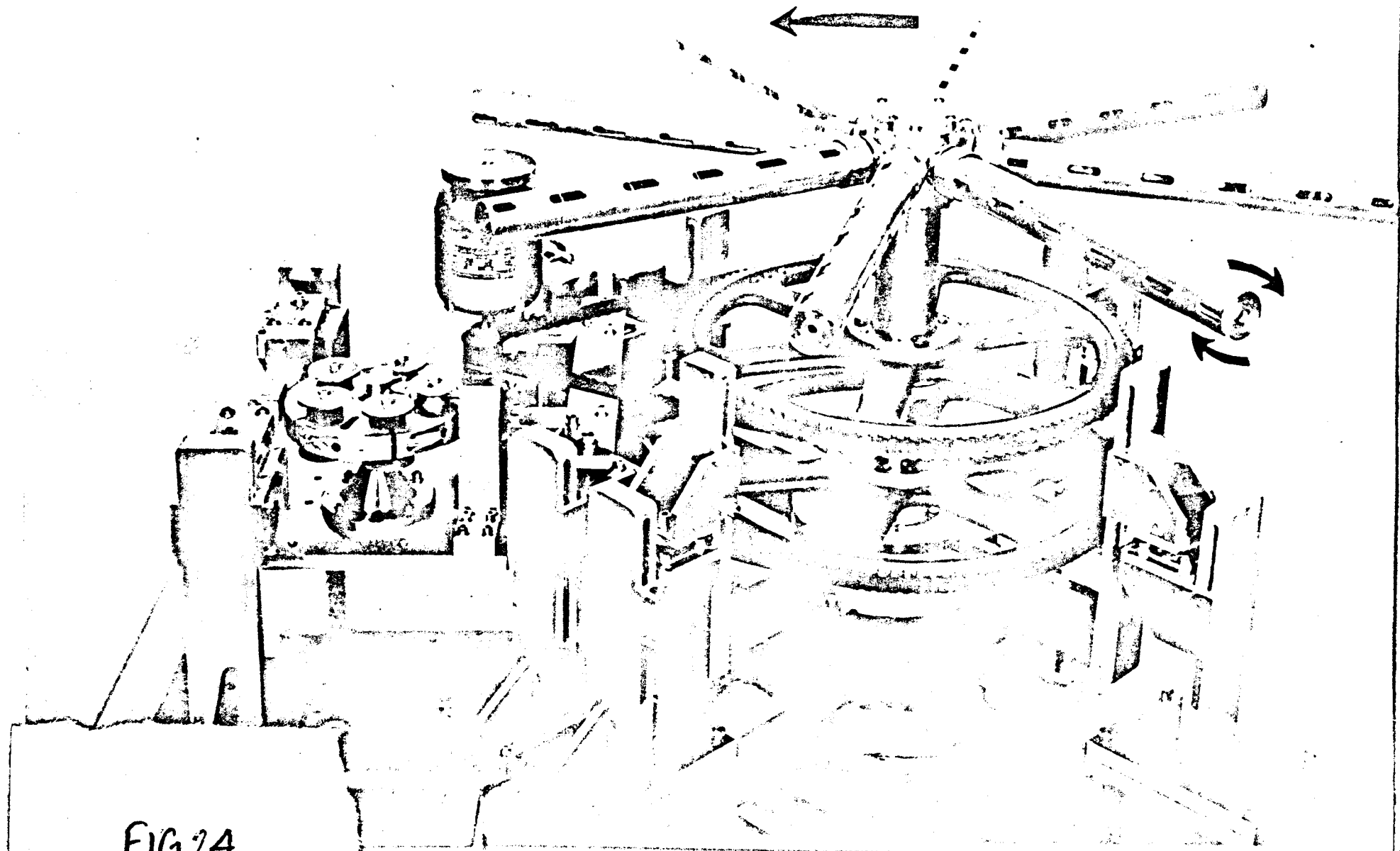

FIG. 24

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GRAVITY COMPENSATION IN OAT SEEDLINGS AFTER 2TT COMPENSATION

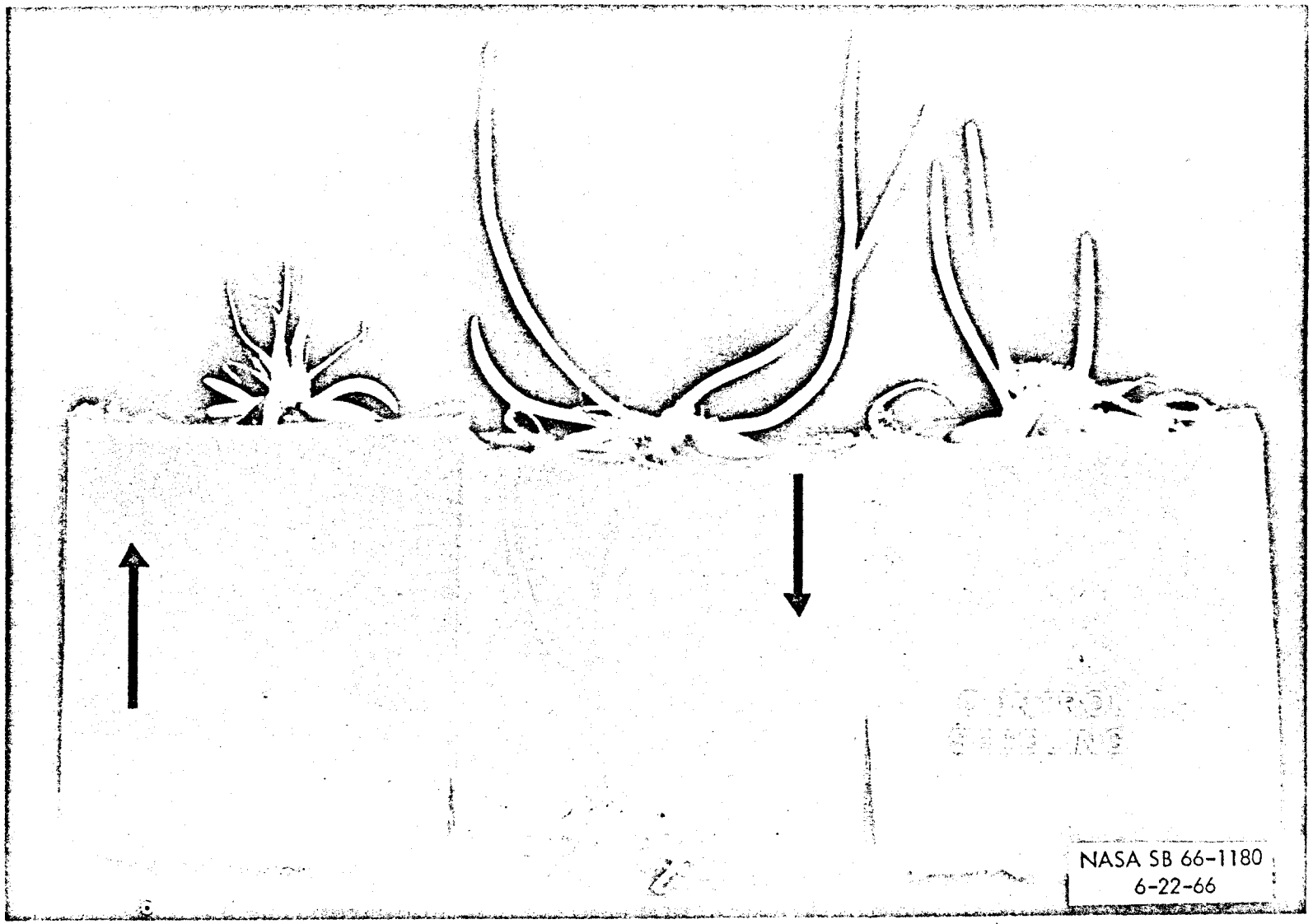

Figure 25.—

7. Weightlessness

Bone Demineralization — Gemini.- The effect of weightlessness on bone demineralization in the crews of Gemini IV, Gemini V, and Gemini VII has been studied (Mack, refs. 201-203) with the technique of radiographic bone densitometry, which measures the percentage of change in bone mass (in terms of calibration wedge mass equivalency). As reported elsewhere in this chapter, bedrest has been found to simulate weightlessness in that bone demineralization also occurs. A bone demineralization study based on radiographic densitometry was conducted to find the effect upon the human skeletal system of prolonged weightlessness and immibilization associated with confinement for a period of days in the Gemini spacecraft.

In the Gemini IV study, the os calcis or heel bone was investigated, as was phalanx 5-2 of the left hand. In the Gemini V investigation, the same bones were examined, with the addition of phalanx 4-2, the distal end of the left radius, and the left talus. In Gemini VII, the os calcis, the talus, and phalanges 5-2 and 4-2 of the left hand were included.

Central Os Calcis Section.- This anatomical site was used in the M-6 experiment in the Gemini IV and Gemini V flights and was repeated

in the Gemini VII mission. The tracing path across the left os calcis in lateral projection runs diagonally between conspicuous posterior and anterior landmarks which, by superimposing successive radiographs, can be reproduced accurately in serial films of the same individual. This single path (1.3 mm in width) is known as the "conventional scan."

Approximately 60 percent of the total os calcis mass is evaluated in the parallel-path system. After making the conventional scan, a series of parallel paths, 1.0 mm apart, were scanned, beginning 1 mm above the conventional path and continuing to the lowest portion of the bone (fig.26). The total number of paths scanned is, therefore, proportional to the size of the bone which, of course, has individual variations. For the command pilot, 38 paths were required to cover the os calcis portion examined, while 42 parallel scans were needed for the pilot.

(Insert fig. 26)

The Talus. - A single scanning path was made through the talus of the left foot, originating at the interior surface and projecting anteriorly to the conspicuous landmark.

Sections of the Phalanges 4-2 and 5-2. - The second phalanx of the fourth and fifth fingers of the left hand was scanned by parallel cross-sectional paths 1 mm apart alined tangentially with the longitudinal axis and covering the entire bone area.

BONE MINERAL LOSS IN GEMINI ASTRONAUTS (HEEL BONE)

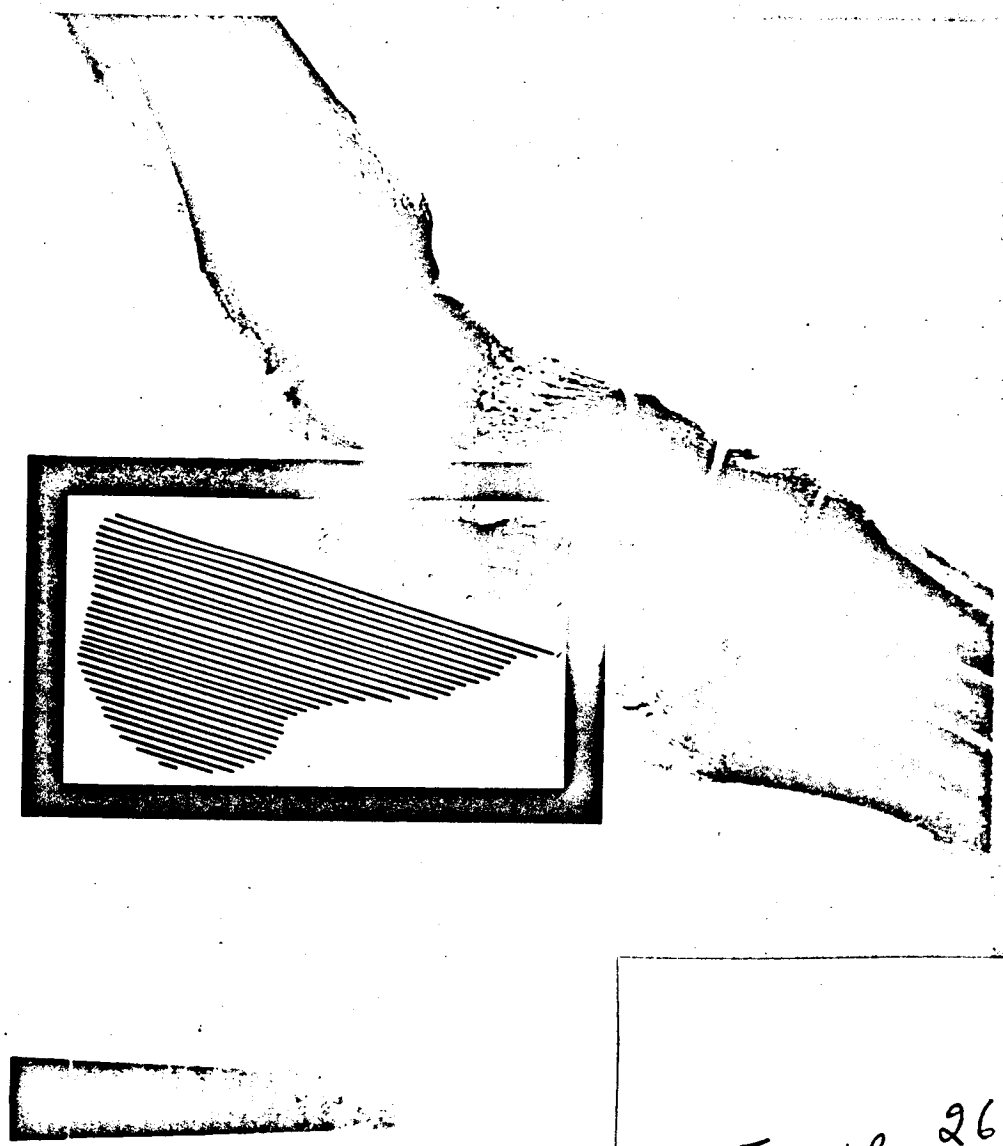

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Figure 26.-

It is interesting to note how the crewmembers of Gemini IV, Gemini V, and Gemini VII have compared with each other as to skeletal changes in three major anatomical sites with respect to changes in skeletal density during space flight. The bone-mass changes in table VII (in terms of calibration wedge equivalency) have been found for the command pilot and the pilot in the "conventional" os calcis section, in the combined sections covering 60 percent of the os calcis, and in hand phalanges 5-2 and 4-2, both for the command pilot and the pilot for the three orbital flights.

TABLE VII.--COMPARISON OF BONE DENSITY CHANGES IN CREWMEN OF GEMINI IV, GEMINI V, AND GEMINI VII DURING SPACE FLIGHT

Position of anatomical site Evaluated	Change in bone mass,* percent	
	Command pilot	Pilot
Conventional os calcis scan:		
Gemini IV	-7.80	-10.27
Gemini V	-15.10	-8.90
Gemini VII	-2.91	-2.84
Multiple os calcis scans:		
Gemini IV	-6.82	-9.25
Gemini V	-10.31	-8.90
Gemini VII	-2.46	-2.54
Hand phalanx 5-2 scans:		
Gemini IV	-11,85	-6.24
Gemini V	-23.20	-16.97
Gemini VII	-6.78	-7.83
Hand phalanx 4-2 scans:		
Gemini IV	**	**
Gemini V	-9.98	-11.37
Gemini VII	-6.55	-3.82

* Based on X-ray absorbency of calibration wedge.
** Not done on this flight.

A comparison of this data with bed-rest studies is shown in Figure 27 ~~28~~.

(Insert fig. 27)

Synergistic Effects of Weightlessness and Radiation in Human White Blood Cells.

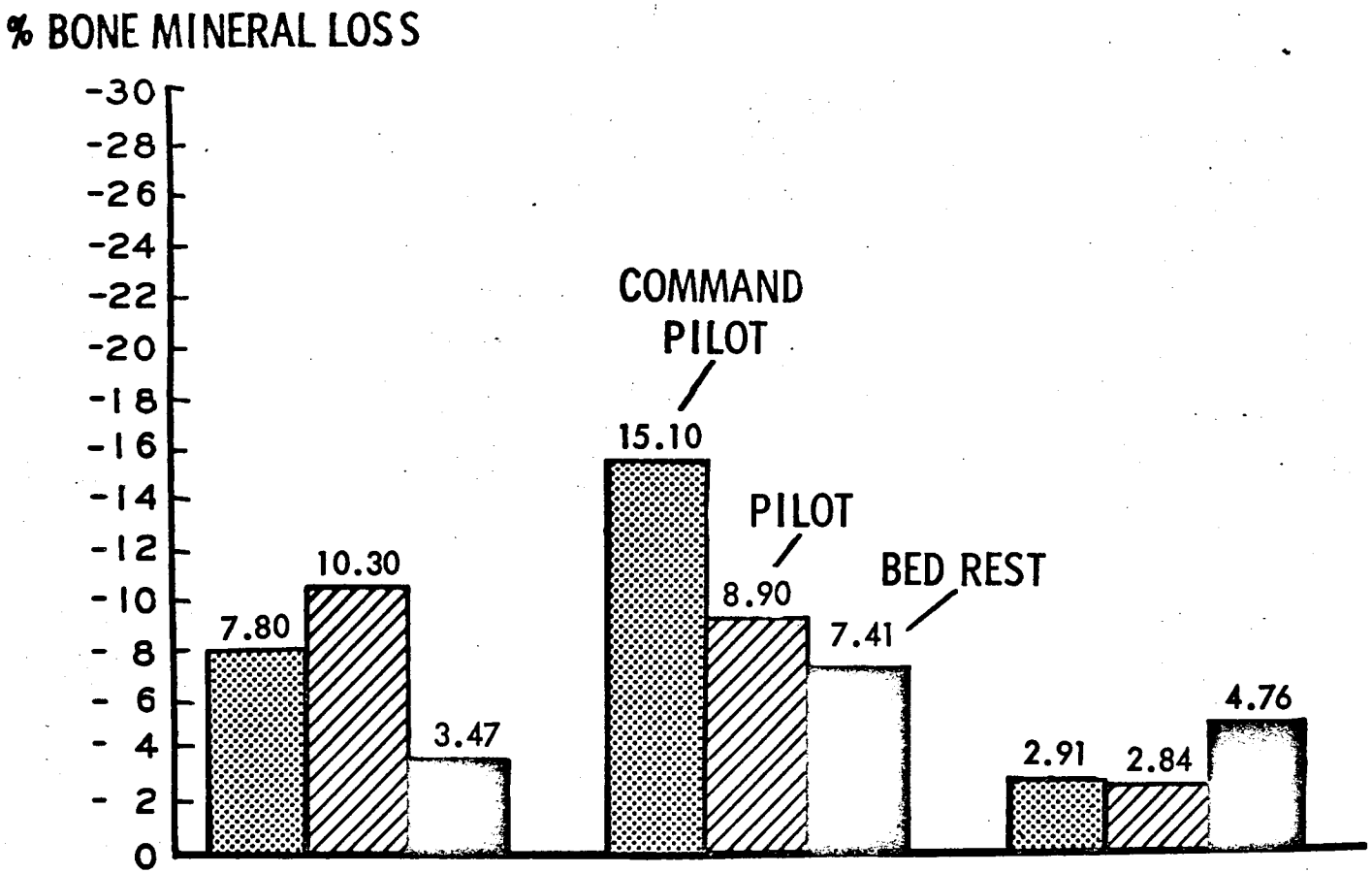
No. 5 Biological effects of the types usually associated with radiation damage have been observed following space flight. These effects include mutation, production of chromosome aberrations, and cell killing. This could be due to either or both of two things: effects of the heavy-primaries component of radiation which is not available for test in terrestrial laboratories, or synergistic interaction between radiation and "weightlessness" or other space flight parameters ~~(37)~~ (ref. 204).

The procedure was to irradiate a thoroughly studied biological material with a known quality and quantity of radiation during the zero-g phase of flight. This, with concurrent and equivalent irradiation of a duplicate ground-located control sample, would yield a comparative set of data and would be evidence of synergism, if it existed, between the radiation administered and some space-flight parameter. Since chromosomal aberration is one of the best-known effects of radiation, it was selected as a suitable response for the study.

The equipment operated properly, and the experimental procedures were successfully completed. The lack of aberrations in the postflight blood samples from the crew makes the possibility of residual effects of radiation encountered on such a space flight very unlikely, at least on genetic systems. The yield of single-break aberrations (deletions) for the inflight sample was roughly twice that seen in

BONE MINERAL LOSS IN GEMINI ASTRONAUTS HEEL BONE

CHANGE IN BONE MINERAL



GEMINI FLIGHT	IV	V	VII
TIME (DAYS)	4	8	14
CALCIUM (GM/DAY)	0.6	0.3	1.0

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Figure 27.—

the ground-control and previous samples. (Fig. 28) All physical evidence contradicts the possibility of variant radiation doses to the ground-control and flight samples. It appears then that some space-flight parameter does interact synergistically with radiation. Although this effect is not large from the point of view of radiation cytogenics, it is of interest. Further experiments will be necessary in order to confirm the synergistic effect and to determine just which space-flight parameter or parameters are involved, as well as the mechanism of the action.

(Insert fig. 28)

Effect of Weightlessness on Frog Egg Development

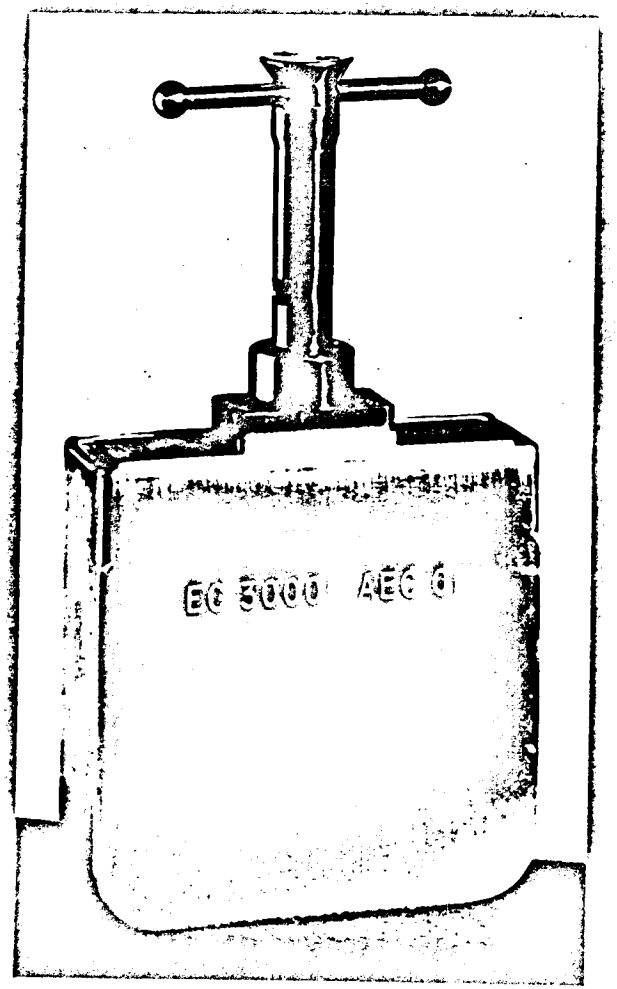
NOT Since frog eggs are known to orient with respect to gravity during their very early development, they were selected for the first Gemini experiments. The effect of weightlessness on the ability of fertilized frog eggs to divide normally and to differentiate and form normal embryos was examined in two experiments which were carried aboard Gemini VIII and Gemini XII respectively.

(Young, ref. 205)

Gemini VIII

The experiment was performed in two identical packages, one mounted on each hatch of the spacecraft. Each package had four chambers containing frog eggs in water, with a partitioned section containing a fixative (5 percent formalin). Each package was insulated and contained temperature-control systems for both heating and cooling in order to maintain an experiment temperature of around 70° F.

CHROMOSOME BREAKS IN HUMAN WHITE BLOOD CELLS



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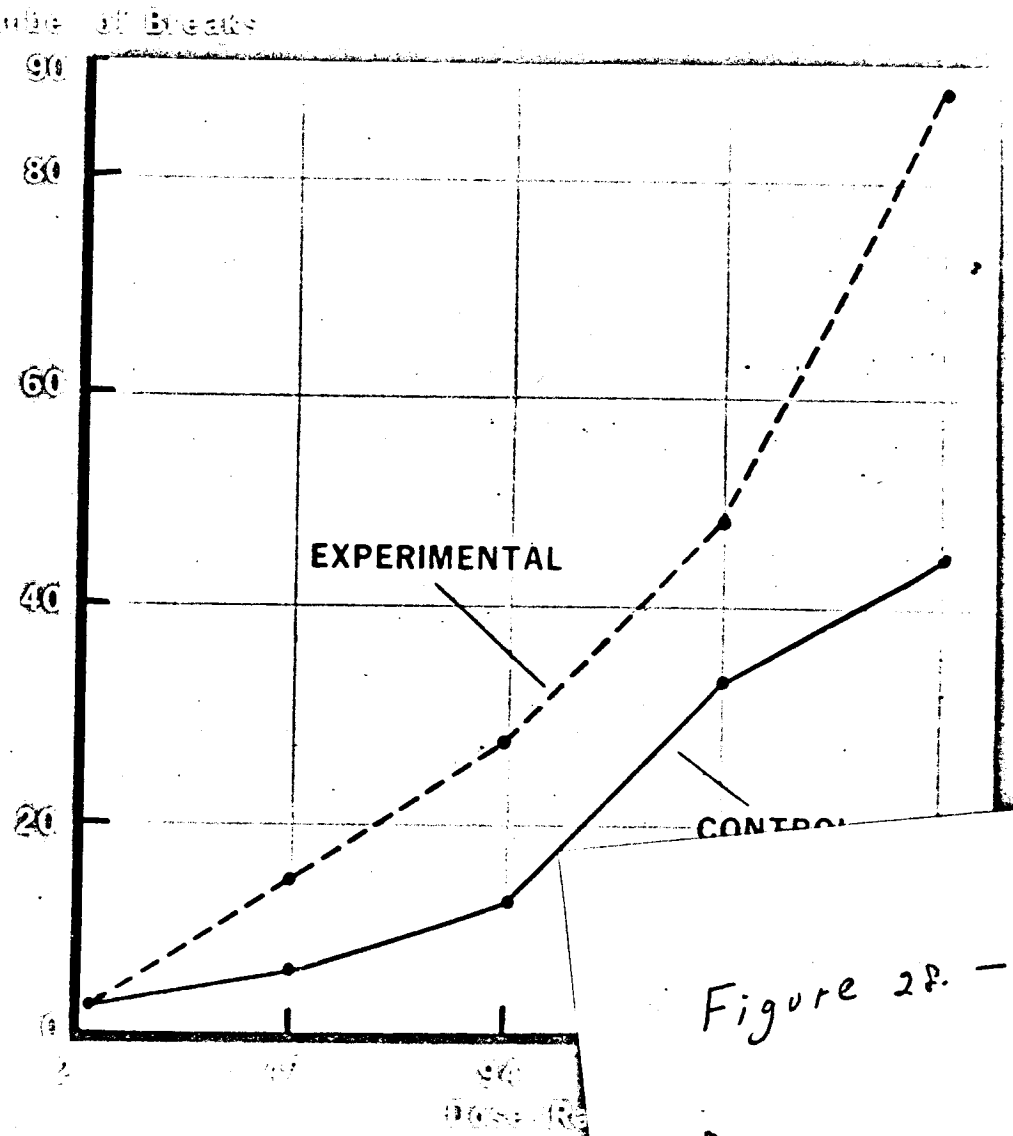

Figure 28. —

The experiment was actuated by handles provided on the outside of each package. These handles and a switch for the heating element were manipulated by the adjacent astronaut either on command or according to a predetermined schedule. Identical hardware was used for control experiments on the ground.

Eggs were obtained from several dozen female frogs (Rana pipiens) by injection of frog pituitary glands about 48 hours pre-launch, in order to induce ovulation. The best of these eggs (from two females) were selected for flight and fertilized by immersing in a sperm suspension made by macerating frog tests in pond water. The fertilized eggs were then removed to a 43° F. cold room and placed in about 10 cc. of pond leak-proof partitions in the chamber. Each chamber received from ~~five~~ 5 to ~~eight~~ 8 eggs, so that a total of 52 eggs were flown. Two sets of controls were set up in identical hardware on the ground. The first was to run simultaneously with the flight, the second was delayed about ~~two~~ 2 hours so that changes in temperature experienced by the flight experiment could be duplicated on the ground more precisely than in the simultaneous control. Since telemetered temperatures were not received instantaneously, such a delayed control was necessary.

The flight experiment was placed in the spacecraft about ~~four~~ 4 hours before launch. By keeping the fertilized eggs at about 43° F. until this time, the first division of the eggs was retarded. It was hoped that this pre-cooling of the eggs would be sufficient to retard first cleavage until the weightlessness phase of the flight.

FROG EGGS WITH EIGHT CELLS AFTER 2.5 HOURS OF WEIGHTLESSNESS

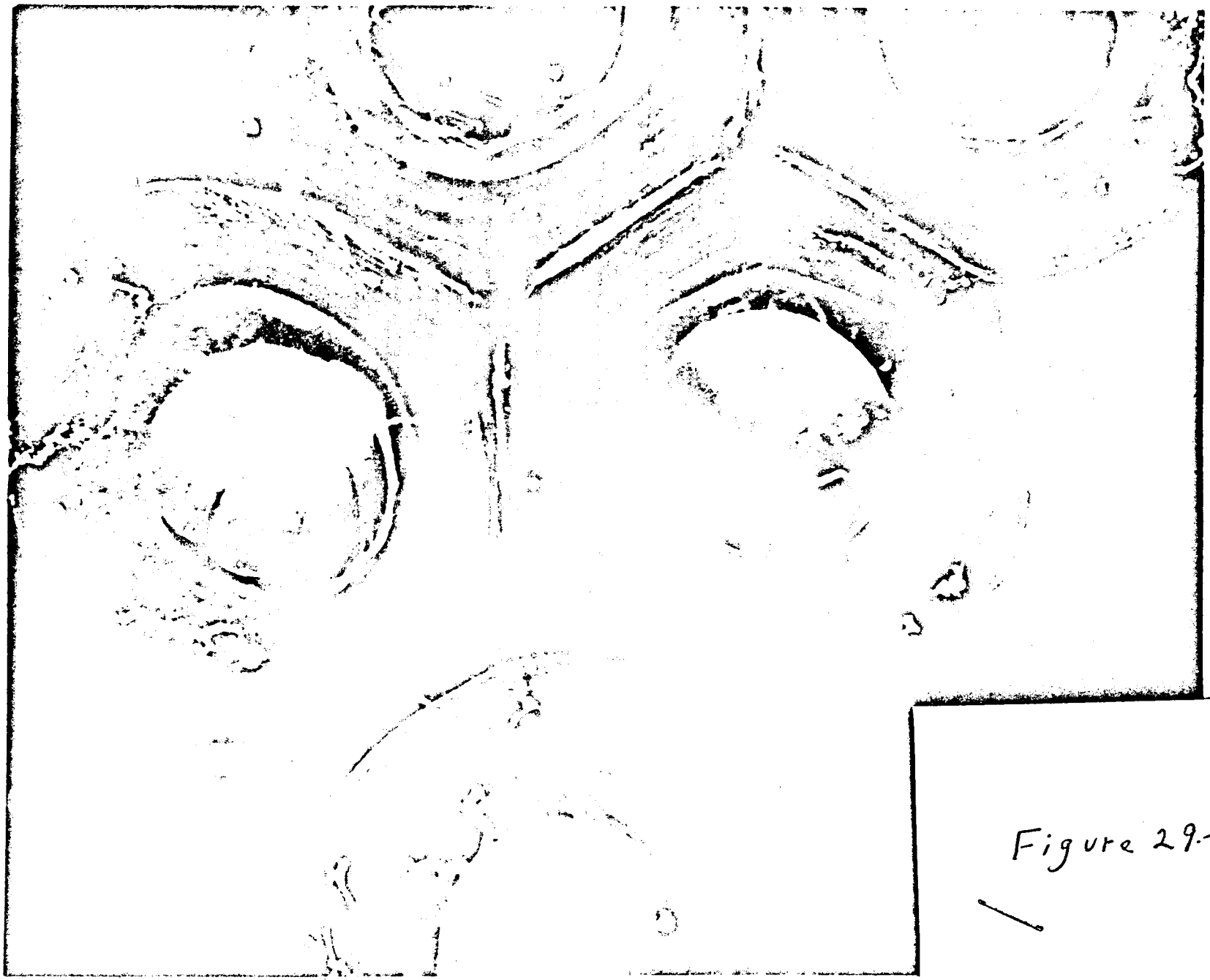

Figure 29.—

Although the cabin temperatures were considerably above the predicted 70° F., the temperature-control system on the experimental packages was sufficient to retard first cleavage until the weightlessness phase of the flight. Thus, the first fixation (at launch plus 40 minutes) was successful in stopping development between first and second cleavages. The astronauts were also able to perform the second activation at ~~two~~ 2 hours and 25 minutes which was at about the ~~eight~~ 8-cell stage of development. Because of difficulties with the spacecraft, the flight was terminated after ~~ten~~ 10 hours, so that the remaining half of the experiment was not accomplished.

The fixed eggs in the first four chambers appeared identical in all respects when compared to the controls. The cleavage planes appeared normal and to have proceed~~ing~~ed on schedule (FIGURE ~~10~~ 29). Histologic and electron-microscopic study might show some abnormalities but this is not anticipated. The absence of a gravitational field does not appear to have any effect on the ability of the frog egg to divide normally during its early stages when such an effect would be most likely to occur because of the large density gradient in these cells. Whether this independence from gravity applies to differentiation and morphologic changes in later stages was not demonstrated because of the short duration of the flight. Whether the egg will divide normally if it is fertilized during weightlessness, so that the egg never has a chance to become oriented with respect to gravity, is also unanswered.

(Insert fig. 29)

Gemini XII (ref. 206) ~~(39)~~

The experimental procedures were similar to those of Gemini VIII. All phases of the experiment were performed on schedule with good results.

In GT VIII early cleavage stages were obtained successfully. However, because of the short duration of the flight later cleavage stages and developmental stages were not obtained. In GT-XII, later embryonic stages were obtained.

The ~~ten~~ 10 embryos in the 41-hour fixation chambers appeared ~~to be~~ morphologically normal as compared to the controls. In other words, no abnormalities were detected by gross observation, which were not also apparent in the controls. The embryos fixed at 85 hours were well-developed and appeared, morphologically ~~to be~~ normal. ~~tadpoles.~~ The ~~five~~ 5 embryos which were unfixed were live swimming tadpoles upon opening the chamber onboard the recovery ship Wasp. Three of these embryos were morphologically normal, ~~two~~ 2 were abnormal. However, the abnormalities were not inconsistent with the controls and no abnormalities can be ascribed to flight. The ~~five~~ 5 embryos died several hours after recovery and were fixed for histologic sectioning. The reason for this death has not been ascertained. It appears that gravity is not necessary for differentiation and morphologic changes in later stages of embryonic development. However, further experimentation, particularly with regard to growing space-flown tadpoles through maturity and reproduction phases, is indicated.

It should be added that the gravitational field in the Gemini XII flight was not a "zero-g" field. There was actually a gravity-gradient experiment performed during this flight about which very little is known. The gravitational field imposed by this experiment and by spacecraft maneuvering must be assessed before additional conclusions can be reached.

7. BEHAVIORAL BIOLOGY

Behavioral biology plays a two-fold role in space bioscience: (1) it investigates how organisms behave under the extreme conditions of space flight; (2) it applies the unique aspects of space to the advancement of behavioral analysis.

Ground-based research on behavior--although imperative--cannot substitute for experiments during space flight. Behavior in space may be influenced by weightlessness, sharp variations in familar modes of sensory stimulation, alterations in day-night cycles, and, especially for higher forms, a drastic form of social isolation. The behavioral biologist, studying these reactions under actual space-flight conditions, seeks better understanding of their influence on perception and sensorimotor control, on learning and motivation, and on sustained vigilance. Perhaps more importantly, he seeks to determine the biological basis for such changes in behavior, thus providing a broader understanding of the total organism and its environmental interactions.

A. Neurophysiology

The search for the mechanisms underlying behavior involves many neurosciences such as neuroanatomy, neurophysiology, and psychology. A new approach--described as "molecular neurology"--has sprung from molecular biology's success in defining some of the ways in which organisms store, read out, and transfer genetic

and immunological information (ref. ~~113~~ 207). However, relating learning and perception to changes in neuronal nets and individual neurons is extremely difficult since there are approximately 10 billion neurons in the brain and many times that number of glial cells, with many neurons synapsing with hundreds or thousands of fibers from other cells.

1. Neurochemistry and Micro-Neuroanatomy

The types of information stored in a neuron are determined by the cell's morphology and physiology. Strumwasser (ref. ~~119~~ 208) believes that since many neural properties are essentially similar in both vertebrates and invertebrates, it is possible to define the distinctive information any nerve cell in the animal kingdom possesses. He distinguishes various ways in which this information affects the structure and functions of a neuron: the development of dendritic and telodendritic patterns; the path of the axon(s) and therefore long-distance conduction; the specific transmitter substance produced by the neuron; differentiation of the surface membrane; the operation and sensitivity of ionic pumps; the presence of endogenous activity; and the storage of information regarding the time of some past environmental experience.

There is some evidence (Schmitt, refs. 207, 209) ~~(refs. 112, 113)~~ of the potential usefulness of immunological methods in studying neuromorphogenesis

and the parallels between psychological memory and immunological "memory." It has been suggested that immunological techniques, using brain fractions as antigens, may illuminate synaptic specificity of function and formation of the engram (ref. ~~112~~ 209).

Antibrain-antibodies have been prepared by the subcutaneous injection of homogenates of caudate nucleus and other brain "gray" substance" in rabbits, sheep, and pigs (Galambos, refs. 210-212). ~~(refs. 54, 55, 56)~~. Introduction of these antibodies into the lateral ventricles of experimental cats initially appeared to produce behavioral depression and a series of electrographic changes at the brain-recording sites (caudate nucleus, hippocampus, auditory cortex). However, recent evidence indicates the caudate nucleus is not as strongly antigenic as previously thought, necessitating large and repeated injections of antibodies. Other work (ref. ~~57~~ 213) suggests that antibodies specific for a particular brain region such as the caudate nucleus cannot be produced by injecting its homogenate with adjuvant into rabbits.

Recent neurochemical studies revealed significant changes in chemical composition of cells in the vestibular system of the brain in animals exposed for ~~as~~ up to 30 days at three different g levels. This study employed the triple toroid centrifuge (fig. ~~1~~ 30), which was designed for essentially continuous operation, and in which groups of animals were exposed to three different levels of gravity concurrently. A sharp decline in the RNA (ribonucleic acid) content was observed in neurons associated with control of posture and equilibrium. However, additional studies are needed

to define their behavioral consequences.

(Insert fig.30)

The use of ultrasonica lesions, which produce degeneration in afferent and efferent tracts and associated nuclei, has permitted precise determination of the total neuron population of the lateral and medical mammillary nuclei, as well as structurally significant subpopulations (Fry, refs. 214-217). ~~(refs. 47, 48, 49, 50).~~ After the cells degenerate, the residual populations are counted, yielding a complete quantitative description of the neural circuitry of brain structures (ref. 218 ~~51~~).

2. Sensory Processes

Recent studies of excitatory processes in receptors, afferent fibers, and central integrative functions have contributed significant understanding of the stimuli to which an organism is sensitive, the coding of sensory impulses, and the response of higher neural centers to spatio-temporal patterns of stimulation. Most of this research has been done on vision and audition, although the cutaneous afferent system has received some attention.

Broad research (ref. 219 ~~124~~) has been undertaken into the neural bases of perception and learning, particularly in terms of the origin and development of sensorimotor coordination and its adaptability and limits during extreme environmental conditions such as those present in space flight. Electrophysiological methods are being used to study changes in receptor fields in response

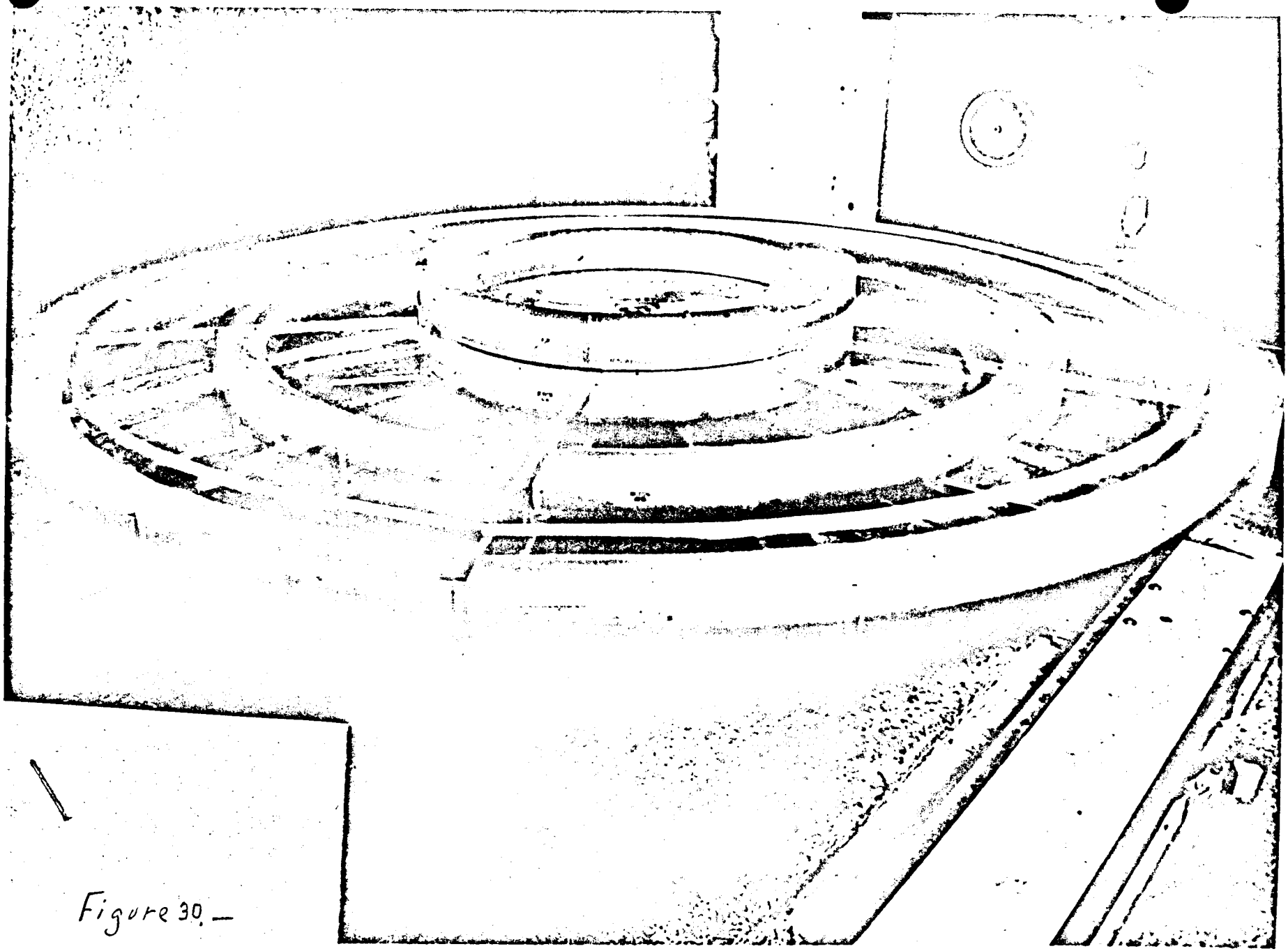

Figure 30. —

TOROIDAL CENTRIFUGE

to various conditions. Comparative and developmental investigations of sensorimotor coordination include use of such animals as fish, birds, rodents (hamsters), kittens, Tupoia, and monkeys. At the general experimental level, the primary concern is with (a) effects of rearrangement and disarrangement of sensory input upon perceptual organization and sensorimotor functions, and (b) closely correlated studies of perceptual constancies and other classical issues in perception.

Vision--Attention has been directed (ref. ~~26~~ 220) to the functions of single nerve cells at various points in the afferent system and the way in which complex signals are received by the receptors and processed by the cerebral cortex in achieving recognition. Although the way in which the organism solves this problem is not yet well understood, recent work with a number of species, including marine mammals and fish, has clarified some of the mechanisms. The central units which integrate impulses from peripheral electroreceptors in the electric fish are being investigated.

The frog's eye, although it has only two layers, represents many functions of the human visual system (including its ability to detect food, danger, and other stimuli related to survival) and has often been used in visual research. The frog's visual system, and its differential response to stimuli, has been modelled by simulation on a digital computer and by the design

of required hardware (ref. ~~121~~ 221). Systems have been developed to simulate the frog's retina and, ultimately, are to be used in a space probe to report the appearance of the surface of Mars. The core of the reticular system has also been modelled to provide a type of decision equipment similar to the frog's decision system. Four primary groups of ganglion cells in the frog's retina, each with distinctive anatomical and physiological characteristics, have been distinguished (ref. ~~87~~ 222): group 1, edge detectors; group 2, bug detectors; group 3, event detectors; and group 4, dimming detectors. The ganglion cells in the second group (bug detectors) are among the most complex in the retinal

- 6 -

structure, and therefore difficult to model. However, simulation of these cells has been partially achieved through the use of logical methods and specially contrived models.

Audition.— Studies of audition in cats have used chronically implanted electrodes in their auditory centers and pathways (ref. ~~91~~ 223). The animals are tested for equivalence between direct electrical stimulation of the auditory system and normal stimulation of peripheral end organs. Results indicate cats can be trained to respond to a 3-volt increase in the intensity of recurrent 1-per-second electrical stimuli. Cats also show immediate transfer when trained to respond to stimulus-intensity changes. Cats have also been trained to respond to changes in the rate of electrical stimulation through bipolar electrodes in the auditory cortex. Significantly more learning trials were required to attain the same performance level when stimulation was applied at the cortical level than at a subcortical level.

The effects of very low frequency tones on human auditory thresholds have been studied (ref. ~~69~~ 224) with ~~nineteen~~ 19 subjects exposed to repeated 3-minute tones, ranging from 2-22cps and 119-144 dB. Temporary threshold shifts of 10-22 dB (between 3,000 and 8,000 cps) were produced in 11 subjects, while the remaining 8 showed no effect. Coats (ref. ~~32~~ 225) has demonstrated that temperature can influence the auditory system.

The green frog (Rana clamitans) has been used (ref. ~~111~~ 226) in recording the response of single units of the eighth(auditory) cranial nerve to acoustic stimuli. All of the units were frequency-

selective, with high- and low- frequency units probably originating in different sense organs within the otic capsule. The evoked responses of the auditory cortex of unanesthetized cats suggested mutiple projection pathways to the cortex with significant functional differences (ref. ~~123~~ 227). Similar evidence has been found in the temporal firing patterns of auditory cortical units as a function of stimulus parameters (ref. ~~58~~ 228). A large number of response patterns were exhibited by different units in response to the same stimulus.

3. Reticular Formation

The behavioral significance of the reticular system has become increasingly evident in neurophysiological research during the last 30 years, particularly in regard to its excitatory and inhibitory influence on the classical sensory system, the cerebral cortex, and the limbic system. Briefly, its rostral effects are implicated in the following functions: the initiation and maintenance of wakefulness; the orienting reflex; sensory control; attention; conditional learning; sleep and internal inhibition, through the pontile tegmentum and midline thalamus; memory, through the temporal cortex and hippocampus; and visceral-endocrine and innate-emotional responses, through the limbic forebrain and cephalic brain stem. Caudally, it is concerned with postural reflexes and motor outflow from the spinal cord. Throughout vertebrate development, the reticular system has shown a capacity to

control an organism's commitment to one mode of behavior rather than another. However, although its anatomy is fairly well known, there is as yet no tenable theory to account for its performance (ref. 229 ~~71~~).

4. Electrophysiology

Research on the reticular system has been largely with electroencephalographic methods, particularly in terms of alpha blocking or desynchronization of the EEG, or through the recording of evoked potentials. Generally, attempts are made to correlate the appearance of certain responses or behaviors with cortical evoked potentials or changes in the EEG pattern. The ascending reticular activating system (ARAS) is known to have a profound effect on both variables; stimulation of the ARAS can result in a blocking or desynchronization of the 8-12 cps alpha rhythm seen in a normal, awake subject, and can also increase the evoked potentials elicited by peripheral stimulation. In studies of attention, reaction time, and perceptual discrimination, marked changes in performance (e.g., a significant increase in temporal resolution) accompany ARAS stimulation, which can selectively and reciprocally lower and raise sensory thresholds for various modalities (ref. 230 ~~78~~).

Cortical Evoked Potentials.--Computer-averaged evoked potentials have been obtained (ref. 231 ~~63~~) from the cortex of three subjects

presented with a visual vigilance task. Nonsignal stimuli (requiring no response) and randomly interspersed signal stimuli (requiring a key-pressing response) were presented to each subject on five trials. Evoked potentials were recorded from the right occipital cortex and fed into a computer. The results indicate that reduction in attentiveness is paralleled by reductions in amplitude of visual, cortical evoked potentials in humans.

Similar results were obtained with visual stimuli of constant duration (Wicke et al., ref. 232). ~~(ref. 132).~~ Here the latency, amplitude, and waveform of the evoked responses varied as a function of the luminance of the stimuli (light flashes), and showed a reciprocal relationship between the luminance and duration of the stimuli.

In studies of cortical evoked responses to visual (light flashes) and auditory (clicks) stimuli presented alternately (ref. 233 ~~115~~), subjects were instructed to attend to one type of stimulus and ignore the other. Responses to flashes recorded from the occipital area were larger when attention was directed toward visual stimuli and responses to click stimuli recorded from the temporal area were larger when attention was directed toward auditory stimuli.

Cortical evoked responses have also been recorded from occipital vertex, temporal, and orbital leads during reaction time trials in which brief light flashes were used (ref. 234 ~~40~~). The subjects performed both with and without knowledge of the results. Faster reaction times

were associated with greater amplitude in average evoked potentials, although knowledge of the results produced a decrease in reaction times and an increase in the magnitude of the evoked potentials. Changes in evoked potentials have also been observed when pairs of light flashes are presented under different conditions (ref. ~~39~~ 235).

Electroencephalography.— EEG data obtained from 50 astronaut candidates were analyzed using display techniques appropriate for medical monitoring and digital computing methods for spectral analysis. On the basis of a number of common characteristics, conscious and sleeping states could be clearly delineated. A common baseline was established during vigilance tasks and in progressively finer discriminative visual performances that simulated closely conditions requiring critical judgment in flight. Recognition of these states by a special-purpose flight computer is considered feasible. The study emphasized the value and superiority of the EEG in detecting shifts in sleep-wakefulness, vigilance, and discrimination when compared to observations of the EKG or respiration.

A simple electronic method for graphing EEG sleep patterns — which circumvents the time and labor problems of conventional techniques — was used to obtain an objective evaluation of the pilot's sleep pattern during the Gemini VII flight (ref. ~~83~~ 236). This sought to identify the factors which might interfere with sleep in an orbiting spacecraft and to show the effects of "weightlessness" on the brain's electrical activity.

5. Stimulation and Self-Stimulation

The finding that direct electrical stimulation of certain areas of the brain acts as positive reinforcement of behavior began with an accidental observation in 1953 by ~~James~~ Olds (ref. ~~96~~ 237). He was using a rat with chronically implanted electrodes in its cortical olfactory area and connected to an electric stimulator by two wires suspended over its box. Previous work by Delgado, Roberts, and Miller had shown that brain stimulation would act as negative reinforcement, i. e., the animal would tend not to repeat behavior immediately preceding brain stimulation (e. g., it would avoid a particular corner of its box). However, following stimulation, Old's rat unexpectedly returned to ~~the~~ its corner, ~~in which it had been,~~ leading to the conclusion that brain stimulation can be positively reinforcing with all the characteristics of a primary reward. This was demonstrated in a number of subsequent learning studies. The animals were also placed in an operant situation and the circuit arranged so that they could, through their own responses, trigger the stimulation (self-stimulation).

The rate at which a rat stimulates its own brain constitutes a measure of the reinforcing properties of the stimulus. Rates vary from 200 to 500 times an hour; the highest rates occur~~ring~~ with electrodes placed in the amygdala, anterior hypothalamus, and septum; ~~and~~ the moderate self-stimulation occurs in the posterior hypothalamus, anterior

thalmus, cingulate gyrus, and hippocampus (Olds, refs. 238 239 240). ~~(refs. 99, 100, 125).~~ Behavior reinforced by stimulation has sometimes been maintained for more than 24 hours, until the animals reach a state of physical exhaustion.

The reinforcing properties of brain stimulation have been extensively investigated (ref. 241 ~~94~~). Studies have been made on the neural mechanisms of appetitive and aversive operant behavior (ref. 242 ~~97~~) and the effects of stimulation of various brain areas in monkeys during the retention interval of a delayed response situation (ref. 243 ~~25~~). Performance on this task was adversely affected with stimulation in both positive and negative areas, and Cianci (ref. 244 ~~28~~) found 23 points in the brains of monkeys where stimulation resulted in severe impairment of their ability to make delayed responses. Tests of the effect of electroconvulsive (ECS) and pain shocks on long-term memory in albino rats (ref. 245 ~~101~~) showed less "forgetting" with pain shock, suggesting that electroconvulsive shock may interfere with all engrams, the effect being inversely related to their age. It was also found that ECS-producing stimulation of the amygdala severely impaired the retention of habits learned

in a Lashley III maze; however, there was no adverse effect when ECS was induced with electrodes in the hippocampus (ref. ~~102~~ 246). When intracranial electric shock was used as positive reinforcement for maze learning, lesions in this area abolished the habit for a period of ~~four~~ 4 weeks. Partial recovery was observed after 7-13 weeks.

Olds (ref. ~~98~~ 247) has also attempted to demonstrate operant control over action potential sequences in order to localize the areas and identify the electric signs of the reinforcement process. He has induced and suppressed hypothalamic self-stimulation by injecting endogenous substances into the self-stimulation site (ref. ~~95~~ 248). Excitatory effects were produced with carbamylcholine, testosterone sulfate, and substances which chelate or precipitate calcium. Solutions of sodium chloride, sodium sulfate, and sodium lauryl sulfate yielded significantly lower response rates (pedal responses). The reticular formation may play an important role in self-stimulation behavior in terms of its exciting or inhibiting influence on specific responses and general organismic states (ref. ~~126~~ 249). It has been found that lesions in the rostral area of the reticular formation have little influence on self-stimulation, but the caudal region will yield some positive effects.

6. Sleep

Space-flight requirements have heightened interest in the neurophysiology of sleep. During the past 15 years, there has been much progress in the analysis of sleep, especially the changes in central neural activity and attendant perceptual, ideational, and

behavioral states (Nauta ref. 250). ~~(ref. 90).~~ Recent work uses the electroencephalograph extensively, and has emphasized the stages of sleep associated with rapid eye movements. Most researchers support the concept that the shift from waking to sleep is characterized by distinctive physiological changes (ref. 250 ~~90~~). Many of these changes can be recorded electrically, and some can be inducted by electrical and chemical stimulation of certain brain areas, suggesting that sleep is actively induced and controlled.

The physiological signs of the transition from wakefulness to sleep include changes in pupil size, blood pressure, skeletal muscle contraction, reactivity to sensory stimuli, and EEG patterns. EEG recordings of the general slowing of the brain's electrical activity and the appearance of spindles have resulted in the establishment of a scale of five stages of sleep, each level characterized by a specific wave pattern. A new sleep phase has recently been identified, in which the EEG changes from slow waves and spindles to fast, low-voltage waves, which closely resemble the activated pattern of an aroused waking subject. This is usually termed paradoxical, desynchronized, or activated sleep, and although all of the other characteristics of sleep are present, this state is always accompanied by rapid eye movements (REM's). The high incidence of dreaming in REM sleep and its infrequent presence during slow-wave sleep has led to the conclusion that dreaming occurs mainly in paradoxical sleep.

7. Learning and Memory

New basic information concerning central nervous system capacity to process and store information, and to integrate behavior, gives promise of providing baseline data for studying highly organized mammalian systems operating in space.

In mammalian learning, certain brain areas manifest "distinguishable activities in distinctive sequences," reflecting the neural actions that accompany learning (ref. 251). Many investigators believe microscopic, sub-microscopic, and molecular events underlying learning may be similar for invertebrates and vertebrates.

Molecular Mechanisms. — Many neurophysiological theories of learning have postulated a dual-trace concept — a recurrent or reverberating neural circuit which carries the memory trace until more permanent structural or metabolic changes take place. Estimates of the length of time for which the trace is carried by such circuits have ranged from 4 hours to several years. Recent evidence (ref. 252), however, suggests the engram is transferred from an electrical or electrochemical mechanism to a structural counterpart in a few seconds. This is based on the fact that the trace survives when all electrical activity in the brain is stopped more than 10 seconds after learning. They suggest a molecular interpretation, including at least three possible mechanisms which vary in the degree of their molecular specificity to the engram. At the highest level of specificity, information would be stored as a subcellular molecular

structure such as in the DNA-RNA sequence, or in the form of a spatial configuration like that in the protein molecule, or in a molecular pattern similar to that in a lipid membrane. A second possibility at the cellular or synaptic level involves the cellular synthesis of molecules which then function as a specific type of switch, thereby modifying ~~inter~~cellular interactions. The third and least specific possiblity ~~is~~ is that molecules may be synthesized to act as a non-specific switch to make or break connections between cells. Research on the genetic cycle and immune response would be quite useful in elaborating the role of the first two mechanisms in the consolidation of the engram.

DNA-RNA.—Research on deoxyribonucleic acid (DNA) and ribonucleic acid (RNA) has provided an extremely promising and useful approach to the problems of learning and memory. Since DNA probably plays only an indirect operational role in psychological memory (ref. ~~113~~ 207), most work has been directed at RNA, which transcribes DNA's information store, supervises the synthesis of specific proteins, and may conceivably be modified in response to incoming impulses.

In a study of some of the neurochemical correlates of learning and memory, particularly in terms of RNA, TRIAP — which alters RNA in neurons and glia — was injected into rats over an extended period. The animals were trained on an operant-conditioning apparatus along several behavioral baselines, ~~and~~

and their terminal performance was compared to that of control rats. Thus far, no differences have been observed in rats given TRIAP and in normal untreated animals. Another study currently in progress involves the measurement of any changes in RNA found in specific neurons or glial cells in rats following training on specific tasks.

Rats trained on a transfer-of-handedness problem showed a significant increase in the RNA of the nerve cells in that part of the somatosensory cortex on the learning side of the brain upon which this ability depends (ref. ~~68~~ 253). Similar increases in RNA have been observed in neurons and glial cells in the brain stem following a learning situation.

A number of questions concerning the DNA-RNA interpretation of memory remain unanswered (ref. ~~52~~ 254). How are synaptic experiences conveyed to the nucleus of a cell, i. e., is there a chemical messenger or a direct electrical influence? Following the synthesis of new RNA, what cytoplasmic changes can it induce, such as an enzymatic modification in the glial-neural metabolic interaction or in the synthesis of the synaptic transmitter substance?

Neural Learning. — Under certain conditions, neurons will exhibit a change in their pattern of discharge on the basis of past "experience," i. e., the cells seems to "remember" (Livingston, ref. 251). ~~(ref. 80).~~ Neurons apparently do not store information; they only exhibit some effects of learning. For example, when a light flash is used as the stimulus, the neurons will fire in a pattern in which the stimulus

frequency is doubled or halved. The cell discharge stops when stimulation is discontinued, but if a single light flash is subsequently applied, it elicits a discharge with the periodicity previously established.

Delocalization--Defining the memory trace or engram presents a number of difficult problems, one of which is its presence in neurons or neuronal nets other than those which originally carried out the afferent impulses or over which the impulses were carried during the consolidation of the trace. This diffusion or delocalization of the engram from one area of the brain to another may occur by means of a spread of impulses through a neuronal network rather than by translocation of engrammatic macromolecules (ref. ~~112~~ 209). One way in which engrams might be transferred from one region to another is through a process of diffusion, in which specific neuronal nets other than those originally involved in the formation of the trace are activated.

Brain Lesions--Recent studies of the correlation between human-brain lesions and memory change indicate that bilaterial lesions or ablation of the hippocampus result in a permanent change in memory, although memory for events in the distant past does not seem affected (ref. ~~92~~ 255). The deficit following loss of hippocampal tissue takes the form of an inability to permanently record and store afferent information. Bilaterial lesions in the

fornix and mammillary bodies also interfere with the recording of ongoing events, but not as severely as do hippocampal lesions. The amygdala, which is located near the anterior of the hippocampus, does not appear to function in memory. Lesions in the cingulate gyrus and the frontal, lateral temporal, parietal, and occipital lobes are not generally associated with permanent memory changes. However, certain neocortical lesions produce a loss of all past memory and the ability to record new information for special functions.

Research has not yet identified the "memory storage mechanism" (ref. 256 ~~89~~). The limbic system, and more especially the hippocampus, although clearly of importance in the storage of memory, cannot itself be the storehouse. The hippocampus seems essential for consolidation of memories, but is not necessarily involved in the recall of such memories if the affective correlates of recall are stored in other limbic or subcortical structures.

Memory and Glia Bibliographies--In 1965, the Neurosciences Research Program published a Memory Bibliography (ref. 257 ~~84~~) containing 430 references to the world literature on memory. The entries were taken from issues of Index Medicus between January 1961 and February 1965. All citations under the heading of "memory" in these issues were included in the bibliography, and each entry was coded in terms of a particular discipline (psychology, psychiatry; neurology; biochemistry, pharmacology;

cybernetics, general or unclassifiable). That research program has published also a bibliography of world literature on glial cells, which is one of only five such bibliographies known (ref. 258 ~~79~~). Approximately 1100 references to glia are listed, with most entries falling in the period between January 1960 and August 1964.

8. Neurophysiological Effects of Environmental Conditions and Sensory Deprivation

In a series of studies (Krech, refs. 259-261) ~~(refs. 72, 73, 74)~~ on the effects of environment on brain anatomy and chemistry, 265 rats were subjected to the following conditions of weaning for a period of 80 days: (1) environmental complexity and training (ECT) on maze and discrimination problems, (2) social control (SC), (3) isolation in extreme impoverishment (IEI), and (4) paired in extreme impoverishment (PEI). Brain dissection, after sacrifice, showed various cerebral effects in the IEI-ECT rats, which were clearly a result of environmental impoverishment rather than social isolation. It was subsequently demonstrated that most, and perhaps all, of the cerebral effects of impoverishment, in terms of cortical weight and acetylcholinesterase activity, are reversible through a program of post-deprivation training and stimulation.

B. Biological Rhythms and Clocks

1. Circadian Rhythms and Space Flight

Although most biologists agree that circadian rhythms have

an endogenous origin, some maintain that the daily rhythmicity of organisms results from an external physical force with a 24-hour period that is a function of the Earth's rotation. It has been suggested that the problem could be resolved by observing the rhythmicity of an animal placed in a satellite orbiting the earth with a 90-minute period (ref. ~~107~~ 262). However, its ultimate resolution would depend on observing the rhythmicity of animals totally removed from the Earth's environmental influences, a condition which can be achieved in spacecraft in solar orbit. The persistence of the circadian rhythm under such conditions would clearly indicate its endogenous origin, whereas failure of the oscillation would point to external causation.

Circadian rhythms are important in dealing with various problems of space flight. Aschoff (ref. ~~3~~ 263) believes the operation and maintenance of both spacecraft and earthbound installations that must be manned continuously at high levels of alertness demand special work-rest cycles for the crew. The basic phenomena of

circadian rhythmicity cannot be disregarded in developing these schedules.

At the Max-Planck-Institut für Verhaltensphysiologie, subjects were isolated in underground bunkers and subjected to changes in dark and light periods, which were analogous to aircraft flights in easterly and westerly directions. Readaptation (reentrainment) following shortening of dark time (easterly flight) occurred immediately, in contrast to the longer time required for reentrainment following "flight" in the westerly direction. Data obtained in France — where two adult human subjects volunteered to spend 3 to 4 months in underground caves in the absence of known time cues — revealed that circadian rhythms were maintained during the entire duration of isolation underground. The rhythms were desynchronized in relation to local time, indicating that they depend, at least in part, upon endogenous factors that characterize the system rather than its environment, and that environmental factors function primarily in a synchronizing capacity.

Considerable data have been compiled on cyclic activity in rats (Bolles, refs. 264-269). ~~(refs. 15, 16, 17, 18, 19, 20)~~. Attempts have been made to determine whether a-diurnal cyclic feeding in rats would result in a-diurnal activity cycles. Evidence seems to indicate that a-diurnal cycles cannot be established through a-diurnal feeding, and that there is an innately determined 24-hour activity ~~cycle~~

cycle to which the rat is bound.

Rats have been tested in a Skinner box to see if they could learn to anticipate a regular scheduled feeding when fed either diurnally (every 24 hours) or a-diurnally (every 19 or 29 hours). Subjects fed on a 24-hour basis exhibited definite anticipatory behavior, i.e., immediately before a scheduled feeding, they pressed the bar at a very high rate, whereas at other times the rate was very low. The a-diurnal rats pressed the bar relatively independent of time. Similar results were obtained when activity wheels were used rather than Skinner boxes.

A record of circadian rhythmicity of locomotor activity of hamsters--shown in figure 31--demonstrates the normal entrainment of activity of a light-dark cycle (areas of highest activity are shown as solid bars). At the point indicated by the arrow, the animal was transferred to constant-dark conditions. The circadian oscillation underlying locomotor activity "free runs" at a frequency greater than 24 hours. Particularly noteworthy is the sharp onset of activity generated by internal timing mechanisms in the absence of an external synchronizer (Pittendrigh, refs. 262, 270). ~~(refs. 107, 108).~~

(Insert figure 31)

Decay of circadian rhythmicity of locomotion in rodents during constant darkness is dramatically illustrated in figure 32. The lack of a light-dark cycle results in the absence of rhythmic activity, probably due to the asynchrony of controlling oscillations.

EFFECT OF CONSTANT DARKNESS ON CIRCADIAN ACTIVITY

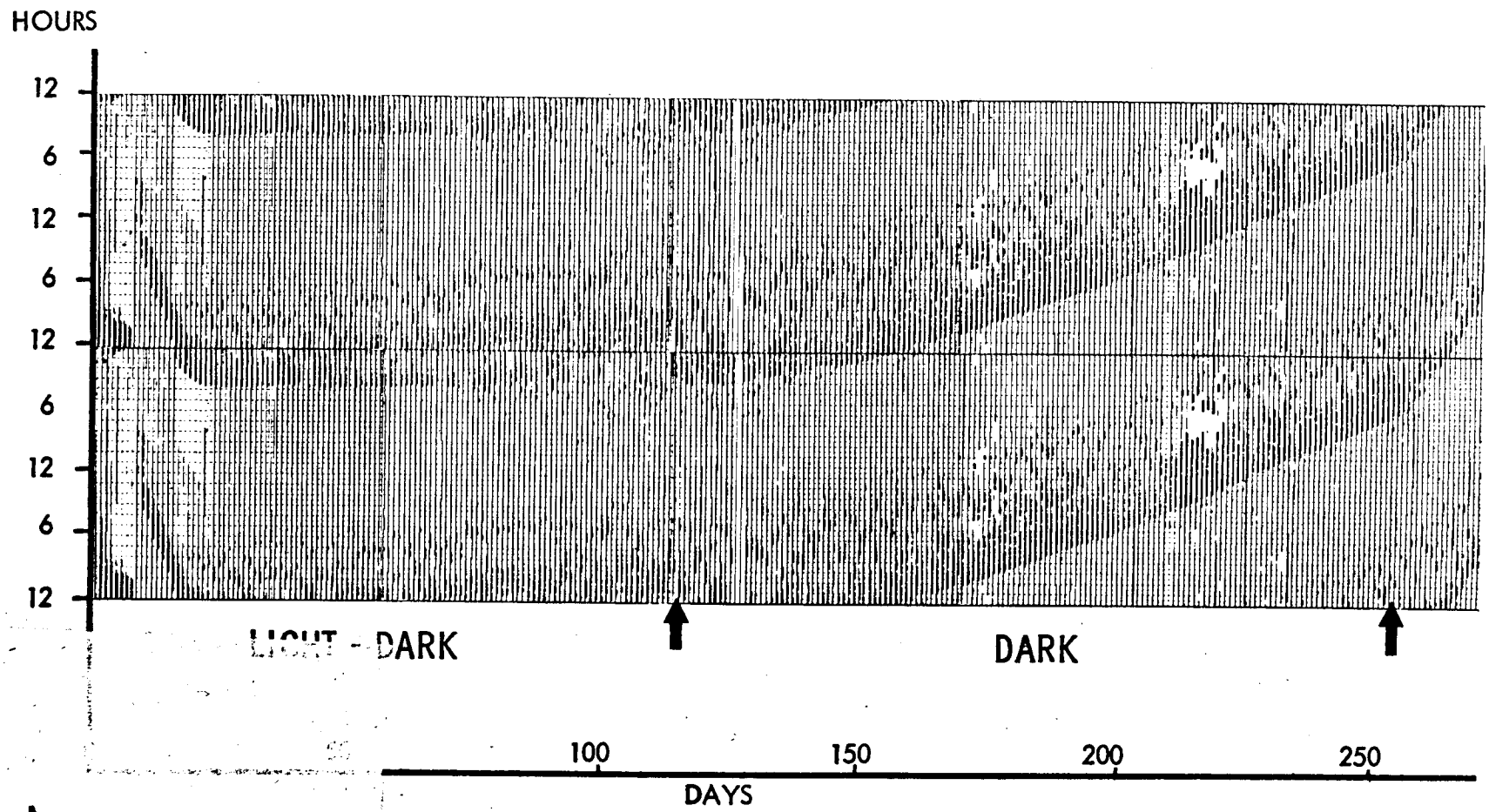

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Figure 31.—

Other studies on both human and animal subjects have provided clear evidence that circadian organization disintegrates into dissociated oscillations when not influenced by an environmental cycle. Experiments with plants have demonstrated that such dissociation is physiologically detrimental.

(Insert figure 32)

Figure 33 shows an unusual phenomenon, the dissociation of activity into two major components which "free run" at their own

DECOMPOSITION OF CIRCADIAN RHYTHMIC ACTIVITY DURING CONSTANT LIGHT

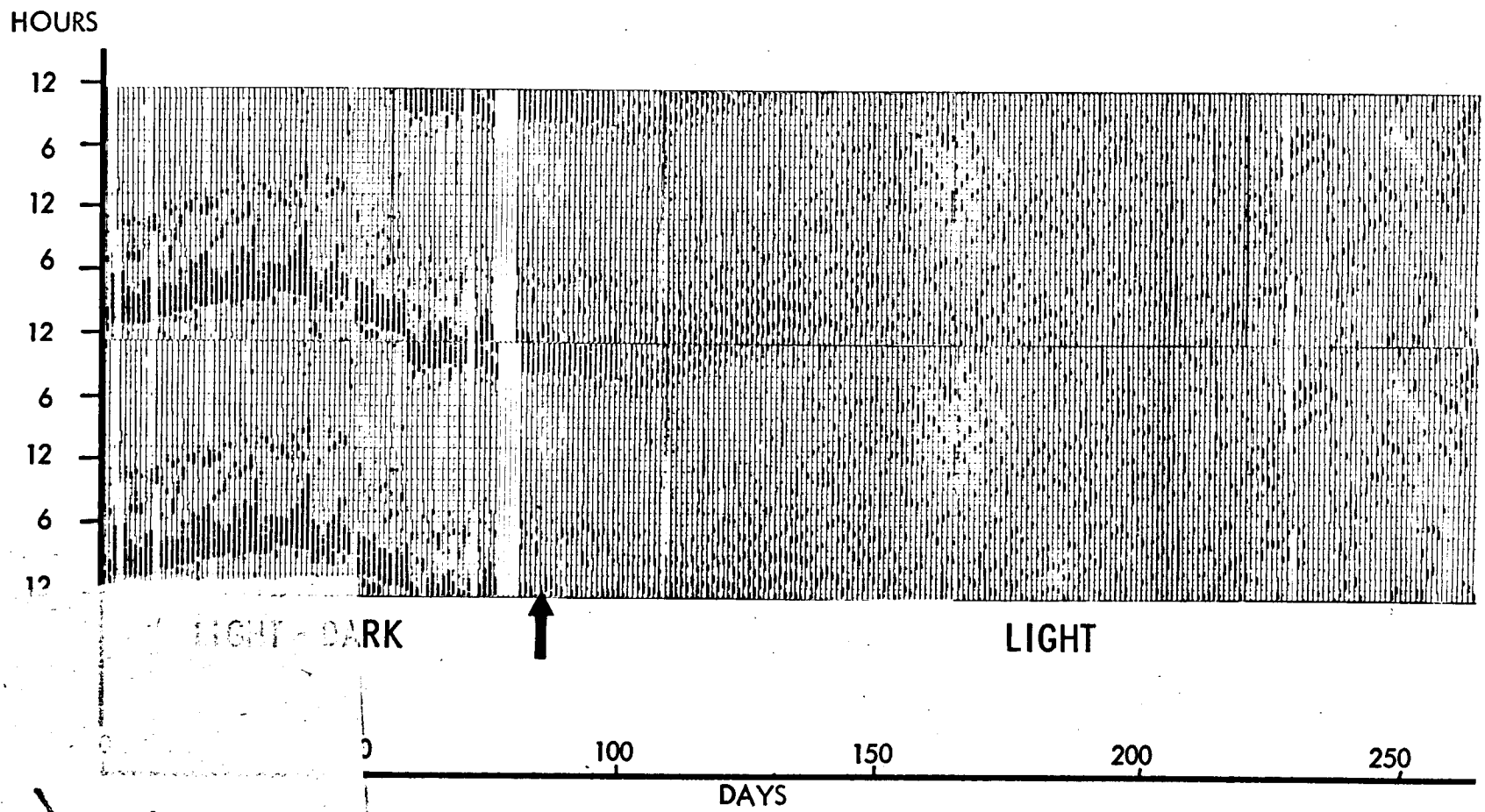

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Figure 32.

natural frequency. ~~That is~~, These data reflect the multiple controlling nature of physiological oscillations, which operate independently and account for rhythmicity in locomotion. These phenomena have been observed for the first time as a result of NASA support of long-term studies using computer collection and computer analysis of enormous quantities of data.

(Insert Fig. 33)

2. Hibernation.

Hibernation is generally defined in terms of energy expenditure during a period of lowered body temperature. The temperature and energy levels vary with different species, but in all cases the result is the conservation of energy resources. Hibernation probably arose independently in several species of mammals as a survival mechanism during periods of food shortage (ref. ~~66~~ 271).

Many hibernators exhibit a cyclical response to persistently low temperatures rather than a sustained reaction (ref. ~~118~~ 272). This cycle seems to be inherent in the organism, and is released by environmental stimuli which set the phase. In addition, during the period of hibernation, there is another internal rhythm or oscillation, possible with a circadian pattern, which intermittently elevates metabolic processes. This has been found in the 13 -lined squirrel, which undergoes intermittent arousal from deep hibernation as a result of circadian oscillation. There is also evidence of a circadian or macro-period oscillation in the brain and in single neurons of hibernators, indicating the important role of neural centers in arousal from hibernation.

DISSOCIATION OF CIRCADIAN RHYTHMIC ACTIVITY COMPONENTS DURING CONSTANT LIGHT

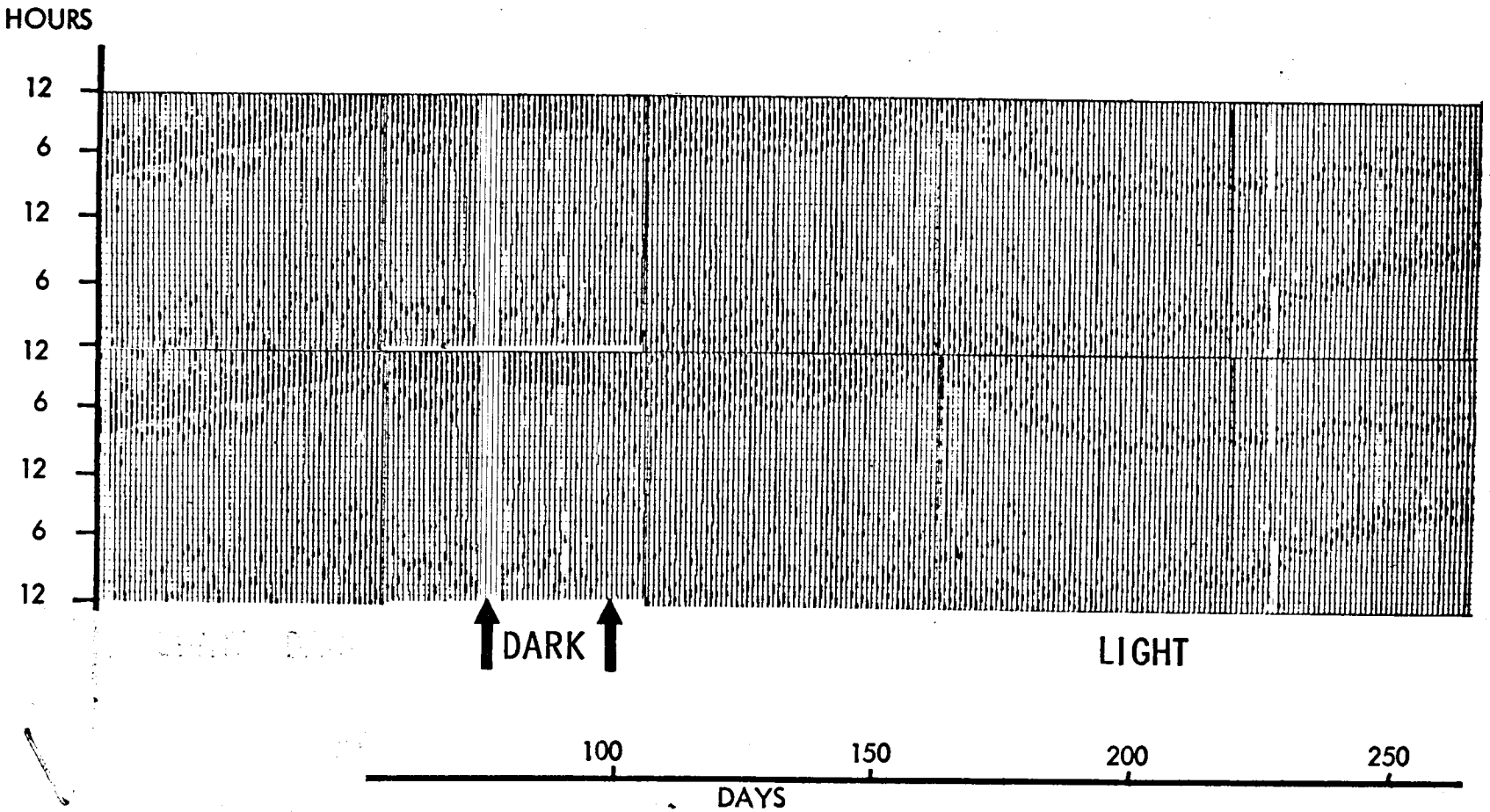

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Figure 33.—

C. Experimental Analysis of Animal Behavior

1. Learning and Perception

Lower Animals. — Two approaches have been used in studying t learning in simple systems (ref. ~~27~~ 273): use of (a) intact, relatively simple organisms, or (b) fragments or simplified systems of either higher or lower species. The first has been used very effectively by a number of ethologists and comparative psychologists, and is described below.

There is considerable evidence that some form of learning is possible in protozoa. For example, when a paramecium is placed in a round container, it tends to swim around the circumference in a circular pattern. If it is transferred to a triangular-shaped container, it continues to swim circularly, although the response is eventually extinguished. The same results are obtained when the organism is moved from a triangular to a circular container, i.e., the triangular swimming pattern persists for a period of time. When ultraviolet rays were directed at one point on the circular path taken by protozoa, they tended to deviate around this area after the rays were discontinued. This type of behavior modification cannot be interpreted as learning, but rather as the result of the presence of a trace substance produced by the organisms following exposure to noxious stimuli (ref. ~~86~~ 274).

Although the nervous system of some coelenterates has been extensively studied, little is known about learning in these animals. One exception is the sea anemone, in which studies of conditioned inhibition have yielded valuable data. This organism has a species-specific response to contact by a starfish, wherein it releases its hold of the substratum and shows vigorous swimming movements. The sea anemone is also sensitive to mechanical stimuli applied to its base, and responds by closing and contracting down onto the substratum. The two stimuli were paired, with the starfish presented first, and then only the starfish. On the first single stimulus trial, none of the sea anemones exhibited a swimming response, and only gradually did they return to the original swimming behavior.

The planarium, well-known for experiments in learning, has continued to interest many researchers (Bennett, Best, Corning, refs. 275-279), ~~(refs. 10-13, 22)~~, particularly in regard to the DNA-RNA hypothesis of learning. Annelids have not been investigated as thoroughly, possibly because of their more limited repertoire of behavior. Bullock states that their "availability, simplicity, phylogenetic position, learning capacity, and tolerance of mutilation suggest that they are well worth new attention"(ref. 273 ~~27~~).

Echinoderms (starfish) are able to learn to select one arm of a Y maze on the basis of substrate texture, and can be successfully conditioned when light (conditional stimulus) and food (unconditional stimulus) are paired (ref. 273 ~~27~~). The lower molluscs have been used infrequently in learning studies, although some of the higher forms

(squid, octopus) have provided some useful data. The octopus has been trained to approach or avoid a crab in response to a perceptual cue, and also seems capable of operant response conditioning.

New methods of preparing arthropods for behavioral, electrophysiological, and biochemical study have asserted the feasibility of permanently implanting electrodes and cannulae in Limulus while permitting it to remain in a behaviorally unrestricted state (ref. ~~34~~ 280).

Studies by the Research Laboratory of Electronics at the Massachusetts Institute of Technology on immature mammalian organisms further illustrate the importance of environmental stimulation upon behavior and physiology. It was found that severe restriction of early sensory experience in dogs produces striking behavioral abnormalities at maturity (ref. ~~124~~ 219). The results support the view that the behavioral effects of early sensory restriction are due to a failure to filter our irrelevant information on the basis of prior experience, thereby producing excessive central nervous system arousal and a disruption of perceptual discrimination and adaptive responses.

The neural correlates of perception, sensorimotor coordination, and other crucial behavioral capacities have been studied at MIT, extending earlier evidence concerning the essential role of neural mechanisms in the origin and maintenance of ~~the~~ spatial organization in perception (ref. ~~124~~ 219).

A central question in perception analysis concerns the stimulus conditions which enable the perceiver to remain properly oriented during

continual transformations of the visual scene and of other sensory stimuli while he moves actively about. Recent studies of this sensorimotor coordination have employed artificial imposition of changes in the spatial or temporal relations between the motor output of the perceiver and the corresponding sensory feedback within the nervous system. Another method employs tracking tasks, wherein the subject works against variable forces, i. e. under conditions similar to radial locomotion in a spinning space capsule or in a decelerating capsule during reentry, when there is an increase in g-forces acting on the astronaut. Important theoretical and practical issues remain to be investigated, particularly the execution of tasks requiring precise sensorimotor control.

Reinforcement and Signal Detection.— The reinforcing value of information in animal behavior has not been generally accepted. One researcher has attempted to show two things: whether information is reinforcing, and whether a negative discriminative stimulus, which is a type of informative stimulus and is generally regarded as aversive rather than reinforcing, is both positively and negatively reinforcing. His results suggest that the reinforcing value of informative stimuli is positively related to the amount of uncertainty in their absence, and that negative discriminative stimuli are most aversive in situations of greatest uncertainty(ref. ~~64~~ 281).

Operant conditioning and signal detection — particularly the effect of the consequences of behavior on the behavior itself — have been studied in human and baboon subjects (ref. ~~59~~ 282). In ~~the~~ signal detection (psychophysical) research, procedures were developed which established and maintained rational behaviors over extended periods in baboons. Such behaviors, when humans are involved, are considered complex decision behaviors, since they are governed by criteria involving net gains that have weighed the consequences of the behavior.

A large number of studies report that (a) signal detection and observing behavior decline as a function of time, and (b)both variables also decline as signals become more/infrequent(ref. ~~30~~ 283). However, there is evidence that observing rates do not decrease as the signal rate declines ~~although~~ although responses are randomly distributed(~~refs. 29, 30~~ (Clark, Refs. 283-284).

2. Behavioral Effects of Full Environmental Control

Several programs are studying various aspects of animal behavior under continuous and total environmental control. This involves long-term isolation, development of complex behavior, aversive consequences of behavior upon ongoing performance, and physiological mechanisms (ref. 285 ~~24~~). These studies are attempting to ascertain some of the effects of brain lesions and drugs on behavior (ref. 286 ~~22~~). Some work has been concerned with the development and maintenance of operant responses under multiple stimulus control, e.g., timing and mediating behavior, and behavior which determines subsequent environmental events (ref. 284 ~~29~~).

Decision making in the chimpanzee has been studied under conditions of extended confinement and isolation (ref. 287 ~~23~~). Results showed that the animals were capable of orderly decision making.

3. Stress Responses

The role of early experience in subsequent stress responses has been investigated at some length (ref. 288 ~~65~~). When mice, which had been reared in either isolation or under crowded conditions, were exposed to intense territorial conflict, the stress effects became evident in several physiological responses: body weight was nearly 30 percent less than normal; ~~there were~~ unsuccessful pregnancies; hypertension; ~~signs of~~ arterial degeneration; and ineffectiveness of anesthesia in reducing blood pressure.

D. Information Systems and Storage

A multi-level series of studies have been carried out on information processing by vertebrates and invertebrates (refs. 289-293 ~~4,5,6,7,8~~). A major goal is to estimate

how many successful processing acts the human brain, acting as a unit, can accomplish during the performance of well-learned tasks. One project is determining the amount of information which a person must process to understand spoken English. Another is checking the time necessary to complete a simple visual processing task.

E. Behavioral Effects of Gravity

Manned space flight has increased the need for knowledge ~~of~~ of the role of the vestibular system and its functional changes during acceleration and altered gravity. With this in mind, investigators (ref. ~~61~~ 294) measured the RNA content of Deiter's cells and associated glia in the lateral vestibular nucleus of rats following exposure to altered gravitational fields. After 1 hour, there was a decline in the RNA content of Deiter's cells, with a subsequent increase at all g levels up to 12 hours; during the remainder of the 24-hour period, RNA decreased. The glial cells showed immediate decrease in RNA at 2.65 g; the increase persisted through 24 hours.

The effects of weightlessness on physiology and behavior have not been clearly defined. Of primary concern is the question of what constitutes an optimal level of simulated gravity for optimal performance and physiological functioning during space flight.

Various aspects of simulated gravity and its behavioral

effects are being studied (Lange, refs. 295-297; Martin et al., ref. 298). ~~(refs. 75, 76, 77, 81)~~. In studies of avoidance of artificial gravity and of gravity preference levels, rats were found to have a prounced preference for Earth's gravity, and to have learned to escape and avoid centrifugally produced increases in gravity of 2.1 g. Results suggested that continued exposure to gravitational and/or rotational stimuli alters the sensitivity threshold to such stimuli (ref. 297 ~~77~~).

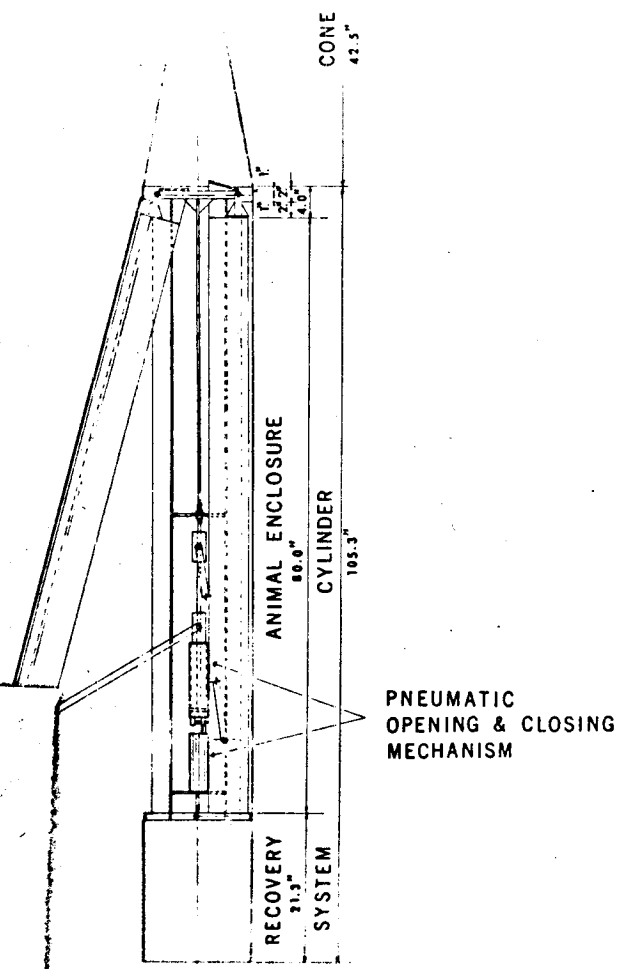
Working with squirrel monkeys under simulated gravity conditions from 1.1 to 2.1 g, it has been demonstrated that centrifugally produced gravity has aversive properties when it exceeds the Earth's gravity. In these studies, monkeys were given access to a lever which controlled the rate of rotation of an automatically programmed centrifuge. Pressing the lever reduced the rate of rotation and therefore the amount of artificial gravity. The avoidance of g-levels above normal Earth gravity was demonstrated over a period of several weeks. Extension of this work to gravity levels below 1 g, however, will require in-flight experimentation.

Figure 34 shows a mock-up of an Aerobee payload designed to determine the feasibility of conducting long-term gravity-level preference studies in space. The experiment utilizes rats which, in ground-based studies, have demonstrated a highly reliable and rapid locomotor avoidance response to gravity levels above 1 g. This flight experiment will be used to determine the preferred

gravity level below 1 g. The planned operational sequence is shown in figure 35, where the payload arms extend to produce the variable gravity field during the zero-g portion of the trajectory, and close prior to recovery. In addition to providing evidence on the minimum gravity requirements of biological organisms, the project may yield data useful in determining the minimum gravity requirements in manned space stations.

(Insert figs 34 and 35)

GRAVITY SELECTION EXPERIMENT IN AEROBEE ROCKET



PAYLOAD ASSEMBLY

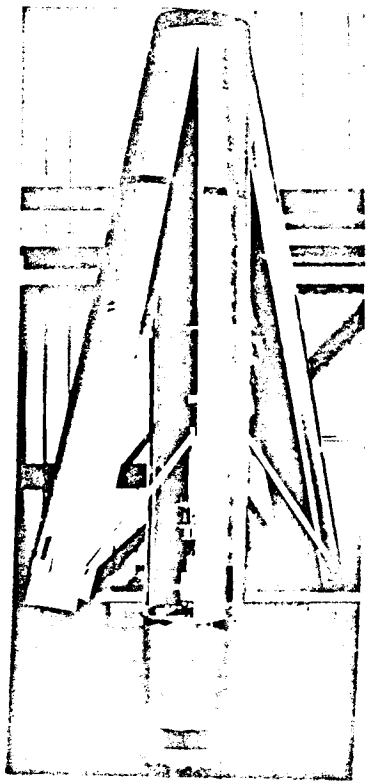


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Figure 34.

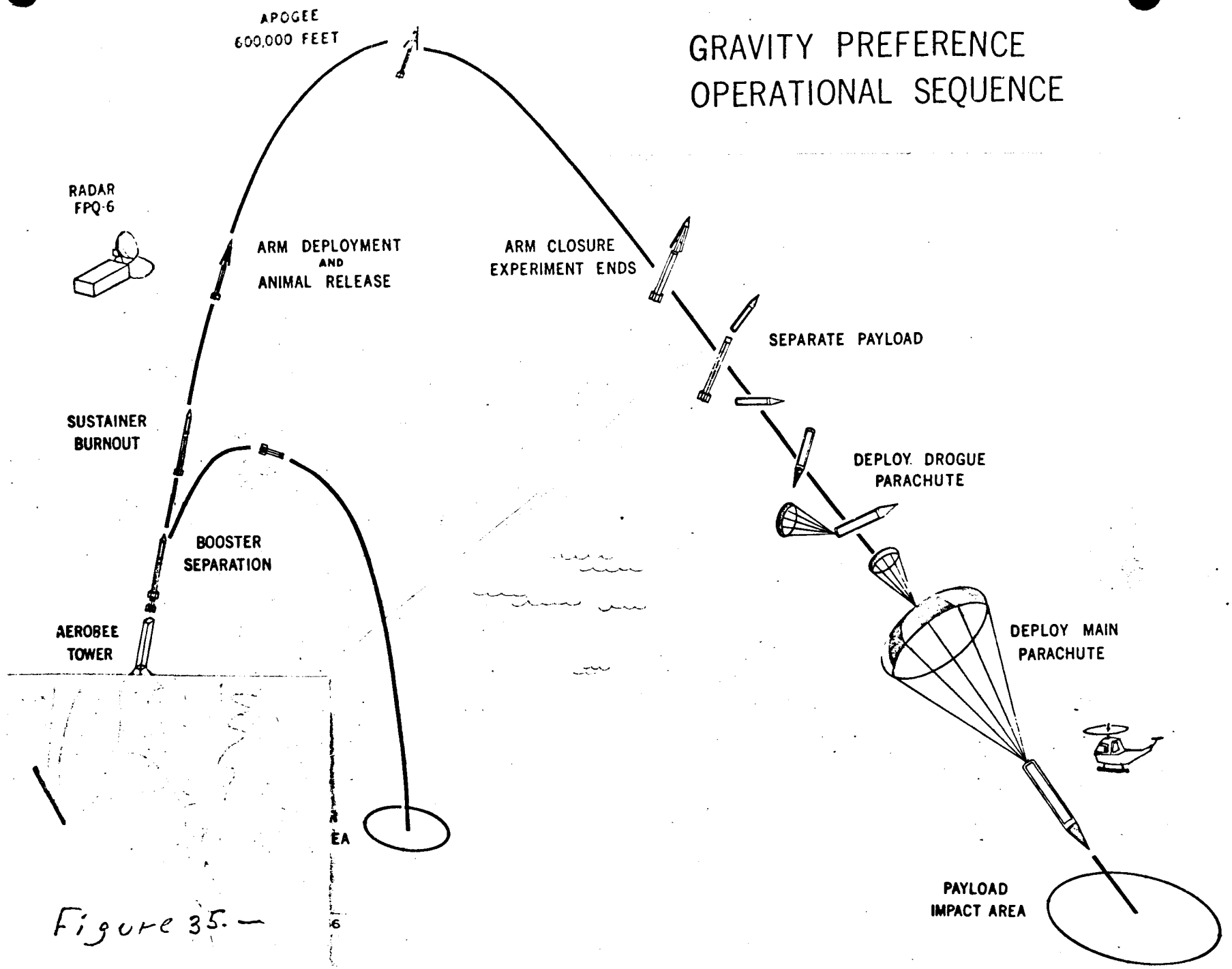

GRAVITY PREFERENCE
OPERATIONAL SEQUENCE
APOGEE
600,000 FEET
RADAR
FPQ-6
ARM DEPLOYMENT
AND
ANIMAL RELEASE
ARM CLOSURE
EXPERIMENT ENDS
SEPARATE PAYLOAD
SUSTAINER
BURNOUT
DEPLOY DROGUE
PARACHUTE
BOOSTER
SEPARATION
AEROBEE
TOWER
DEPLOY MAIN
PARACHUTE
PAYLOAD
IMPACT AREA

Figure 35.—

8. FLIGHT PROGRAMS

A. Biosatellite Program

In the summer of 1962 the Space Science Board of the National Academy of Sciences recommended "...an exploitation of special features of the space environments as unique situations in the general analysis of the organism-environment relationships...." (ref. 279). This recommendation ushered in the NASA Biosatellite Program. By February 1964, the first experimental payloads for a Biosatellite were selected and a spacecraft contract was awarded to the General Electric Company (ref. 300).

The Biosatellite Program, of NASA's Office of Space Science and Applications, under project management of the Ames Research Center, Calif., seeks to determine and analyze the unique components of the space environment and their effects on various life processes. Of special importance to man's successful performance and survival in space exploration are the environmental characteristics of weightlessness (greatly decreased gravity) and disengagement from Earth's 24-hour rotation and its concomitant influence on biorhythms. The effect of exposing organisms simultaneously to weightlessness and radiation, will be studied to determine if these factors work together (synergistic) or in opposition (antagonistic); or, indeed, if their effect is insignificant in contrast with controlled laboratory tests on Earth (ref. 301).

The intense interest of the scientific community in the Biosatellite Project is reflected in the 187 separate experimental proposals that were submitted. Experiments were selected to elicit data on all relevant factors, and include studies at the cellular, tissue, and organism levels. They will record biochemical reactions, genetic variations, growth and development, and will embrace six categories:

primates; mammals (non-primate); animal, cellular, and egg; plant morphogenesis, photosynthesis, and growth; biorhythms; and radiation. The many contributions to knowledge expected from this program include: (1) basic knowledge of the effects of weightlessness on living systems; (2) biological changes related to prolonged manned space flights, including cardiovascular changes, loss of muscle tone and physical capability, mineral losses in the skeletal systems, changes in fluid balance, and effects on behavior and performance of continued sensory deprivation; (3) a broad spectrum of experience and data for use by bioscientists in further development of their discipline; and (4) development and testing of new instrumentation, surgical, prophylactic, and therapeutic techniques.

Many preliminary studies were needed to ascertain what kinds of experiments, what types of instruments, could best be exploited in a biosatellite. After exhaustive review, by panels of experts and consultants, 19 experiments (involving a variety of animals and plants) were selected and distributed among three primary biosatellites for flights of 3, 21, and 30 days, respectively. The flights have been categorized by their nominal time in orbit and the aims of their respective experiments. Experiments for the biosatellites are contained in the re-entry vehicle, while all support equipment is in the adapter section. The latter is to be separated from the re-entry vehicle before the retro maneuver. After re-entry, the capsule will be caught, before hitting the water, by Air Force recovery aircraft, and delivered within ~~six~~ 6 hours to the laboratory at Hickam Air Force Base, Honolulu, for examination of the results (ref. 302).

The primary aim of the biological investigations to be carried out in the biosatellites is determining whether conditions of space flight, and especially prolonged weightlessness, change an organism's functional state. As an extension of this, will return to the customary gravity of Earth pose excessive strain or disability on the organism?

1. The 3-day biosatellite mission seeks to determine the effects of weightlessness and radiation — alone and in combination — on animal and plant systems.

In the two years preceding the launching of the 3-day biosatellite, numerous investigations were carried out in an effort to insure the success of the mission. Many of the studies were ground-based; the biological specimens intended for experimentation were extensively studied in ground laboratories. Thirteen general biology experiments were selected for the first satellite, including such specimens as pepper plants, spiderwort plant, wheat seedlings, amoeba, frog eggs, fruit flies, flour beetles, wasps, bacteria, neurospora mold, and viruses (fig. 36).

(Insert fig. 36)

The thirteen experiments were prepared before launch, in a definite time sequence, assembled, and brought to the launching pad ~~four~~ 4 hours before take-off. Such precise timing (before and during launch, and on-flight) is required because biological materials deteriorate, and because they grow and mature in precise sequences.

To evaluate gravity's role in basic biological processes and thus help anticipate possible effects on astronauts, this satellite includes experiments to illustrate zero-g effects on gravity-dependent organisms

BIOSATELLITE EXPERIMENTS PAYLOAD ASSEMBLY
(BEHIND RADIATION SHIELD)

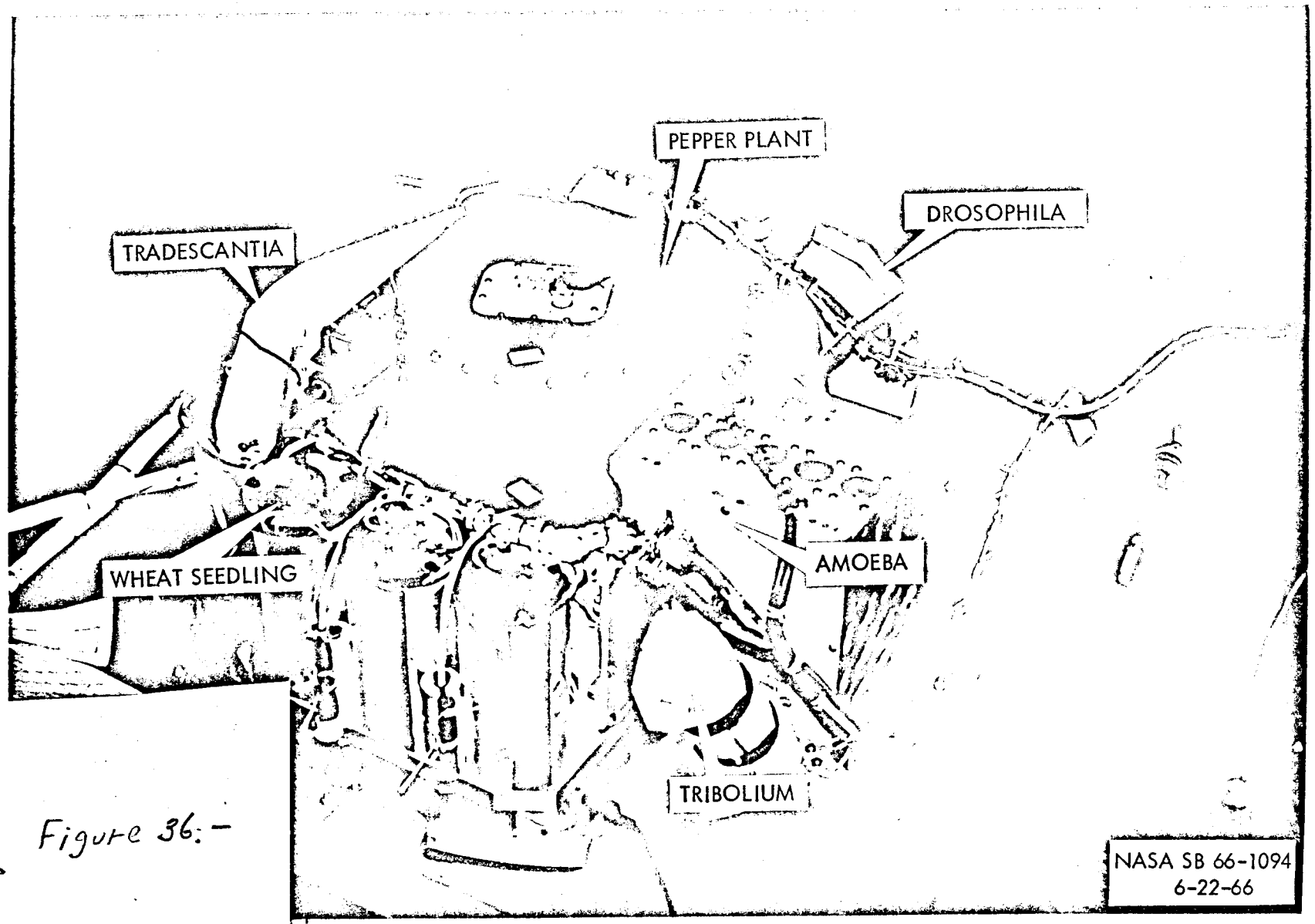


Figure 36:-

(frog eggs), structure and function of the amoeba, and plant growth and development.

Experimentally exploring the possibility that radiation is modified by some yet unidentified space factor, the 3-day flight repeats reliable ground-level radiation studies using a known source of radiation (fig. 37). ~~(Insert fig. 2)~~ The flour beetle has been selected for one of these studies because of the sensitivity of its larvae to radiation and the known wing defect provoked by radiation interference with its embryonic development.

(Insert fig. 37)

The effect of controlled radiation on weightless lysogenic bacteria will be monitored by exposing the bacteria (which harbor a virus in an inactive state), to specified radiation amounts which cause the virus to break loose, replicate, and attack other cells.

An important preliminary study of the effects of gravity, radiation, and temperature has already revealed that optimal temperature for growth of wheat seedlings is 25° C; at this temperature, centrifugation and radiation have maximum anomalous effect. At temperatures from 21° to 25° C, elevated gravity stimulated growth, and a force of 150 g reduced seedling sensitivity to radiation.

Bodily and reproduction effects — particularly chromosomal and other mutations that can be precisely scored — will be a particular concern of seven experiments with molds, bacteria, spiderwort plants, fruit flies, beetles, and wasps. Identical experiments — not irradiated — will be used as controls to separate out the weightlessness factor from the combined effects (ref. 300).

2. The 30-day biosatellite mission will investigate weightlessness

STUDY OF COMBINED WEIGHTLESSNESS AND KNOWN RADIATION

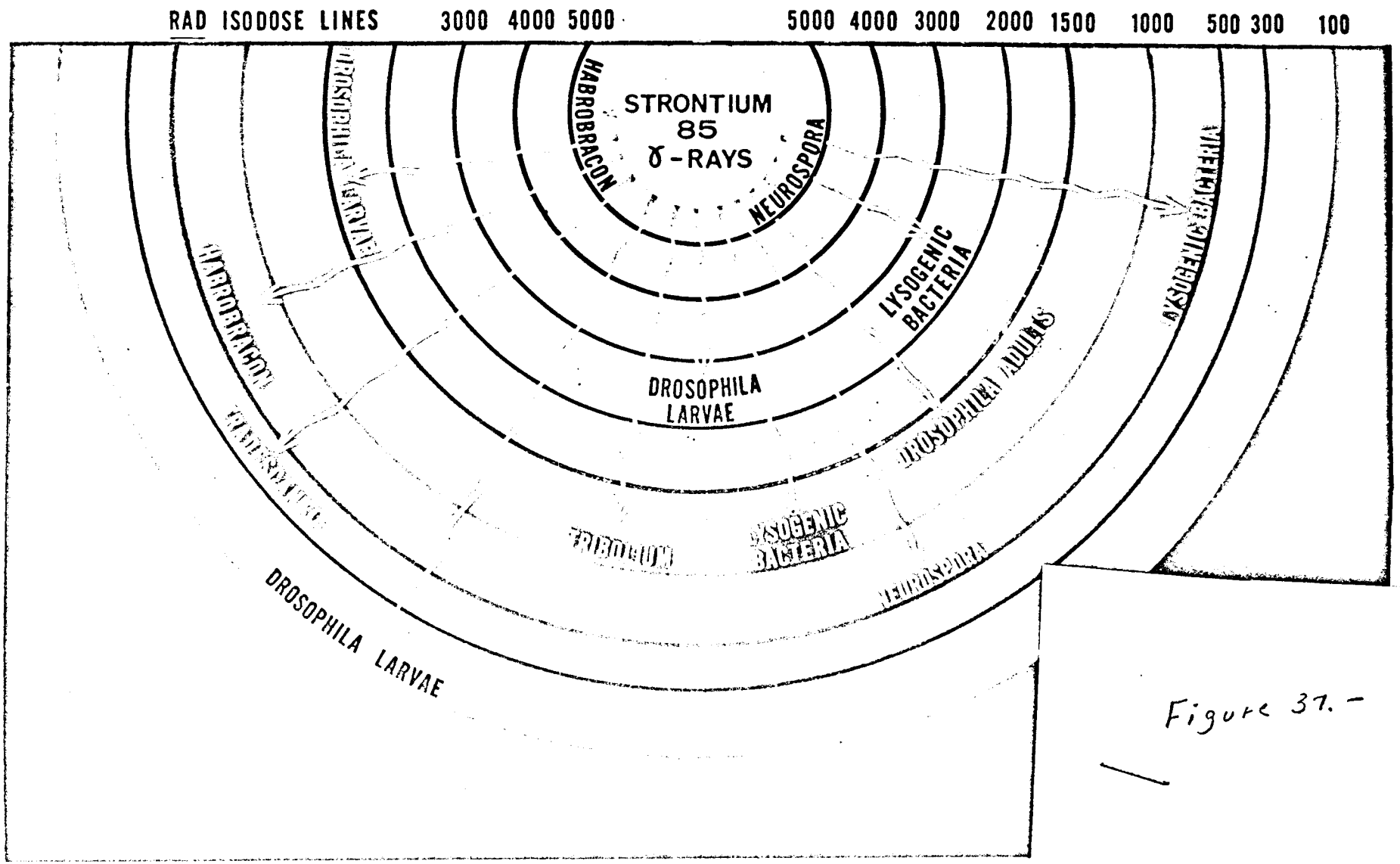


Figure 37.-

effects on the blood-circulating, cardiovascular, nervous, and skeletal systems of primates, as well as on their metabolism and behavior. In terms of instrumentation and life-support, this has the most demanding payload and is very significant for longer manned flights.

The primate experiments are such as cannot feasibly be undertaken with humans, and are intended to reveal effects not observable in the intact human. Thus, electrodes deep in the primate brain will transmit clues to effects of flight on the orientation mechanisms of the nervous system or on changes in the sleep-waking cycle.

In other tests, central nervous system experimentation will check alertness, decision-making, and fatigue. General metabolic tests will cover dietary utilization, energy, and biochemical tissue changes. Performance and behavior will be monitored through a variety of special-purpose devices, such as a psychomotor test chart.

The Jet Propulsion Laboratory has developed a fully automated system for in-flight chemical analyses of urinary biochemical constituents of physiologic significance. Planned for use in this flight, the system (5600 cm^3 in size, 4.5 kg in weight) will monitor urinary excretion of specific chemical constituents in primates. Once every 6 hours it will analyze urine samples for calcium (fluorometrically), and creatine and creatinine (colorimetrically). Data are stored and telemetered to tracking stations (fig. 38). (Insert fig. 38)

Experiments on this flight will focus, also, on those systems which, in man, will most likely be affected by weightlessness (ref. 300). For example, it is postulated that biochemical and microradiographic analytical tests on the primates undergoing varying degrees of activity will yield data on mineral loss during space flight that cannot be

AUTOMATED URINE ANALYZER

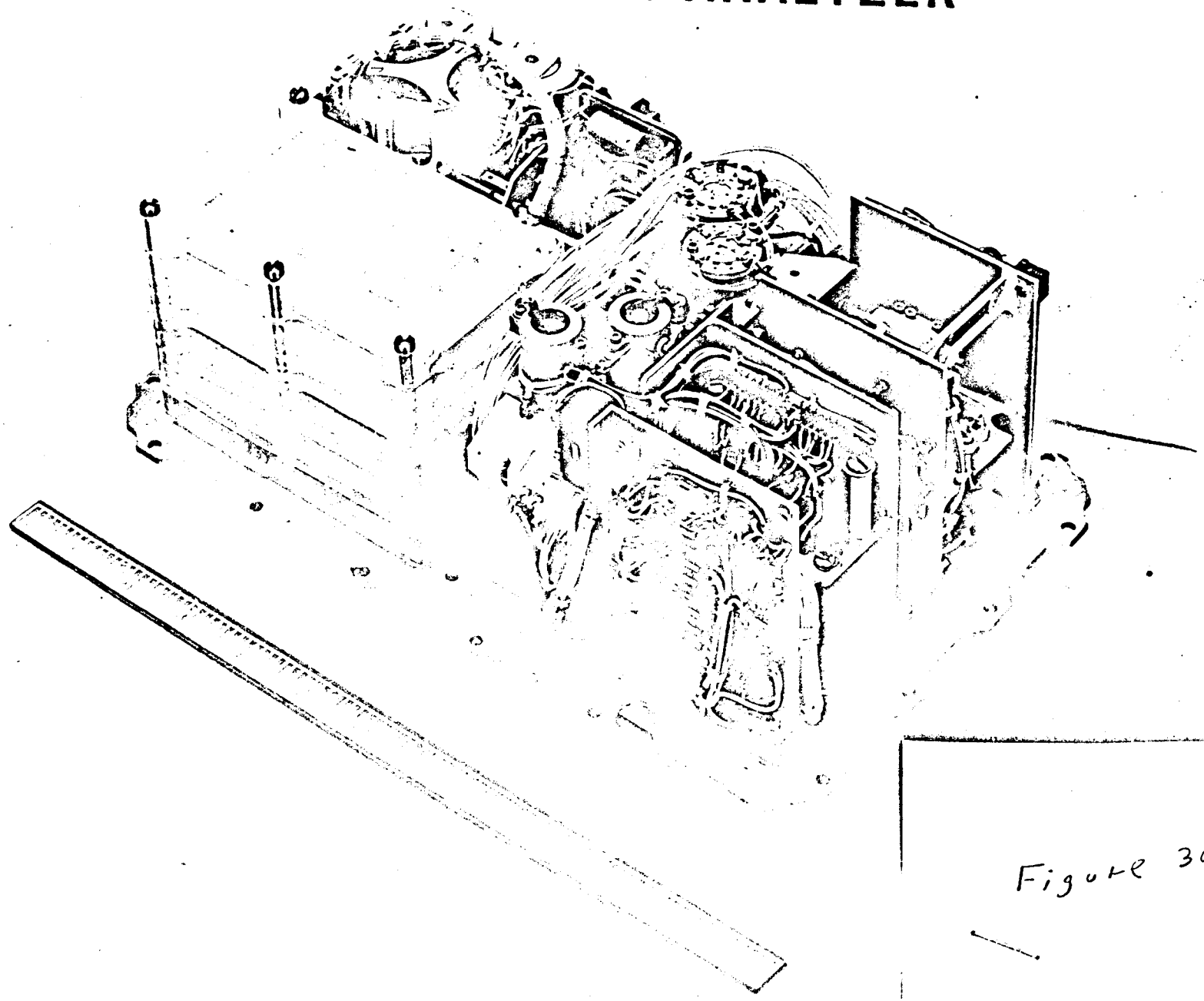


Figure 38.—

gathered from human subjects. In this experiment, measurements of calcium intake and exretions will accompany radiographic measurements on bone and will provide information on total calcium loss, as well as on the parts of the skeletal system affected.

3. The 21-day biosatellite mission deals with the effects of weightlessness on body composition and cellular functions; on circadian rhythms; and on structural, developmental, and functional patterns in gravity-dependent plants (ref. 300).

This flight will test the influence of zero gravity on cell structure. Two biopackages of isolated human liver cells in culture, completely automated, will be observed for capacity of the cell to maintain its cytoplasmic membrane and its constant reversibility of the cytoplasm sol-gel, and to undergo normal mitosis. Results of this test are important for space flight, since derangement of cell components — as occurs with low doses of X-radiation — may produce profound disturbances in multicellular systems (ref. 303).

One experiment on this biosatellite will study structural, developmental, and functional patterns in plants that may be dependent on gravity. Another will investigate the effects of space flight on the highly significant biorhythms that exist in all living cells, and affect all creatures, including man. These rhythms (called "circadian", because they occur about every 24 hours), follow the day-night cycle in any given geographic area (ref. 300). Moved to a new longitude, an organism's cycle goes out of phase and requires a period of time for adjustment to the new milieu. In the experiment, a sensor implanted in a rat's abdomen will permit recordings of diurnal variation in body

temperature. The gross activity and food intake will also be monitored. The same animal will provide information on how weightlessness modifies body weight and composition of such organs as muscle and bone.

The 3-day biosatellite's payload consisted of 13 general biology experiments. Animal cells and insects were exposed to precise doses of gamma radiation provided by an 85 strontium source. Genetic effects of the combined radiation and weightlessness stresses were to be evaluated by determining frequencies of chromosome breakage and of mutation of many different genetic loci. Non-irradiated experiments were designed to study basic mechanisms of cell division, cell growth and development in the frog embryo; structural changes in protoplasm and enzyme patterns; and orientation with respect to reduced gravity of leaves, roots, and shoots of a mature plant as well as developing seeds. Thirteen identical experiments were set up on the ground as controls, subject to conditions as close as possible to those of the flight experiments, except for weightlessness.

Within the recovery capsule, the experiments are located in two groups. The forward group includes the seven radiation experiments, located ahead of the Strontium 85 radiation source. The source is isolated in a tungsten-nickel-copper sphere, which is opened on command by spring mechanism in orbit (releasing a 180° cone of radiation) and closed by command prior to entry or automatically as a result of entry forces.

The radiation experiments are placed concentrically around the source so that they get one of nine radiation dose levels (from 200 to 5000 r) during the 3-day mission.

The forward section contains dosimeters to check total radiation. It is walled off from the rest of the capsule by a laminated aluminum-tungsten-aluminum backscatter shield.

The aft section contains the general biology experiments and control versions of the radiation experiments.

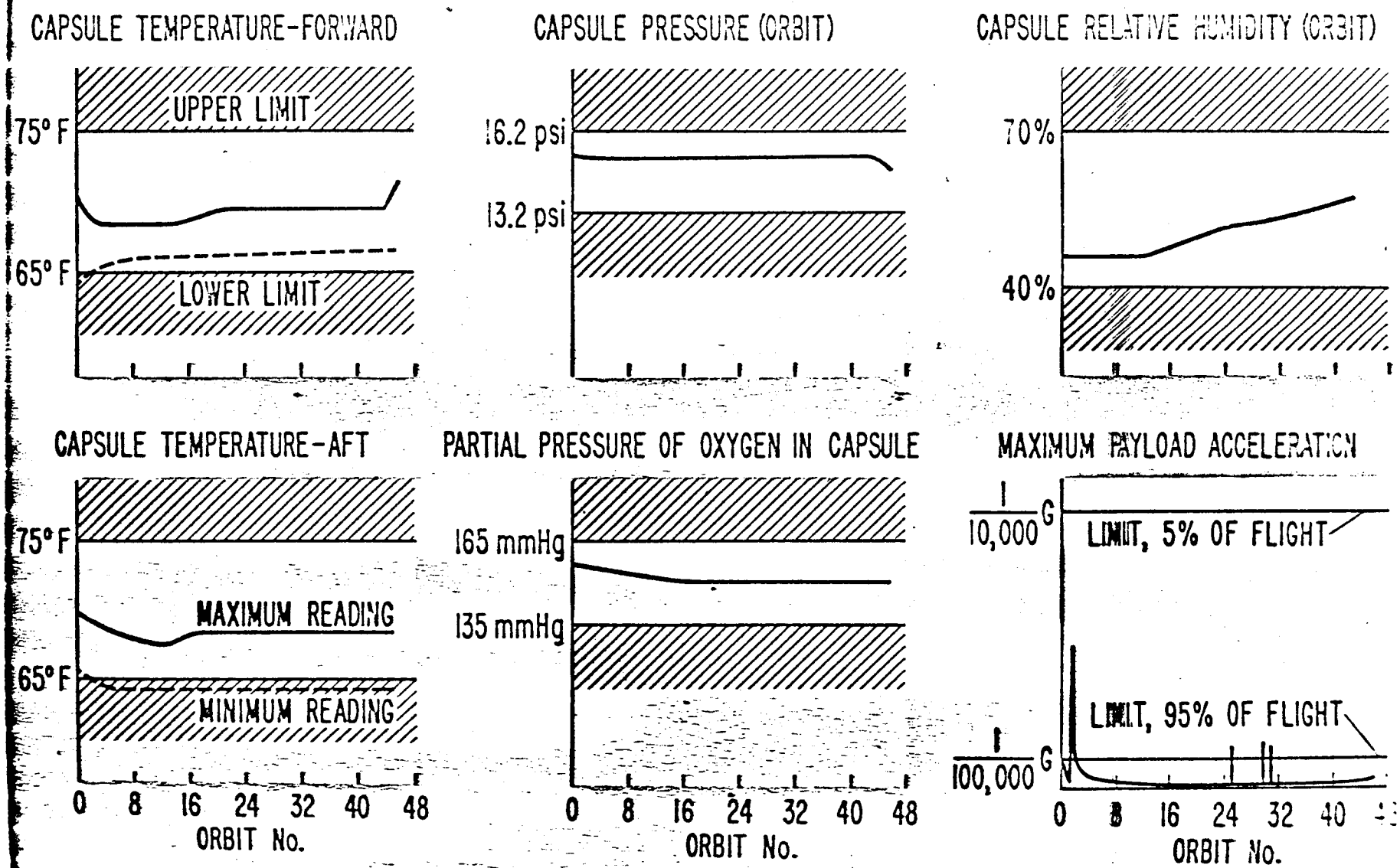
The life-support system consists of a high-pressure sphere containing air, a circulating fan, metering and pressure regulating system. Relative humidity is controlled at 40-70 percent by silica-gel absorbers.

Biosatellite I was launched from the Eastern Test Range on December 14, 1966 by a two-stage thrust-augmented Thor-Delta booster and successfully placed into orbit with a perigee of 159 nautical miles and an apogee of 178 nautical miles. The environmental conditions (temperature, humidity, pressure, and acceleration) were within specification during most of the orbital flight time, as shown in figure 39. (Insert fig. 39)

During orbit 47, the deorbit events were initiated. All events occurred in sequence including separation of the thrust cone and de-spin of the capsule even though the retro rocket did not fire. De-orbit did not occur as planned and the capsule eventually decayed from orbit on February 15, 1967. A search was organized along the orbital track near Australia by the USAF search and rescue aircraft, during the expected time for de-orbit, using both electronic and visual means, but no trace of the satellite re-entry vehicle was discovered. A visual search was made by light Australian aircraft with no success.

Changes in the Biosatellite de-orbit system have been incorporated so that the critical circuits are duplicated from the de-orbit programmer to the retro motor, in order to increase the system's

ORBITAL PERFORMANCE OF BIOSATELLITE I



functional capability. The second launch is planned for late 1967.

It is generally agreed that, despite the recovery failure, Biosatellite 1 demonstrated that space bioscience experiments can be successfully carried out. Except for the retro-rocket system, all systems and the engineering aspects of the spacecraft responded effectively (ref. 301).

It is expected that the variety and range of experimentation on the biosatellites will yield biological knowledge and principles applicable to many unsolved problems on earth, and crucial to man's preparation of himself and his spacecraft for successful extra-terrestrial ventures.

B. Gemini Program

The Gemini Program was the second phase of the U.S. manned spaceflight program, serving as a preliminary stage towards the goal of landing a man on the moon. Utilizing flights up to 14 days, this fully successful venture provided prerequisite experience for the Apollo-Saturn systems in maneuver, rendezvous and docking, extravehicular activity, and manned scientific investigations in space.

Early in 1965, investigations with biological specimens were begun with Gemini 3 and 4 flights, and continued with success during succeeding flights (ref. 304). A number of experiments carried out on these flights were of significance for the bioscience program.

On Gemini 3, human white blood cells, exposed to a known source of beta radiation (32-phosphorus) during weightlessness, showed a greater increase in the number of single chromosome breaks than did controls

on the flight (ref. 305). Weightlessness and radiation, apparently, worked together to yield more chromosome breaks than either by itself. On a later flight, however, the same experiment showed no increase over controls.

Gemini 8 provided conditions for study of the effects of weightlessness on a biological specimen (ref. 306). Fertilized frog eggs (frog eggs orient themselves with respect to gravity during early development) were biologically fixed (killed and preserved) at certain intervals by the astronauts. Eggs arrested during first and second cleavage were normal; those stopped at 2 hours and 25 minutes in the 8-celled stage were also normal, with typical cleavage planes and rate of development on schedule. The low gravitational field apparently exercised no effect on the ability of the froggegg to divide normally during its early stages, when such an effect would be most likely to occur because of the large density of the gradient in these cells. When some of these experiments were repeated on Gemini 12, a twin larva was found, but the ground controls also contained a twin larva, believed attributable to genetic twinning.

On several long flights, X-ray densitometry studies of the bone-mineral losses among astronauts showed greater loss from the skeleton than during complete bed immobilization in shorter flights; this is discussed under Environmental Biology.

Gemini 5 and 7 astronauts carried out visual ability experiments (ref. 307). Seeking to determine how well objects on the ground could be discerned and whether weightlessness affected acuity, these tests used a device that permitted evaluation of visual sightings from space in relation to baseline values on earth, and a photometer that measured

light attenuation through the spacecraft window caused by scattering. During the Gemini 5 flight, ground targets were assigned on scheduled passes and some data were read out, but difficulty in site identification and cloudy weather limited results. During Gemini 8, there was no difficulty in making out the site, and in-flight vision tests made by the astronauts revealed no eye damage under low-gravity conditions.

An experiment measured the effects of space on such earth organisms as the T. phage virus, Bacillus subtilus, mosaic tobacco virus, and pencillium roquefortium. These specimens were found capable of withstanding all effects of space environment except radiation, and even against that, there was evidence that only a thin shield might suffice. Balloon-sounding rocket experiments with these organisms failed to achieve complete kill. In one instance, when a container was cut in two, and one-half mounted on the other, insulation provided by the top layer of microorganisms was enough to increase survival rates of the lower layer by 10-100 percent.

Micrometeorite collection packages carried on Gemini 5 were designed, also, to collect living microorganisms in space — if any were there. An organism-collection plate, in the package, was fitted with a velocity filter so that any organism emanating from the spacecraft itself presumably would not have had enough force to penetrate the shield.

C. Apollo Program

In 1961, the United States declared its intention to achieve a manned landing on the moon by the decade's end. To meet that goal, the Apollo-Saturn system has been developed and brought to the point of initial tryout of the Apollo spacecraft. It is planned that manned

flights — scheduled as 14-day earth orbitals — will carry basic bioscience experiments intended to confirm results of tests carried out on Gemini missions.

The possibility of "organogenic" substance on the Moon has derived from speculative examination of many excellent lunar photographs taken by Surveyor 1 and Orbitors 1 and 11. Investigators in many institutions have prepared techniques, procedures, and analytical methods for examining samples expected to be returned from future Moon landings. Experiments have been defined for detecting in these samples evidence of extra-terrestrial life. A scientific analysis facility (the Lunar Receiving Laboratory) is being completed at the Manned Spacecraft Center, Houston, Texas, to quarantine returning astronauts, and to receive lunar samples and make preliminary examinations of special time-dependent analytical tests on such samples before distribution to investigators. Other laboratories in various parts of the country have planned specialized tests for organic materials bearing on the origin of life (ref. 307).

Two significant experiments developed for Apollo missions involve the effects of weightlessness on human performance during space flight. One study will determine whether zero gravity has any demonstrable influence on living human cells kept in tissue culture within the time limits of exposure in a manned orbital satellite (Montgomery, ref. 308). Employing chang liver cells maintained in enriched Eagles media and Hank's B.S.S, it differs from the comparable biosatellite experiment in both breadth and depth, and in astronaut availability to manipulate equipment and inject the living-cell cultures as required.

The influence of gravity on growth and development of biological organisms has been studied since the early 19th century, and data are available on gravitational effects on uni- and multi- cellular organisms. The advent of space flight now makes exposure to zero gravity a matter of unique and immediate concern. Very low gravity may affect man, at the cellular level, and conceivably with the same subtle and profound effects of small doses of X-radiation on cells. Such derangements in cell structure — with their genetic implications — may best be studied in individual living human cells in a zero-gravity milieu (ref. 308).

D. Bioscience Apollo Application Program (BAAP)

This program, whose goals have been projected through the 1970's up to 1985, has been planned for space biological investigations in which spurious accelerative and other periodic effects introduced by man's presence will not influence results, and where the recovery volume of the experiments can fit into the limited return capability of the command module. Utilizing Apollo hardware, BAAP seeks, in addition, to exploit man's ability to perform, repair, recover, and reorient in space, and to note and report on bizarre and unforeseen events beyond the power of instrumentation to record (ref. 309).

The following experiments have been developed under this program:

(1) Primate studies (60-90 day flight):

(a) complete metabolic assessment

(b) central nervous system studies

(c) behavior and performance

(d) endocrine balance

(e) Cardiovascular functions

(f) Neuromuscular functions

(2) Small Mammals

(a) metabolic studies using radio-active tracers

(b) cardiovascular functions

(c) growth and reproduction

(d) biorhythms

(3) Plants

(a) geotropism

(b) growth and development

(4) Biorhythms

E. Manned Space Station

It has been recognized for some time that there are biological questions about the space environment to which biosatellites, Gemini, and Apollo craft will not provide answers. What is needed is a facility that will provide the specimens and equipment for long-duration, in-depth studies of biological organisms in the space environment (ref. 310).

Five Regional Study Councils — organized by the American Institute of Biological Sciences — have recommended a program of bioscience research in a Manned Space Station. The manned station has been strongly recommended to achieve best results and especially in light of the apparent requirement for a 1-g control environment aboard the vehicle. Artificial gravity through slow rotation has been advanced as a solution to the debilitating effects of weightlessness, especially cardiovascular deconditioning (Hoover, ref. 311). Planning has been undertaken to provide such a laboratory, in which a trained investigator will be able to conduct, control, and evaluate sophisticated biological experiments. It has already been demonstrated that such a facility is a "stepping stone"

to manned exploration and exploitation of space.

The following have been defined as goals for bioscience research on, and uses of, an orbiting space station (ref. 310):

(1) Study effects of space residence on forms of earth life, including man.

(2) Develop and test bioregenerative life-support systems. (For man, prolonged space missions will require such a system. The most probable system will employ microorganisms to regenerate food; the mass behavior of these organisms may strongly condition the design of future space vehicles.)

(3) Remote sensing of terrestrial biology. (Spacecraft that can stay aloft a long time open new opportunities to study biogeophysical events and global biology, and will give new insight into the world man inhabits.)

(4) Study space environment effects on prebiotic synthesis.

(5) Astronomical studies pertinent to exobiology and for development of remote-sensing, life-detection techniques.

(6) Collect micrometeorites containing possible extraterrestrial evidences of life.

It is generally agreed that the biologist's task includes exploitation of special features of the space environment for general analysis of organism-environment relationship. Placing earth organisms in the space milieu, a space station provides opportunities for critical testing of biological relationships now thought to be understood, for confirming suspected relationships not yet detected on earth, and for discovering totally unforeseen relationships.

Establishing manned space stations poses problems of weightlessness, water, food, waste disposal, thermal management, communication with the

earth, solar energy, danger from radiation and meteors, and construction materials (Lyapunov, ref. 312). ~~(ref. 3).~~ Prime requisite is probably satisfaction of the astronaut's physiological needs, but the most efficient use of man in space presumes that all of the environmental requirements for human existence have been provided. These needs can be satisfied through appropriate design, established early in the development program (Celentano, ref. 313) ~~(ref. 4)~~.

9. LIFE SUPPORT

Maintaining a man in space for prolonged flight requires, in addition to protection from a hostile environment, provision of oxygen, food, and water, and removal of deleterious products, such as carbon dioxide, water vapor, and body wastes. All these elements must be sustained in requisite equilibrium.

The longer the mission, the greater the advantages of a regenerative life-support system. On Earth, oxygen and food derive from a highly intricate balance between plants and man. In space, the problem is to compress this Earth ecology to the dimensions of a capsule – a major scientific challenge. Best suited, apparently, to the bioengineering requirements of such a system are two groups of microorganisms: (1) photosynthetic algae, and (2) hydrogen-consuming bacteria.

In order to develop reliable engineering systems involving these organisms, it is necessary to understand completely their biochemistry and physiology so that provision can be made for their optimal, sustained growth (ref. 314). It is a well-known fact that micro-organisms respond differently to varying environmental conditions. Consequently, it has become important to secure data which will permit a prediction of performance in typical culture systems which may be adaptable to space flight.

A. Bioregenerative Systems

1. Photosynthesis:

Proposed first in 1951, the photosynthetic closed ecologic system uses single-celled algae in an interaction of light energy with CO_2 and H_2O to produce O_2 and plant cells. Chlorophyll--contained in all green

plants--picks up the light energy that thermodynamically converts CO_2 and H_2O into a carbohydrate that is later reconverted into other substances, such as protein and fat. In this process, CO_2 is consumed and an approximately equal amount of O_2 is liberated.

A good deal of study has gone into these organisms proposed for support of man in space. Inasmuch as man's survival will depend on his mutual co-existence with them, it is essential to understand these organisms more fundamentally within the context of their role in space flight. It is essential to examine their growth rates, potential yields, and secretions in continuous recycling devices similar to those which must be employed in space flight. Data concerning the organic components of the algal product are needed. Their performance must be understood under illumination with different wavelengths and intensities of light. It is necessary, too, to understand shifts in metabolism with different nutrient and carbon dioxide concentrations in conditions of light and darkness. Finally, we have only scratched the surface in our understanding of the physical chemistry of the photosynthetic process.

Early studies of algae were done with cultures that did not involve recycling. Since a system in space will require recycling of the water and the medium, a Recyclostat (fig. 40) was devised to provide continuous cultures (Krauss, ref. 314). A system of this type, while imposing rigorous demands on the performance of the culture and requiring sterility and a nutrient-replacement formula, nevertheless is most suitable for space.

(Insert fig. 40)

RECYCLOSTAT FOR CONTINUOUS CULTURE OF ALGAE

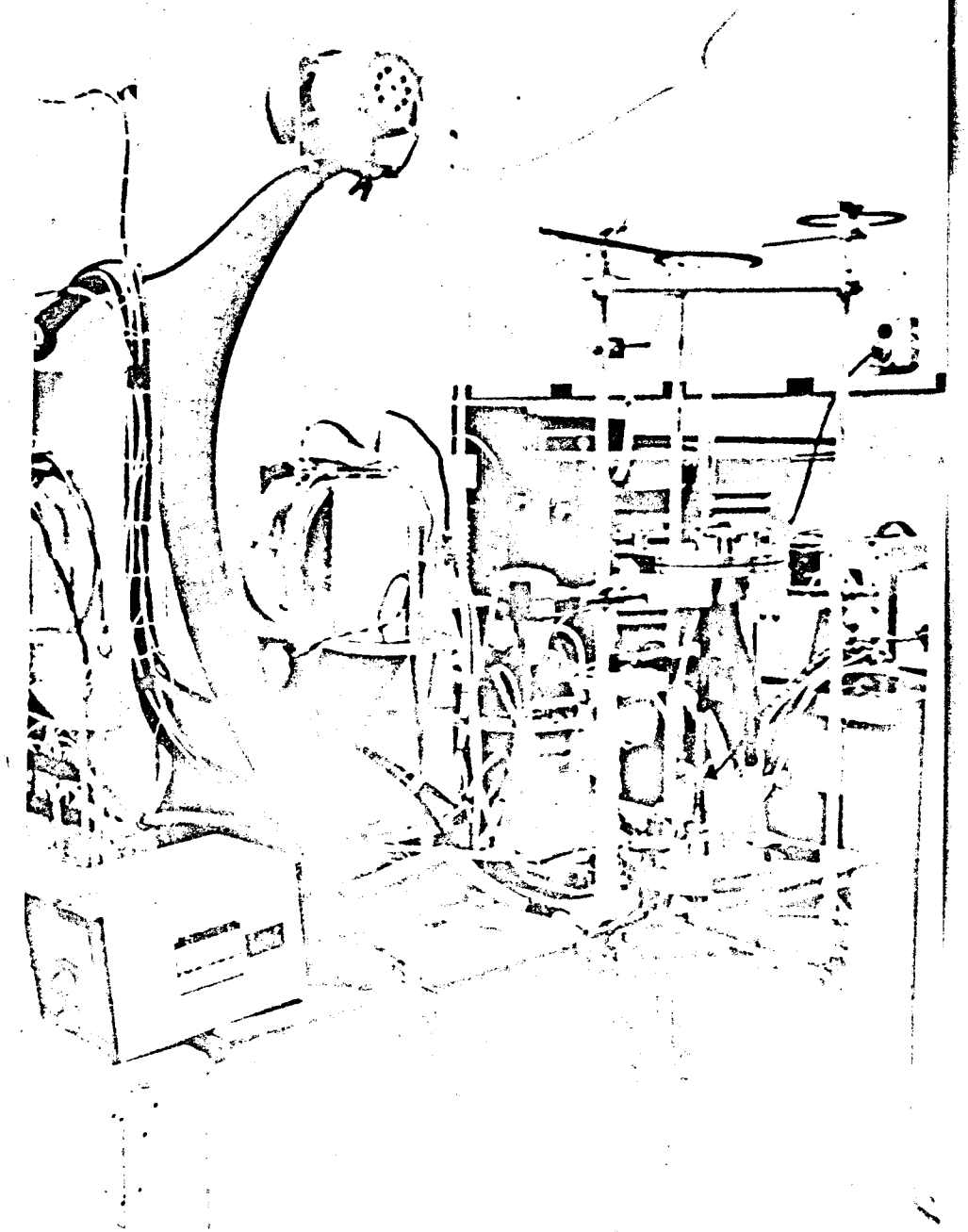


FIG. 10

NASA SB 66-141
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The optimal long-term cell production of Chlorella in the Recyclostat has been not more than 1 gram per liter per day. In order to supply the oxygen requirement of a man, a volume of 436 liters/man is required. Engineering design and modifications are envisaged which would increase the yield without diminishing process efficiency.

The results with a colorless, non-photosynthetic alga, Prototheca zopfi, have shown that a light-induced inhibition indeed does exist (ref.315). In the presence of light from cool-white fluorescent lamps, Prototheca growth rates dropped linearly with increasing light intensity of 1200 ft.-c. Incandescent light of equal intensity was less inhibitory. The action spectrum for this inhibition of growth was localized in the near - ultraviolet region of the spectrum as well as in the blue end of the visible spectrum (420 mμ). The most likely receptor appears to be a cytochrome and the inhibition appears to be confined to cell division. This inhibition may be a major factor in the phenomenon of light-dark-induced synchrony in green algae.

The Recyclostat has permitted studies of other factors which affect optimal growth of algae. An important parameter is the gas mixture with which CO_2 is supplied to the cultures. Sorokin (ref. 316) emphasizes that CO_2 may affect cell metabolism directly and/or through its effect on pH. In certain circumstances, the pH effect is obscured by the dramatic action on cell division of the dissolved undissociated ~~carbon dioxide~~ CO_2. Two factors were shown to cause (ref. 317)

deviation in the buffering capacity of cells. One is metabolically produced CO_2. With an increase in cell-population density, the effect of CO_2 evolved by the cells will add to the effect of the initial acid concentration and, under certain conditions, may become by itself a decisive factor in limiting cell activity. The other factor is acid concentration high enough to suppress the secretory activity of the cells, thereby exposing cells to the harmful acid for a longer time.

Synthesis of organic matter was studied in cells of the green, high-temperature alga, Chlorella 7-11-05, separated from a non-synchronized population into fractions of predominantly small or large cells by centrifugation (ref. 318). It was found that synthetic activity of the smaller (younger) cell fraction was invariably higher than that of the larger (older) cell fraction. Occasionally, this difference was not obvious and was traced to cell division taking place at the time of observation.

It was later shown (ref. 319) that growth studies indicated that even under most optimal external conditions, the capacity for organic synthesis declines in older cells. The declining portion of the metabolic activity during each growth-division cycle was identified as cellular aging. Speculations concerning the nature of rhythmicity in biochemical and metabolic events during the cell-division cycle include assumptions as to the accumulations of poisons and inhibitors, feedback mechanisms, immobilization due to molecular cross-linkages, and dependence of timing of enzyme activity on the timing of the duplication of specific genes responsible for the synthesis of specific enzymes.

Patterson and Krauss ~~(8)~~ (ref. 320) examined the characteristics of the sterols occurring naturally in three species of Chlorella. C. vulgaris contained three sterols, one of which was chondrillasterol. Porifera-sterol was identified as the principal sterol of C. ellipsoidea and C. saccharophila. It has been shown (ref. 1) ~~Patterson (in ref. 1) has shown~~ that C. vulgaris produced significant quantities of n-paraffins ranging from 17 to 36 carbon atoms in length.

Most recent research in photosynthesis has begun to look into the interesting phenomena correlated with photophosphorylation, pH change, light scattering, and chemiluminescence induced by pH-jump or redox shift. Studies have been made of the association between fluorescence and chemiluminescence, indicated by chlorophyll being the source of emission spectra from each process. Based on earlier work on fluorescence emission, a study has been undertaken of luminescence induced by reducing agents ~~(8)~~ (ref. 321).

An earlier hypothesis held that an unknown substance in the chloroplasts — reduced either by a pH shift or added reductant — reacted with another chloroplast intermediate to liberate energy used to emit light quanta. New data make the process seem less complex, possibly involving molecular oxygen as one of the reactants in the light-producing reaction. Modifications of apparatus are permitting a better exclusion of oxygen from the system to test this hypothesis. If, indeed, oxygen is involved, the chemiluminescence of chloroplasts would become entirely analogous to the many other bioluminescence and chemiluminescence reactions, all of which involve molecular oxygen as a participant. (Ref. 23).

Kok and Rurainski (ref. 322) have shown recently that in photosynthesis, plastocyanin and cytochrome f function "in parallel" in the chloroplast. Both donate to Photosystem I electrons generated in the second oxygen-evolving act.

Based on the voluminous data evolving from physical biochemistry, present concepts visualize photosynthesis as the result of two cooperating light reactions (ref. 323), Photoacts I and II. In each photoact, light absorption is assumed to be followed by the formation of a primary oxidant and reductant.

In Photoact I, the following reactions occur:

$$2\,V + H_2O \xrightarrow{\text{Chl } h\nu} 2VH + \tfrac{1}{2}\,O_2$$

$$2VH + O_2 \longrightarrow 2\,V + H_2O_2$$

$$CH_3\,CH_2\,OH + H_2O_2 \xrightarrow{\text{catalase}} CH_3CHO + 2H_2O$$

$$\overline{CH_3CH_2OH + \tfrac{1}{2}\,O_2 \longrightarrow CH_3CHO + H_2O}$$

Photoreduction of substrate V (a semiquinone compound) by photosystem I, which generates photooxidant P_{700}^{+} and photoreductant X^{-}, proceeds according to:

$$P_{700} + X \xrightarrow{h\nu} P_{700} + X^{-}$$

$$X^{-} + V \longrightarrow V^{-} + X$$

The great stability of photosystem I, for which no specific poison has been found and which survives aging, mild heating, and detergent treating, might suggest an essentially simple system of small molecules. P_{700} and its reaction partner X might be chlorophylls.

Whereas photosystem I generates a strong reductant (X^-) and a weak photooxidant (P_{700}^+), photosystem II generates a strong oxidant (yielding O_2) and a weak reductant ~~(12)~~ (ref. 324). Coupling between the two photosteps presumably occurs between the weak reductant and a weak oxidant. As depicted in series scheme, O_2 is evolved from water in system II while a substance of intermediate potential (Y) is reduced. In complete photosynthesis reduced Y is reoxidized by photooxidant (P_{700}) made in photoact I along with low potential photoreductant X.

$$H_2O \xrightarrow{h\nu\ II} Y \xrightarrow{dark} P_{700} \xrightarrow{h\nu\ I} X$$

For some time, research has indicated involvement of manganese in photosynthesis. The extent of this involvement has not yet been fully determined. According to some investigators, most observations of manganese deficiency have been made with relatively aged tissues and it thus may be argued that its effects are secondary rather than primary. There are, they say, no completely convincing indications that manganese directly participates in photosynthesis, although many photosynthetic schemes dogmatically include manganese as a catalyst on the oxidant side ~~(13)~~ (ref. 325). Because of this, most research in the past few years has been devoted to critical evaluation of manganese participation in photosynthesis and to examining how manganese is bound within the chloroplast.

Other studies reveal both support of and disagreement with this view. Electron micrographs of manganese-deficient cells (≃ 90 percent deficient by growth measurements) revealed no striking dissimilarities between these and normal cells ~~(14)~~ (ref. 326). Minor differences such as decrease

in thickness of cell wall, absence of starch grains, and increase in cell size were seen in the deficient cells, but the lamellar-stacking of the chloroplast was scarcely affected by the deficiency.

In contrast, it has been reported that photosynthesis is restored by addition of manganese in manganese--deficient Scenedesmus (ref. 326). This restoration is independent of protein synthesis, but peculiarly dependent upon light. In addition, a small but significant (10-15 percent) increase in photosynthesis can be observed with dark pre-incubation of deficient cells with manganous ions. This small increase in the dark appears to be an effect on the CO_2-fixing enzymes.

2. Chemosynthesis

The chemosynthetic bioregenerative system, first proposed in 1962 (ref. 327) is a closed life-support system directed to conversion of carbon dioxide and human metabolic wastes into breathable oxygen and a food supplement. In essence, the system functionally couples electrolysis of water and Hydrogenomonas bacteria (fig. 41).

(Insert fig. 41)

Electrolysis--carried out in a closed unit containing an electrolyte (KOH solution) with anode and cathode--produces oxygen for the cabin supply, and hydrogen for the bacteria. Preceding electrolysis, CO_2, produced by man, is recovered from the cabin atmosphere by a regenerable absorber so that relatively pure CO_2 can be fed into the gas phase of the closed-culture vessel. The CO_2 is assimilated by the bacteria, which in turn furnish protein, fat, and carbohydrate required for human nutrition. Thus, a considerable portion of man's metabolic wastes would be processed and recycled in the closed ecologic system (refs. 328-330) (fig. 42).

(Insert fig. 42)

A number of advantages derive from this system: (1) waste products

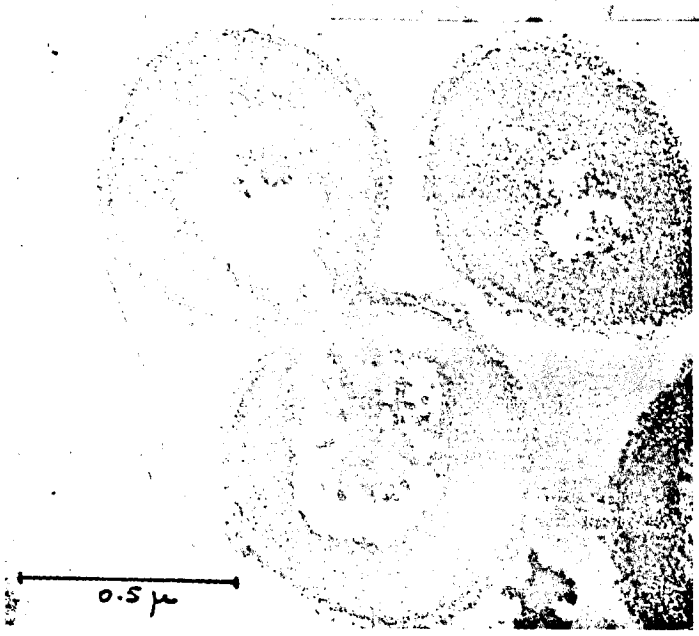


Fig. 6. Electron micrograph of normal *Hydrogenomonas eutropha* cell dividing

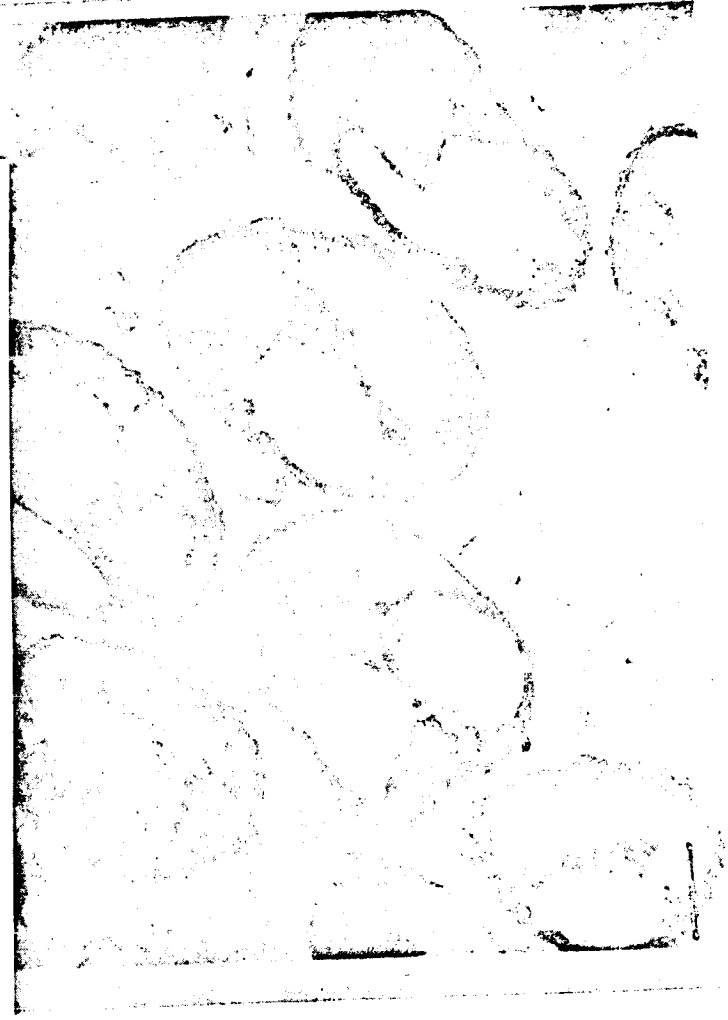
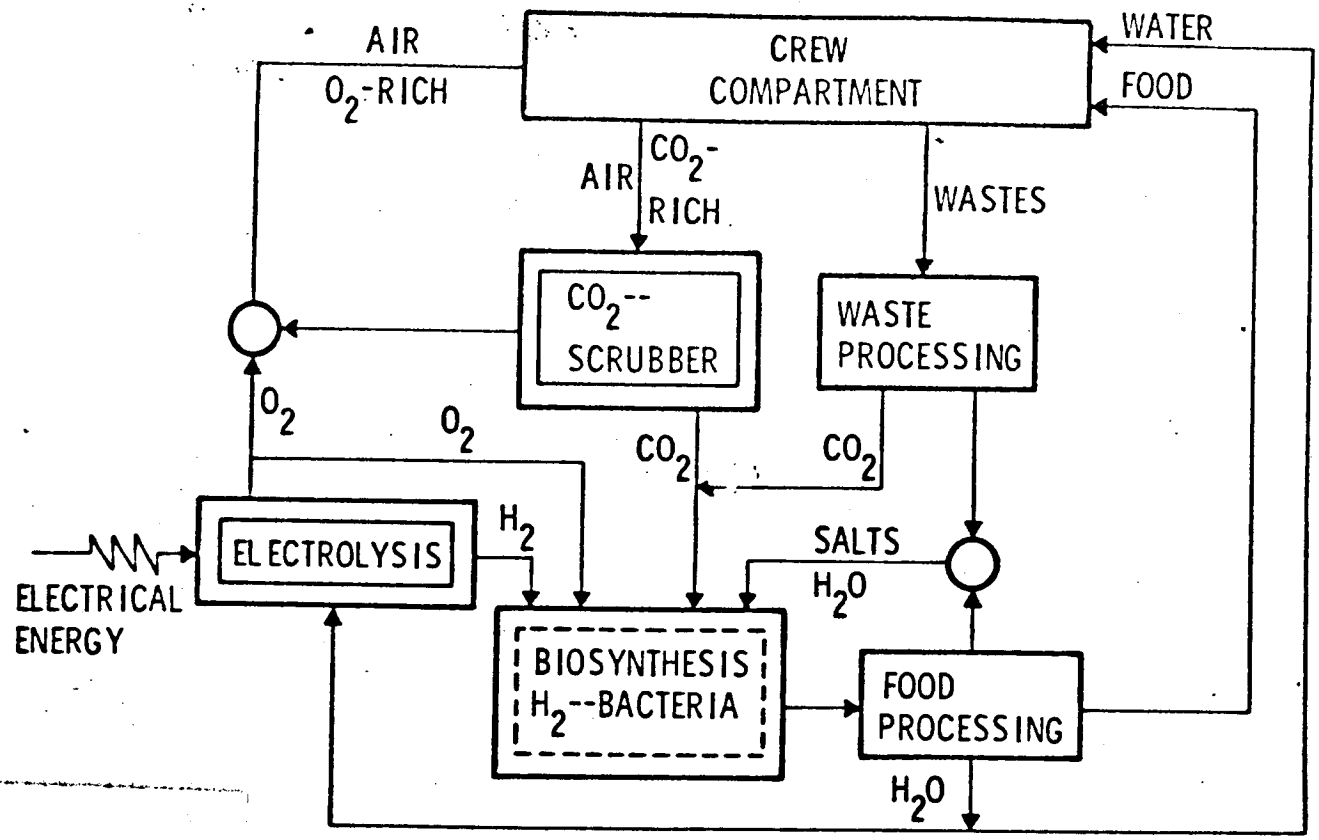


Figure 41.- Hydrogenomonas bacteria.
Left: normal cell dividing.
Right: cells with fatty inclusions.

CHEMOSYNTHETIC ECOSYSTEM



HUMAN RESP: $CH_2O + O_2 \longrightarrow CO_2 + H_2O$

BIOSYNTHESIS: $6\,H_2 + 2\,O_2 + CO_2 \longrightarrow CH_2O + 5\,H_2O$

ELECTROLYSIS: $6\,H_2O \longrightarrow 6\,H_2 + 3\,O_2$

ELECTRICAL ENERGY (600 to 1000 WATTS CONT)

Figure 42.—

provide growth needs for the bacteria--urea yields nitrogen and carbon dioxide provides carbon and molecular oxygen; (2) a by-product of electrolysis--hydrogen--is a source of energy; (3) the harvested excess of cells from a steady-state culture is a potential food supplement for man; and (4) simpler equipment than in photosynthesis, which requires confining the culture in thin layers to permit penetration of light.

Much work has gone into defining optimum conditions for continuous culture and a batch-culture system that will yield a maximum quantity of pure and fresh *Hydrogenomonas eutropha* for nutritional studies (refs. 328, 331).

To speed up *H. eutropha* growth in batch cultures, experiments have varied the gas mixtures, liquid volume, and growth medium constituents. Best gas mixtures in batch culture proved to be 67-70 percent H_2 ~~hydrogen~~, 20-22 percent O_2 ~~oxygen~~, and 10-11 percent CO_2 ~~carbon dioxide~~. When using the growth medium described by Bongers (ref. 331) (Table 1) and a gas mixture of 70 percent H_2, 20 percent O_2 and 10 percent CO_2, the larger batch-culture apparatus will yield an average harvest of 2.0 to 2.5 grams dry weight of cells per liter of medium in a 48-hour growth period.

TABLE VIII. GROWTH REQUIREMENTS OF H. EUTROPHA

Temperature	33 C. - 35 C.
Hydrogen	50 - 560 mm
Oxygen	120 - 150 mm
Carbon Dioxide	70 - 80 mm
$CO(NH_2)_2$	0.5g/liter
$MgSO_4\ 7H_2O$	0.1g/liter
$Fe(NH_4)_2(SO_4)_2.6H_2O$	0.008g/liter
Phosphate (Na,K)	0.03 M
pH	6.5 - 7.5

A mathematical model containing all the characteristic features of batch microbial growth (lag, exponential growth, stationary and decline phases) has been developed. ~~(20)~~ (ref. 332). The model is rigorously treated and based on the assumption that the growth is controlled by the amount of some critical intermediate produced by the organism and the consequences of the assumption that the production of this is so regulated as to produce the maximum cellular mass by the time the limiting nutrient is exhausted. In spite of its simplicity, this model gives a sound qualitative agreement with observations of the developments that occur in microbial cultures, the lag phase being the result of the cell first building up its supply of the critical intermediate. The model was later applied and satisfactorily corroborated laboratory data on the dynamics of microbial cell populations, ~~(21-24)~~ (refs. 333-336).

Continuous culture differs from batch culture in that its steady state of growth persists and its various parameters (pH, concentration of nutrients, oxygen, hydrogen, and carbon dioxide) are in continuous unchanging balance. On the basis of performance recently obtained with growth in steady-state cultures, it appears that the chemosynthetic approach to life support is indeed promising.

A continuous or steady-state culture in which the rate of growth is controlled by a single factor (e.g., the carbon source) is defined as a chemostat. In such cultures, the concentration of nutrients other than the limiting ones, ~~the concentration of nutrients other than the limiting ones~~, the concentration of products excreted into the medium in the course of cell reproduction, and the pH are more or less independent of dilution rate. A continuous culture in which the dilution rate is

set by growth rate is called a turbidostat (ref. 331) or product-limited culture. Figure 43 shows an adaptation of a unit fabricated by the Battelle Memorial Institute (ref. 328).

(Insert fig. 43)

Considerable effort has been expended on developing a chemostat continuous reactor capable of growing pure cultures of Hydrogenomonas. Scientists at Battelle Memorial Institute, working from a 2-liter model (ref. 328), have developed a 20-liter continuous-culture system in which a number of experimental runs have been made (ref. 337). Since investigations with continuous cultures have shown urea concentration as a substantial influence on Hydrogenomonas growth and urea assimiliation, they have designed apparatus for automatic urea analysis that can be integrated with the system. Incorporation of pH control, as well as urea control, into the system now permits studies that promise major increases in growth rate at high-cell density.

Experimental studies of continuous cultures have demonstrated a number of important considerations. Initial growth period is critical in determining the course of active growth in denser cultures produced later. A change in mode of growth is signalled by a sharp minimum, then rapid,rise of pH. It is not yet known how to revert a culture rapidly, once it has changed. Understanding of culture growth requirements has increased: a cell density of 5 grams per liter--using a dilute inoculum-- has been attained in batch culture in about 24 hours; this is a shorter period than heretofore attained (ref. 338).

TURBIDOSTAT CONTINUOUS CULTURE EQUIPMENT FOR HYDROGENOMONAS BACTERIA

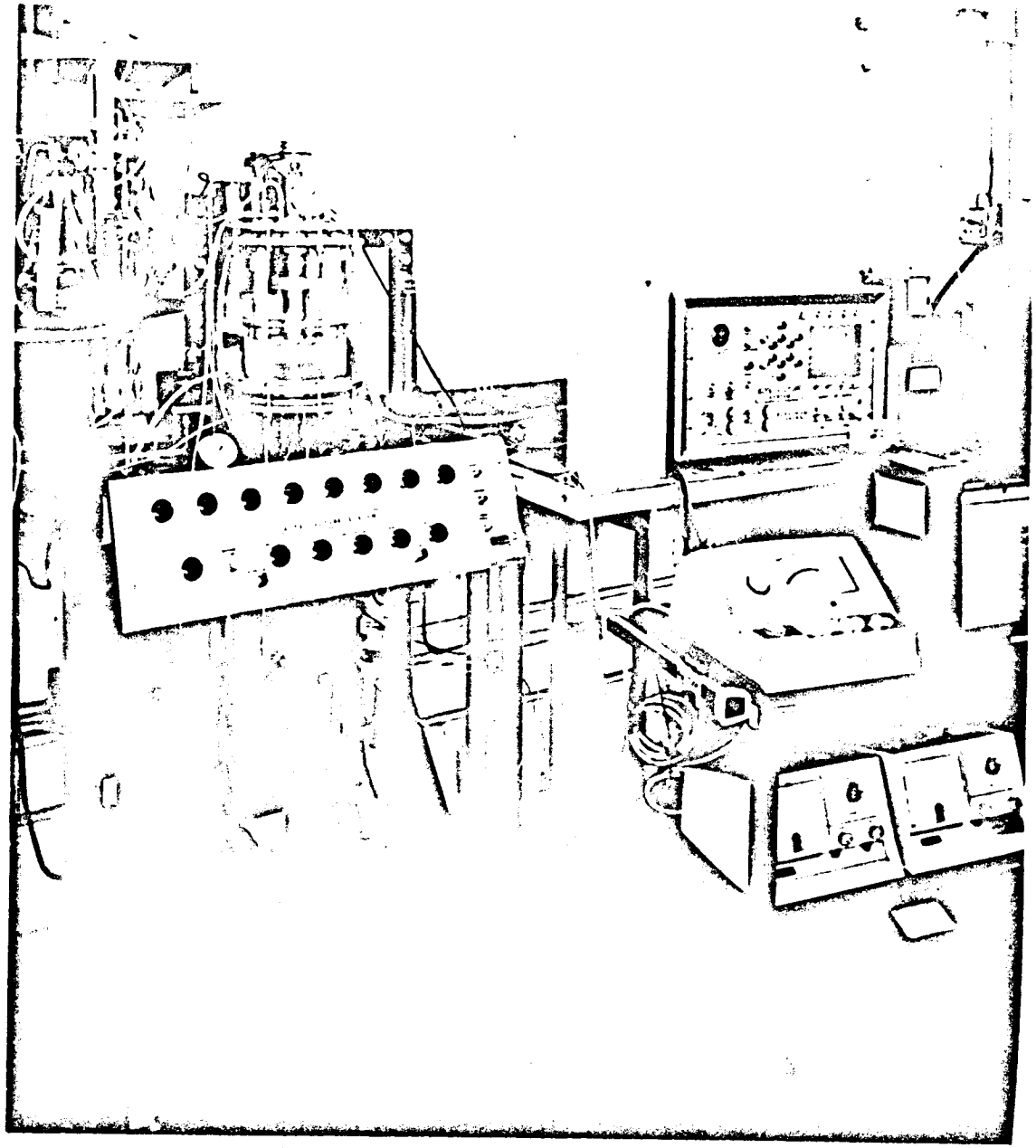


Figure 43.—

Other experiment~~al~~s ~~runs~~ have studied such items as pH control as a means of producing a dense culture from a dilute inoculum, effects of ammonia concentration on growth, and conversion of urea into intermediate compounds (ref. 339) ~~(27)~~. Rate of gas consumption has been found to be a meaningful measure of growth activity of Hydrogenomonas cultures. Comparisons of maximum rates of mixed-gas consumption in parallel cultures have been used to evaluate effects on growth of differences in mixed-gas or liquid-medium composition. A higher gas-consumption rate has been interpreted as indicating better growth activity or less inhibition by the culture environment, as compared to a control culture.

Control of the environment of the continuous culture is requisite to an indefinite steady state maintained at maximum efficiency. Attainment of such efficiency demands a satisfactory merging of three items (whose relative importance would vary with specific space missions): (1) carbon dioxide conversion efficiency — the ratio of CO_2 conversion rate to hydrogen and oxygen conversion rates; (2) urea-conversion efficiency — the ratio of protein harvested in the cells to the urea converted; and (3) rate of production of harvested cellular material potentially useful as food per unit volume or weight of the continuous culture in its steady state ~~(26)~~ (Foster, ref. 338).

A number of scientists have felt some uncertainty about the nutritional adequacy of Hydrogenomonas cells as food supplements. In nutritional studies — in which rats were fed H. eutropha — a high biologic quality of the protein has been demonstrated in one sample of the bacteria ~~(28)~~ (ref. ~~340~~ 340). In other samples, the tests showed good protein

digestibility, poor fat digestion, and great variability in mineral content. There was no evidence of toxicity.

A chemosynthetic bioregenerative system has a high requirement for supplemental inorganic salts. To find a way of reducing this need, human urine was studied as a nutrient medium to support autrophic growth of H. eutropha (29) (ref. 341) Investigation has shown that human urine can give such support for the organism's concomitant utilization of H_2, O_2, and CO_2.

Efficient cultivation of hydrogen bacteria in a urine-supplemented medium under an atmosphere of H_2, O_2, and CO_2 requires a knowledge of the metabolism of the bacterium under these conditions. High organic content of urine necessitates study of the effects of organic compounds on the culture's autotrophic processes, and the effect most in need of study would be hydrogenase (hydrogen-utilizing) activity and the CO_2-fixing systems. Investigation of the Hydrogenase system of H. eutropha has shown it at least partially active in the presence of a number of organic compounds, and fully active when H_2, O_2, and CO_2 are associated with an organic compound (30) (ref. 342)

Investigation into the effect of trace metals on gas-uptake rates of H. eutropha have demonstrated that 15% urine diluted with redistilled water is lacking in trace metals nutritionally necessary for optimum growth and gas uptake of the organism, (31) (Goldner & Dittman, ref. 343). Most inorganic media used for the culture of this organism are supplemented with trace metals or have sufficient trace metals supplied as impurities in the reagents used for preparing the media.

Many investigators have observed H. eutropha sensitivity to O_2: both an insufficiency or a high concentration of O_2 will inhibit its growth. O_2 concentrations below optimum cause storage of poly-betahydroxybutyrate; high levels inhibit hydrogenase activity (ref. 343).

Studies of metabolic products of H. eutropha cells grown in culture have isolated four sugars: glucose, ribose, arabinose, and xylose. Those, along with galactose, lyxose, mannose, rhamnose, sedoheptulose, sorbose, and sucrose, could not be oxidized by either autotrophically or heterotrophically grown H. eutropha cells, nor could they support the growth of this organism. Fructose, which was not detected in the medium, was the only sugar tested which could be metabolized by H. eutropha. Although H. eutropha was unable to utilize glucose, the organism did grow abundantly in a medium containing glucose which had been autoclaved in the buffer before adding the other components of the basal medium (ref. 344).

An ~~Apparent~~ apparent variant of H. eutropha (ref. 345) shows slightly larger cells and tends to be more rod-shaped than the typical coccobacilli morphology. In liquid media, growth of the variant results in a great deal of foaming, suggesting production of a surface-active substance. The limited number of biochemical tests performed thus far have revealed no difference in sugar metabolism. Although the new organism is probably not a contaminating species (it would be unusual for a slower-growing contaminant to completely replace the faster growing H. eutropha), its implications

for the entire program suggest the need for full characterization of the variant so that any similar developments can be readily recognized. To minimize recurrence of variants, stock cultures are now maintained in a frozen liquid medium from which a new working culture is prepared at weekly intervals.

The discovery of bacteriophages active against H. eutropha (Fig. 44) has demonstrated the possibility of a phage infection of this bacterium in a life-support system (DeCicco, ref. 346). Important questions to consider include the effect of infection in a continuously growing culture, and possible measures to reduce its likelihood. Under a given set of growth conditions, the infectious pattern would be of chief importance. Since four phage types have been isolated, an initial need is to determine similarities or differences in patterns; after that, the effect of an infection can be predicted and control measures employed.

(Insert Fig. 44)

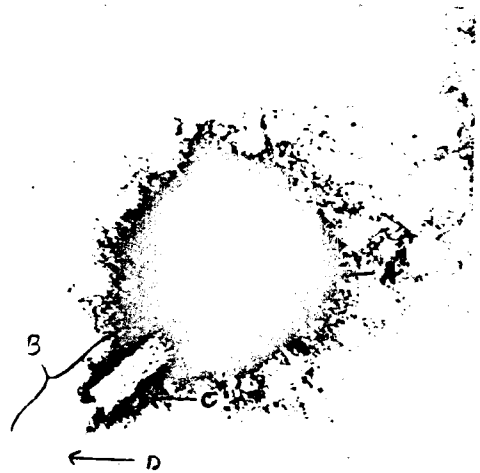
B. Depressed Metabolism

A large volume of research has been devoted to the depression of metabolism, which is regarded as a possible means of facilitating astronaut survival on, and successful completion of, prolonged space flights. Depressing the astronaut's metabolism means reducing his nutritional and metabolic needs and, for a voyage lasting several months, permits considerable reduction in a spacecraft's storage requirement (and weight) for food.

Metabolic rates in animals depress in response to the reduced energy expenditure that accompanies lowered body temperature. Two means of reducing temperature — hibernation and hypothermia — have received much attention. Hibernation — a cyclic, stress-free physiologic

HYDROGENOMONAS eutropha PHAGE

Bacteriophage Type HeMa



Bacteriophage Type HeMb

Bacteriophage Type HeC

Bacteriophage Type HeV

Figure 44.

state, usually seasonally linked, and characterized by reduction to minimal levels of many body processes — has been shown to have gradations of inactivity from very light to deep torpor (~~35~~ ref. 35). Hypothermia lowers the body temperature by direct application of cold to a point where desired metabolic depression occurs. Extensive study in animals has been carried out to determine the feasibility of these metabolic depressants in man.

1. ~~A.~~ Hibernation

A large mass of facts is available ~~of~~ on how hibernation affects the various body systems of animals (~~35~~ ref. 34). A well-known distinction between hibernators and non-hibernators is the continuance of the heart beat at very low body temperatures without onset of ventricular fibrillation. Studies of isolated perfused rodent hearts have shown that in non-hibernators the heart stopped beating at 10°-15°C; in hibernators, the hearts maintained heat until 7° to -1°C. The heart rate of the hibernating hedgehog has been found to be 10% or less that of the non-hibernating animal. Exposed to 2.5% concentration of CO_2, hibernating animals remained in deep torpor but showed increased heart and respiration rates.

Hibernating hamsters and ground squirrels have shown increased oxygen and red-blood-cell concentration in the blood, with a diminished occurrence of tissue hypoxia, a frequent result of reduced cardiac and respiratory activity. Reduced prothrombin in hibernating ground squirrels has been accompanied by increased clotting time; this is at variance with the delayed blood clotting characteristic of hibernation.

Hibernators show an important distinction from homoisothermic animals in maintenance of irritability even when body (or brain)

temperature is greatly reduced. Study of nerve conduction has shown that in hibernating golden hamsters it does not cease before 3.4°C; in rats, it stopped at 9°C. Citellus beecheyi, although showing a 90 percent reduction in brain-wave amplitude at 6.1°C, continued discrimination, attention focusing, sound localization, vocalization, and motor coordinations at that temperature.

Histological examination of ground-squirrel and hamster kidney has shown, during hibernation, pronounced vasocongestion of the glomeruli and increase in kidney size. Shortly after cold exposure, there is an increase in mitotic activity, but after 8 weeks of cold, mitosis has stopped altogether and the kidneys resemble those of a renal hypertensive rat.

Histological changes in the digestive tract of the hibernating arctic ground squirrel are those which would normally be expected in an animal which is not ingesting food. Secretion and storage of mucus in the cells of the superficial epithelium increases and this, possibly, prevents adhesions between the walls of the collapsed gut during hibernation, or prevents autodigestion of the mucosa by enzymes and acid remaining in the stomach.

Hibernation and Radiation Protection:

No?! It has been demonstrated that ground squirrels irradiated while hibernating show a marked degree of radio protection when compared to irradiated active squirrels. This protection occurs over a large range of doses, embracing the three main syndromes associated with radiation lethality (Hematopoietic, gastro-intestinal, and central nervous system).

Comparison between squirrels irradiated during winter and summer showed most effective protection during winter hibernation, with little difference shown for active groups. Results indicate radio-protection depends upon degree of activity and the "hibernating" temperature. Activity in all groups reduces protection; winter hibernation affords greater protection than does summer protection, although in a series of temperatures — 5°, 13°, 23°, 36°C — the greatest protection occurred at 13°, ~~(37)~~ (Musacchia, ref.348).

Despite some reports that hibernation in the ground squirrel does not provide any lasting decrease in radio-sensitivity, most studies confirm the decrease. It appears that, apart from experimental differences related to the radiation facilities and irradiation procedures, the period of hibernation before and after, and the depth of hibernation during, irradiation are possibly the most significant factors responsible for differences in response. The mechanics of radio-protection in hibernation are, as yet, not understood, but it seems likely that several physiologic factors operate, especially reduced mitotic activity, hypoxia, and endogenous chemical protection ~~(37)~~ (ref.349).

Experiments in which animals were first irradiated, then placed in hibernation, support the conclusion that: (1) irradiation does not necessarily suppress intestinal absorption of glucose; and (2) in post-irradiation hibernated animals, the transmural movement of glucose is enhanced. Although still difficult to assess the role of hibernation in radio-protection, evidence shows that in the hibernator, absorption

and transfer of sugar is sustained following exposure to lethal levels of ionizing radiation (ref. 350).

Concomitant with the establishment of the physiologic features of hibernation, detailed biochemical studies are in progress (ref. 351). Extensive work has been done on brown fat in hibernators, which is considered by many to resemble functionally the glands of internal secretion. Studies were made on the effects of cold acclimation culminating in hibernation on the weight, mitochondrial fat, and liver of ground squirrels (Citellus lateralis). Micochondria from brown fat and liver of hibernators showed a higher degree of oxidation of various substrates of the glycolytic pathway. It was concluded that the increased oxidative activity of brown fat, together with the increased ratio of brown fat to body weight, indicates that the thermogenic capability of brown fat is enhanced in the process of preparation for hibernation. Further work by Smith, et al. (ref. 352) substantiated these findings, by confirming essentially the physiologic role of brown adipose tissue as a thermogenic effector organ both in cooled or cold-exposed mammals and in hibernators during cold-induced arousal.

2. Hypothermia

Hypothermia, although easy to apply, causes stress reactions. Unlike hibernation, it is not a normal event for the animal; the latter is never found ready for it. Thus, in hypothermia, at 25° C., ventricular fibrillation occurs in the dog, the rat, and in man as well (ref. 347). Most hibernators normally hibernate at 2° - 5 °C., without fibrillation.

Studies of whether tolerance to cooling can be developed have monitored resposes of rats subjected to deep hypothermia (0°C.) for

increasing durations. Indices of adaptation used were: (1) ability of animal to thermoregulate one hour after reanimation when placed in successive environments of increasing temperature; (2) restoration of body weight; and (3) absence of neurologic signs. Percentage weight losses reached maximum values after the third induction of cooling. Thereafter, weight losses were progressively less, males improving faster than females. Neurologic signs (partial paralysis, hyper-irritability) markedly diminished. After each trial, the thermoregulatory test revealed progressive improvements. The results firmly demonstrated increased tolerance to induced hypothermia on repetitive exposures (ref. 35.3).

A very promising method of inducing hypothermia has come out of an extensive series of studies in which hamsters, placed in specially designed isolation chambers, were exposed to a synergistic 80:20 helium-oxygen combination at a low temperature. The hamsters readily tolerated confinement for periods of 2-3 days. Hypothermia occurred within a range of temperatures (5° to 12°C.). At 5°C. they became hypothermic within 12 hours, with respiration and heart beat, although reduced, both well sustained during the hypothermic physiologic depression. The most important feature, however, was that recovery from hypothermia occurred merely with a return to room temperature. These results are very encouraging, especially since the "helium: oxygen and cold" combination is easily obtained and is suitable for use in a flight system (Musacchia, ref. 354).

Hypothermia and Radiation Protection:-

No 9 Some pilot projects with hypothermic hamsters at 5.5°C. have shown an increase in radio protection, and further investigation of this

type is planned for future biosatellite flights. The role of deep hypothermia (about 0°C.) and radioresistance in rats and hamsters is being studied in another series of experiments.

To compare aspects of radioprotection, active and hypothermic animals were exposed to whole-body gamma-irradiations of 1000 r and 1500 r (^{60}Cobalt). All the active animals died within a few days, while most of the cold-exposed animals irradiated at 1000 r survived well beyond 30 days, many still alive months later (fig. 45).

(Insert fig. 45)

Rats and hamsters exposed to whole-body gamma-irradiation (^{60}Cobalt) during deep (0°C.) hypothermia, showed considerable reduction of radiation sensitivity. Protection is apparently due to a combination of both low temperature and free radicals in the radiation chamber (ref. 355).

3. Cold Acclimatization

Unequivocal evidence for the thermogenic function of brown adipose tissue (fat) in both cold-exposed and hibernating animals has been obtained in a study which observed--in intact deer mice under hypoxia and hypercapnia--brown fat temperature and concurrent effects on hypothalamic temperature during exposure to various environmental temperatures (Smith, ref. 356).

It has been shown that mitochondria from brown adipose tissue of rats cold-acclimated at 6°C. oxidize alpha-ketaglutarate at a rate twice that of controls at 26°C. In addition, both respiration and phosphorylation are unaffected by DNP (2, 4-dinitrophenol), bovine serum albumin, or glutathione. Endogenous respiration of tissue slices is not stimulated →

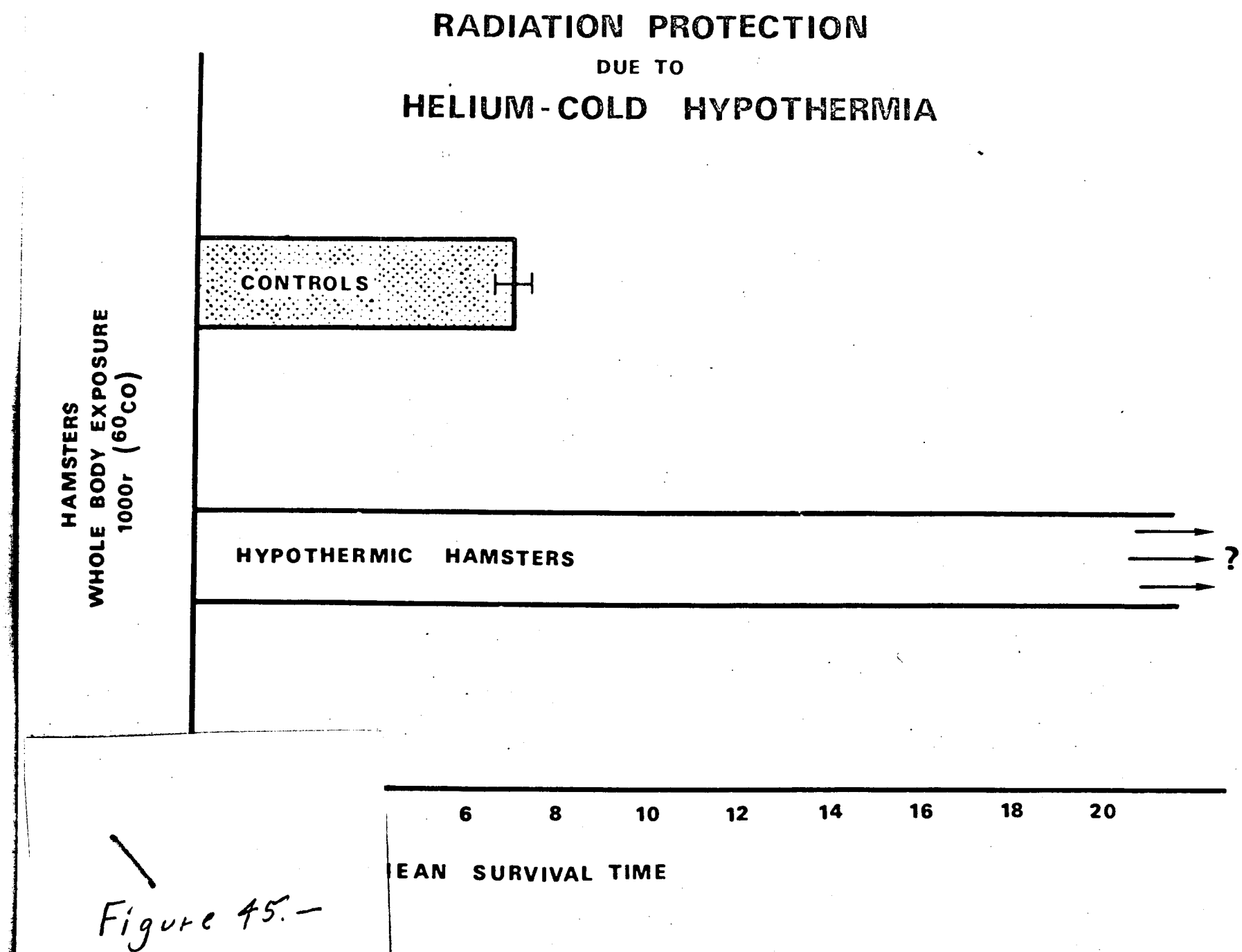

RADIATION PROTECTION
DUE TO
HELIUM-COLD HYPOTHERMIA
HAMSTERS
WHOLE BODY EXPOSURE
1000r (^{60}CO)
CONTROLS
HYPOTHERMIC HAMSTERS
?
6
8
10
12
14
16
18
20
EAN SURVIVAL TIME

Figure 45.—

by DNP. This evidence suggests that brown-fat mitochondria are not capable of oxidative phosphorylation, but do phosphorylate at the substrate level. These findings provide an unusual example of electron transport via an energetically non-conservative pathway and are especially significant to thermogenesis by brown adipose tissue (~~40~~ ref. 352).

Recent studies of deer mice moved from their normal altitude to other altitudes have shown that on initial exposure to altitude, both absolute and relative weight of brown fat rose to levels found in native high-altitude controls, demonstrating that body cooling, whether induced by hypoxia or cold, is associated with compensatory thermoregulatory responses in brown fat. On exposure to cold or arousal from hibernation, lipid metabolism in brown fat is greatly enhanced. Thus, the pyridine nucleotides would seem extremely important in this tissue, not only as terminal oxidative substrates, but also as adjuvants to the highly active biosynthetic and degradative reactions involving the lipid stores and processes of cell proliferation and growth (~~45~~ ref. 357).

In a number of studies, adrenal glands of both control and translocated mice were removed, weighed, and assayed for adrenal corticosteroids. Changes in total adrenal corticosteroids roughly paralleled the changes seen in brown-fat heat production, but not necessarily the changes in brown-fat mass after translocation. It has long been accepted that hypoxia or translocation to high altitude represents a stressful situation. The marked increase in adrenal corticosteroid of mice translocated from high altitude to sea level would seem to indicate that

"down-hill" transfer also represents a stressful situation.

Seasonal changes in metabolic rate differ markedly with altitude. At sea level, the response of deer mice to seasonal cold appears ascribable to an increase in physiologic isolation. At 3800 m, where summer metabolic rate is low, the response to seasonal cold is a metabolic one, that is, an increase in metabolic rate with no change in body temperature. It is interesting that at sea level, metabolic responses to temperature and altitude during winter cold differ in magnitude and direction from responses to experimentally imposed constant cold. There appears to be no clear-cut relationship between body temperature and altitude, and a number of factors other than hypoxia undoubtedly influence not only body temperature, but also the thermoregulatory ability of mice from different altitudes (~~46~~ ref. 358).

C. ~~X~~. Cabin Atmospheres

Two widely different types of habitable atmospheres have been used in manned spacecraft. The USSR has favored an atmosphere whose composition and pressure is very similar to that at normal sea level, whereas pure O_2 at a pressure of 258 mm Hg has been used by the USA in the Mercury and Gemini Programs. Other approaches will be studied for future use, but they will be faced with the potential problems of hypoxia, oxygen toxicity, dysbarism, the need for inert atmospheric gases, etc. "Due to the limited number of actual space flights to date, examination of the experience in ground-based testing of various atmospheres for application to manned space flight affords a guide for prospective gas mixtures" (~~47~~ ref. 359).

The physiologic effects of the absence of nitrogen or a decrease in the nitrogen level of the breathing atmosphere on living systems can be studied through the creation of a nitrogen-free environment. This has been done by using a 100-percent oxygen or hyperoxic atmosphere, or by substituting an inert gas such as helium for the nitrogen, thus establishing a helium-oxygen atmosphere.

1. Hyperoxic or 100 Percent Oxygen Atmosphere ¶ Wright et al. (ref. 360) (48) tested mice, rats, and chicks under varying conditions of a hyperoxic environment. They found that mice were able to survive the toxic effects of one atmosphere of O_2 if they were periodically exposed to air. If the animals were kept in 100 percent O_2 at one atmosphere continuously, half of them died within 5-6 days, but with an exposure ratio of 16 hours in O_2 to 8 hours in air, or ~~two~~ 2 days in O_2 to ~~one~~ 1 day in air, there were no deaths. When the 2:1 proportion was changed so that relatively less time was spent in air, the mortality ratio also changed, but the lethal effects were not prevented. This led to the question of whether the "benefit of the period in air lay simply in that the O_2 tension in the respired atmosphere was decreased to normal levels or whether the N_2 in the air played some specific protective role". The results of further study indicate that the reduced oxygen tenion in the respired atmosphere was the essential factor in protecting mice from oxygen toxicity. The presence or absence of N_2 did not seem to affect survival time.

In contrast to mice, Weiss et al. (ref. 361) found that white leghorn chicks are much more resistant to the toxic effects of 100 percent O_2 at atmospheric pressure. No morbidity or mortality was evident when 2-7-week-old chicks were exposed tohyperoxic conditions for a period of four weeks, although there was a reduction in growth rate (75 - 25 percent),

feed intake (75% of normal) and slowing of respiratory rate by 31%. Hematologic changes occurred. Two hypotheses have been proposed to account for the chick's resistance. Initially, it was thought that the unusual morphology of the avian respiratory system and its deficiency of surfactant might explain the resistance to O_2 toxicity (61) (ref. 362) Some researchers regard surfactant as the primary area for the effects of high oxygen tension. However, surfactant has been found in the lungs of birds and mammals with signs of O_2 toxicity, suggesting that this resistance might be a function of the immaturity of the chicks. Weiss and Wright are currently testing adult chickens, but the initial data does not support this interpretation, i.e., some of the adult subjects show the same resistance to the lethal effects of one atmosphere of O_2 as the chicks.

The difficulties associated with extracting and measuring surfactants in chicks and rats following oxygen toxicity have led to the development of a controlled quantitative approach to lung lipids. The results of this work point to a correlation between pulmonary surfactant lipids and compliance in O_2-poisoned rats. "Adult rats exposed to 100 percent oxygen at one atmosphere for 60 hours showed low compliance curve values and only one-third the amount of surfactant lecithin as control rats" (50) (ref. 363). The adult Japanese quail (Coturnix japonica) also appears to be more resistant to O_2 toxicity than either rats or mice, and about equal to the adult chicken.

When 100 percent O_2 is used at a pressure of 5 psia, rats will exhibit signs of marginal oxygen toxicity at first, but many of the symptoms disappear after 12 weeks of exposure (51) (ref. 364). The subjects appear normal and slightly larger and heavier than control animals, which

cannot be explained in terms of edema or an increase in the deposition of fat. The "greater carbohydrate content of both tissues and carcasses after 12 weeks of exposure together with a smaller amount of lipid found both in the tissues and in the carcass suggest a difference in the energy source utilized by these experimental animals". This may have been a result of the rats using carbohydrate as their major energy source, since the turnover rate of carbohydrate was significantly greater in the experimental subjects. A potentially important finding was that the concentration of serum and cholesterol increased as a function of the exposure period, and although the levels returned to normal by the 12th week, heart cholesterol concentration remained high. Acetylative capacity of tissues (Coenzyme A concentration) was observed to decrease to 50~~%~~ percent of control values during the first 4-5 weeks.

Lipids have been found in various visual structures, and although their functions in the eye are not well understood, they seem to be involved in the relationship between visual reception and the solubility of visual pigments. Since the eye comes into direct physical contact with the atmosphere, it is probably highly sensitive to the toxic effects of a 100-percent oxygen environment. When rats were subjected to high oxygen-low pressure conditions for ~~four~~ 4 weeks by Jordan et al. ~~, Allred, and Bond~~ (ref. 365) ~~(52)~~ there was a 33-percent decrease in their eye lipid. This change involved all classes of lipids, and though the eyes of the experimental rats weighed less than those of the controls, the authors do not believe that this was a result of the lipid difference ~~(53)~~ (ref. 366).

The metabolic changes which occur in animals exposed to a 5 psia of 100-percent O_2 take the form of a shift in the relative importance of specific metabolic response sequences and pathways (ref. 367). It appears that the pentose cycle assumes greater significance for glucose metabolism in these animals. At the present time, Allred is attempting to quantify this effect. Little change has been observed in the acetate pool size, but some adrenal cortical stimulation has occured in high oxygen atmospheres.

An improved system has been developed (ref. 368) for exposing small animals to a low-pressure--high-oxygen environment with several unique features such as (1) pressure control, (2) an electronic watering device, and (3) a constant environmental temperature control. The system is simple, reliable, relatively inexpensive to operate and environmental conditions are highly reproducible. It is adaptable to a number of gaseous environments.

2. Helium - Oxygen Environment.

Recent evidence indicates that when rats are placed in an atmosphere of 79 percent He - 21 percent O_2, they exhibit a higher level of metabolism and therefore an increase in urine volume and water consumption in response to the helium. Weiss and Wright (ref. 369) believe that this could complicate the weight and space requirements of a helium-oxygen atmosphere. They attempted to clarify the problem by studying rats in a closed environment containing 79 percent He and 21 percent O_2 for a period of 24 days. Water and food consumption, urine volume, and urine osmalarity were

recorded and compared with data from a control group in air. Contrary to the earlier reports, no significant differences in urine volume or water consumption were found, but there was a definite increase in food consumption which they attribute to ~~evelated~~ elevated metabolism resulting from greater heat loss in He-O_2.

Efforts have also been made to determine whether the decreased number and smaller size of chicks obtained from eggs incubated in He-O_2 are the result of a direct biochemical effect of He on embryogenesis or indirectly as a function of helium-related changes in the physics of the incubation environment. Thus far, normal hatches have been produced in only 2 of 10 trials in He-O_2, and it appears that helium begins to depress embryonic metabolism as early as the first week of incubation. Although the factors responsible for the poor hatches have not been identified, it is though that some of helium's physical effects such as higher heat conductivity and evaporative loss may account for these results (ref. ~~390~~ 370).

Wright, et al. (ref. 371) have used chick embryos in a He-O environment to determine the effects on both metabolic rate and x-ray sensitivity. When homogenates from 8-day embryos incubated in a 79 percent He - 21 percent O_2 atmosphere were exposed to 1000 r x-irradiation, there was no change in respiratory activity. However when the irradiation level was increased to 2,000 r, a significant metabolic depression was observed in the He-O_2 and control groups.

Several studies have also been done with human subjects in a He-O_2 atmosphere. In order to separate the effects of surface-heat conductivity factors from the biochemical and pharmacologic influences of He (ref. 363), four environmental conditions were used: (1) breathing air, skin in contact with air; (2) breathing air, skin in contact with 79 percent He- 21 percent O_2; (3) breathing 79 percent He - 21 percent O_2, skin in contact with air; and (4) breathing 79 percent He - 21 percent O_2, skin in contact with 79 percent He - 21 percent O_2. They found that the primary and possibly the sole effect of He is mediated through heat loss from the skin. Under conditions (2) and (4), where the skin was in contact with 79 percent He - 21 percent O_2, lower skin temperatures were recorded regardless of what was breathed. No differences in O_2 uptake ~~was~~ were observed in any of the groups, indicating that the "human subject compensates for lowered skin metabolism by vasoconstriction, rather than increased metabolism as seen in rats and chicks". However, the ambient temperature used throughout the study was 85°F., which may have been inadequate in producing a metabolic reaction since the helium depressed skin temperature by only 0.5°F.

Research is also currently being done on O_2 consumption, heat and respiratory rates, and skin, tail, and rectal temperatures in rats in He-O_2 and air over a wide range of ambient temperatures. Preliminary results indicate that rats in 79 percent He - 21 percent O_2 at 28°C show an increase in oxygen consumption and heart and respiratory rates, and a decrease in skin, tail, and rectal temperatures. At 23°C, the temperatures of the He - O_2 animals continued to decrease, but O_2

consumption and heart and respiratory rates remained at the same level relative to the control group in air. However, when the ambient temperature was increased to 33 C., rats in the He - O_2 atmosphere had lower skin, tail, and rectal temperatures and heart rate, but no changes were observed in O_2 consumption of respiratory rate. Weiss and Wright believe that at this temperature the animals in air may be showing the first signs of heat stress.

Human thermal responses were studied (ref. 372) by Fox et al. during rest and moderate exercise in a helium-oxygen atmosphere at 95°F and 90 percent relative humidity, at 95°F and 40-60 percent relative humidity, and at 71°F and 45-50 percent relative humidity. They report that the only time a He - O_2 environment facilitated heat loss was when skin temperature exceeded ambient temperature. Conduction and convection are thought to be the primary mechanisms accounting for heat loss differences between He - O_2 and air, with evaporation having little or no effect. They also conclude that the helium-oxygen atmosphere is more effective in heat dissipation during exercise than at rest.

3. Transfer of Animals to and from a Helium-Oxygen Environment

When chicks or rats have been maintained in a 79 percent He - 21 percent O_2 atmosphere for a period of time and then moved to air, their oxygen consumption falls below that of control animals which have been kept in an air environment. In addition, when helium-raised animals are placed in 100-percent O_2, their O_2 uptake decreases to the level of the control subjects (ref. 373). It appears that "animals living in an N_2-low environment may be

unusually sensitive to the depressing (narcotizing?) action of N_2 " (ref. 369). The metabolic depression of such animals on their first exposure to air (N_2) may be comparable to the nitrogen narcosis at higher N_2 partial pressures in subjects accustomed to air.

It has been found (ref. 374) that germfree rats and mice tend to die sooner and within a narrower time span than conventional animals on exposure to 100 percent O_2 at 1 atm. The suggestion has been made that chronic respiratory conditions may increase resistance to O_2 toxicity. Lung pathology of germfree animals dying in O_2 appeared much like that of chronic respiratory infection, indicating that inflammatory cell infiltration of the alveolar wall can occur in animals presumed free of disease.

10. BIOSCIENCE COMMUNICATION

One of the most important aspects of scientific research is the field of information science--who is doing what and where. Major aspects of information science embrace collection, analysis, storage, retrieval, and dissemination of the published literature both from the primary and secondary sources. There are many ways of disseminating scientific information: by personal contacts among scientists; by specialized conferences, committee meetings, and scientific meetings; by training courses; and by publication in the open literature. All these forms of communication are supported by the Bioscience Communication Program Branch.

A. Advisory Panels, Conferences, and Supporting Activities

During 1966, advisory panels were established under contract with the American Institute of Biological Sciences (AIBS). These panels have two major purposes: (1) to evaluate the scientific merits of research proposals submitted to them by the bioscience program chiefs, and (2) to act in an advisory capacity upon additional and special matters as requested. Five advisory panels have been established, concerned with the following subjects: behavioral biology, environmental biology, exobiology, spacecraft sterilization, and theoretical biology. Members of the panels were selected from universities and non-profit institutions by the AIBS in consultation with the bioscience program chiefs, and with concurrence of the Director of Bioscience Programs, Office of Space Science and Applications. The panels meet on an average of three times each year. While all final decisions regarding funding of proposals rests soley with NASA, it is already apparent that these experts, drawn from the nation's most capable men →

in the many areas of biology significant to the space program, insure impartial and expert evaluation of each request for the limited funds available to support research and technology in the Bioscience Programs.

The AIBS continues to plan and coordinate ad hoc conferences as required for NASA research contractors and grantees, providing a forum for these individuals wherin current research in space biology can be presented and discussed. During 1966 a new activity was included, namely, continuation of the Regional Council Advisory Program initiated under the Division of Manned Space Sciences, Office of Space Science and Applications.

The crisis in scientific and technical communication results in part from the progressive splitting off of specific areas within single scientific disciplines. This procedure leaves the microbiologist, for example, who is hard pressed to keep abreast of advances in his own field, hopelessly without time to keep sufficiently acquainted with developments in such fields as behavioral biology or environmental biology. To counteract this situation in biology, all aspects of which are implicated in the exploration of space, NASA supports a conference program through the Smithsonian Institution, which has developed a special technique for management of small, informal discussion groups with multi-disciplinary orientation and inclusion of multi-professional participants. The fact that the goals of the conference are kept to specific subjects with wide application, and the fact that attendants include only actual participants has resulted in more efficient communication at each meeting. Additionally, the

conferences allow for extended discussion, a facet of scientific and technical interchange increasingly excluded from the usual present-day scientific meeting, made up by formal presentations of many short papers.

Jointly with other program offices of the Office of Space Science and Applications, Bioscience Programs funds are provided for the annual operating costs of the Space Science Board of the National Academy of Sciences. Specific international congresses are sponsored and/or conducted by the Academy which attack problems inherent to biological aspects of the space program.

B. Education and Training

Postdoctoral and senior postdoctoral resident research associateships in space biology provide to investigators of unusual ability and promise an opportunity for basic research in the various areas of the life sciences which play a significant role in the space program. Investigations are both ground-based and/or in-flight experiments. These research associateships are supported by the Office of Space Science and Applications, NASA, and administered by the National Academy of Sciences - National Research Council. Postdoctoral (regular) and Senior Postdoctoral Resident Research Associateships are open to citizens of the United States and to foreign nationals. Applicants must have training equivalent to that represented by the Ph.D. or Sc.D. degree, or must have completed the requirements for one of the above degrees at the time of entering upon the associateship. The associateships are tenable at those NASA field centers where the laboratories provide the necessary experimental and computing

facilities for the program of the Research Associate.

In the summer of 1965 a Bio-Space Technology Training Program was established, supported by ~~the National Aeronautics and Space Administration~~ NASA under contract with the University of Virginia and conducted for three weeks at NASA's Wallops Station, Wallops Island, Virginia. The program is intended for life scientists from government installations, universities (graduate students and staff), and other research organizations. The general purpose of the program is to acquaint biologists with the engineering and operational aspects of biological research in space. Practical experience is provided the participants by several launchings of an instrumented space probe incorporating biological payloads. The trainees participate directly in preparing the specimen and following the mission from launch through to recovery and examination of the specimen. Contractors and instructors for the training include engineers and scientists from universities, government organizations, and private research laboratories who are involved in biospace research or ancillary programs. Participants are paid travel and $12 a day during the instruction period. Selection of participants is based on interest and competence in the life sciences. Previous training or experience in biospace technology is not required. The first summer (1965) there were 35 participants; in the summer of 1966 42 participants; and for this current summer 50 U.S. citizens and 5 foreign nationals were selected from approximately 350 applications.

C. Publication

Under an agreement, signed in October 1965, by representatives of the Soviet Academy of Sciences and ~~the~~ NASA, work has begun on the

preparation of a joint review of space biology and medicine to be titled "The Foundations of Space Biology and Medicine," US/USSR. The agreement calls for a U. S. - Soviet joint editorial board to accomplish the work, and the NASA contingent of the editorial board held its first meeting at NASA Headquarters on January 29, 1966. Dr. Melvin Calvin, University of California, Berkeley, was appointed ~~as~~ the U.S. Co-Chairman, and the other six members of the U.S. Board are: Professor Loren D. Carlson, University of California, Davis; Dr. Robert W. Krauss, University of Maryland; Professor Robert E. Leighton, California Institute of Technology; Dr. John P. Marbarger, University of Illinois; Professor Wolf Vishniac, University of Rochester; and Dr. Orr E. Reynolds, NASA. Chapter titles and outlines of their contents have been exchanged with the Soviet Academy of Sciences. The plan allows that, in deciding upon the compilers for the chapters, the Board composed of both U.S. and Soviet members will select approximately half from the Soviet and half from the American authors. It is believed that this agreement is a significant step toward a real and valuable collaboration between the two countries in the scientific exploration of space. The schedule, at present, calls for publication of the volumes in 1968. The administrative work of the U.S. Editorial Board is accomplished by the staff of the Bioscience Communications Program.

By agreement, each of the two countries may publish in other forms the material collected for the USA/USSR publication. NASA has an arrangement by which those reviews prepared by the U.S. compilers will be published in a separate monograph series. It is planned to have a series of 19 monographs dealing with the following subjects:

1. (a) Theories of the Origin and Nature of the Universe

(b) Physical Characteristics of Interplanetary Space

2. (a) The Moon and Its Nature

(b) Planets of the Inner Solar System (Mercury, Venus and Mars)

(c) Planets of the Outer Solar System and Their Satellites, Asteroids, Minor Planets, Meteorites (Including Cosmic Dust) and Comets

3. Biological Effects of Extreme Environmental Conditions (Including Laboratory Simulation)

4. Theoretical and Experiemental Prerequisites of Exobiology

5. Search for and Investigation of Extraterrestrial Forms of Life

6. Planetary Quarantine: Principles, Methods, and Problems

7. Effect on the Organism of Radiant Energy from Cosmic Space

8. Effect on the Organism of the Artificial Gaseous Atmosphere of Spacecraft and Stations

9. Principles of Gravitational Biology

10. Effect on the Organism of Dynamic Flight Factors (Chapters 2, 3, 4, 5 and 6)

11. Psychophysiological Problems Connected with Flight and Stays in Spacecraft or Space Stations

12. Combined Effect of Various Flight Factors

13. Methods of Investigation in Space Biology and Medicine

14. Biological Indicators for Space Flight Profiles

15. (a) Selection of Astronauts

(b) Training of Astronauts

16. Methods of Providing Life Support for Astronauts

17. (a) Air Regenerating and Conditioning

(b) Integrated Characteristics of Life - Support System Complex

18.(a) Astronauts' Clothing and Personal Hygiene

(b) Isolation and Removal of Waste Products

(c) Habitability of Spacecraft

(d) Individual Life-Support Systems Outside a Spacecraft Cabin, Space Suits and Capsules

19. Protection of Man Against Adverse Flight Factors

Authors have been selected for all of the monographs, and agreements reached as to specific scientific content. Contracts have been let for most of the nineteen. This series will be a significant contribution to space bioscience.

D. Scientific Communication Research

As the scope of NASA's exploration of space increases, the requirement to keep abreast of new developments, ideas, and areas of promise in biological research appropriate to the NASA program also increases. It becomes vital to make sure of the maximum flow of scientific information to and ~~between~~ among all interested persons and groups. ~~parties~~. Not only the scientists at the bench must be kept informed but ~~the scientists~~ those in administration must have up-to-date scientific information on a continuing basis.

George Washington University's Biological Sciences Communication Project is utilized under contract to conduct studies in scientific communication in all areas of space biology which relate to current NASA programs, and those areas of the subject which indicate future program needs such as sterilization of spacecraft, back contamination, etc.; to compile comprehensive bibliographies and reviews of literature regarding effects of space environment on biological organisms at the cellular and molecular levels; and other specific subjects of current concern in the

biological aspects of space exploration.

A recent effort analyzed all of the work published as a result of NASA support of research. This analysis was conducted for each of the Branches of the Bioscience Program Division, and served as a detailed program review as well as a source of information on all published literature.

In the area of sterilization of spacecraft, an information exchange center is ~~being operated which~~ supplying information to the 23 contractors of the Planetary Quarantine program. This exchange supplies to all contractors not only copies of pertinent report material received from any of the group ~~23~~, but also supplies any new research information available.

The value of this program is in having biomedical scientists working closely with the staff scientists so that the exchange of information is on a daily basis.

II. SIGNIFICANCE OF ACHIEVEMENTS

Biologists have for some time now been aware of the unique opportunities space provides for testing biological materials under new and heretofore unavailable conditions. Fresh insight into physiological and biological mechanisms is coming from experimentation in a milieu of zero gravity and removal from Earth's rotation. Beyond this, the extensive and profound experimentation and development of new materials and techniques necessary for successful penetration of space is yielding a splendid harvest of resources with applicability in many areas vital to man.

NASA's Assistant Administrator for Technology and Utilization has been given the task of making relevant technological developments available — at the earliest possible time — to the general economy. In purely economic terms, these "fringe" benefits will match the costs of the space endeavor. In ultimate humanitarian and spiritual returns, it is safe to say they will outstrip the mere money outlay.

A. Health and Medicine

The surgeon is the recipient of many technological improvements that have originated in space research.

No 77 Cryogenic surgery — for treating Parkinson's disease, brain and pituitary tumors, detached retinas, and cataracts — has

benefited from new probes which have incorporated ultrasmall thermocouples derived from space medicine. Other surgical techniques — with potential for lowering infection rates in operating rooms — have received improvements suggested by the planetary quarantine program. Propellant research has contributed a decontaminating liquid plastic highly effective in sterilizing surgical instruments just before operating.

The cardiac specialist has much to be grateful for from space investigations. A new tool for studying body changes that precede heart attacks is emerging from studies of metabolism under pure oxygen conditions. Advances in theoretical fluid dynamics are promising large usefulness in cardiovascular studies. Dosimeter measurements — carried out in manned space-flight research — of the effects of external irradiation of the heart on cardiac output, venous pressure, and arterial pressure have produced insight into the degree of right-and left-heart failure from this cause. Research on the most efficient types of man-made atmospheres for spacecraft passengers to breathe is expected to lead to improvements in modified atmospheres ~~heres~~ for heart patients and for post-surgery therapy. Knowledge of space-vehicle control systems has contributed to the bio-engineering aspects of an artifical heart, now one of the supreme goals of biomedicine.

The Biosatellite program has made possible many practical applications. One of these — an automated system for continuous urinalysis — is being applied to ~~the~~ monitoring uremic patients. In the course of developing lightweight apparatus for in-flight biological experiments, it was discovered that plastics sometimes are toxic to biologic materials — a finding of importance to surgical prosthetics. Advanced electrometers contrived for use in space flight may monitor — by noting electromagnetic variations in circulation — changes in character and flow of blood.

Cellular studies in space biology have extensive usefulness. An automated system of tissue culture, developed for studying growth of human cells, makes use of time-lapse photography that holds promise for study of growth and the characteristics of isolated normal and abnormal body cells. The investigative potentials of the systems include metabolism of cancer cells and cellular effects of radiation. Other studies of cell formation of new compounds from reserve materials have applicability to bodily adjustments in metabolic disease.

Biological telemetry is providing the medical researcher with devices for monitoring physiological and behavioral responses pertinent to cardiac and psychiatric patients. An allied development ties in with indentification of extraterrestrial organic life — an important aim of the bioscience program — which is employing research on coding biochemical information. A more

immediate benefit of this process lies in its potential help in understanding the human genetic code, with ~~consignent~~ consequent insight into molecular defects and the tragic inherited abnormalities they produce.

Gas chromatography, together with mass spectrometry, is being developed for detection of extraterrestrial life. These techniques are providing rigorious means for identifying disease-related components of blood. Improved ultraviolet and immuno-fluorescent microscopy — also techniques for space-life detection — ~~is a~~ are rapid and reliable ways to identify disease-producing micro-organisms.

B. Food and Agriculture

Space science research is producing new knowledge vital to understanding and control of many natural phenomena.

No ¶ Unraveling the complex genetic codification within organic cells holds promise not only for increasing human happiness and well-being, but also for improving the quality and yield of many food crops. Control of genetic biochemistry may indeed help produce new animal and plant foods, plant textiles, and forest products — all geared to predetermined needs.

The array of benefits is indeed impressive. Advanced methods for studying slow chemical reactions have application in food-spoilage prevention. High-quality color photography — as Gemini V photographs demonstrated — can be used to detect

the state of soil moisture over large areas, and to record the progress of various crops — especially cereal grains — thus giving the alert to drought areas, insect infestation, or fungal attack. Over the long run, satellite photography could monitor growth or shrinkage of glaciers, thus furnishing clues to where floral and faunal transformations may occur.

The gathering of global meteorological data from celestial instrument platforms furnishes information helpful to the oceanographer and marine biologist in plotting ocean temperatures and determining marine breeding habits, which, finally, benefit the commercial fisherman in increasing mankind's food sources.

Research on hydrogen - fixing bacteria — for use in a life-support system — has demonstrated the nutritional adequacy of their protein and lipid content. Methods are being developed for extensive cultivation of the organism, particularly to alleviate food shortages in underdeveloped areas of the world.

C. Industry and Manufacturing

Evaluation of efficiency of air and liquid filters in removing biological particles led to ~~th~~ discoveries of use to the drug and food industries.

Developments in the kinetics of thermal inactivation of microorganisms have been shown to have relevance for thermal degrading of electronic components and should help the engineer to isolate failure modes when these components begin to age.

The space program's rigorous testing procedures for sterilization, qualification, and evaluation have revealed information about component failures that should help manufacturers achieve trouble-free operations of devices for the commercial market.

Tests to determine degree of sterilization possible in components designed for planetary landing capsules have already made available many high-temperature plastics and rubbers useful for surgical gloves and equipment, and for insulating electrical wires in high-temperature environments. Continuing tests are expected to prove out about 150 varieties of heat-resistant plastics in the next four years.

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373. RHOADES,R.A.; WEISS,H.S.; WRIGHT,R.A.; and HIATT,E.P.: NASA Grant NsG-295-62, Prog. Rept., Sept. 1966.

374. WRIGHT,R.A.; HIATT,E.P.; and WEISS,H.S.: Mortality and Histopathology of Germfree Rats and Mice Exposed to 100% Oxygen. Proc. Soc. Exp, Biol. Med., vol.122, pp. 446-448.

Foundations of Space Biology and Medicine
Monograph Outline

For clarity, the BSCP has reorganized and condensed the Russian outline into monographs. For a detailed outline of each part and chapter, please refer to the Outline of Contents of March 22, 1967.

Monograph #1

Theories of the Origin and Nature of the Universe
Compiler: E. M. Burbidge and/or G. R. Burbidge
Suggested length 100 pages*

Physical Characteristics of Interplanetary Space
Compiler: J. A. Van Allen
Suggested length 30 pages*

Monograph #2

The Moon and Its Nature
Compiler: H. C. Urey
Suggested length 30 pages*

Planets of the Inner Solar System (Mercury, Venus and Mars)
Compiler: D. G. Rea (H. Strughold)
Suggested length 30 pages*

Planets of the Outer Solar System and Their Satellites, Asteroids, Minor Planets, Meteorites (Including Cosmic Dust) and Comets
Compiler: C. Sagan, (John A. Ward)
Suggested length 35 pages*

Monograph #3

Biological Effects of Extreme Environmental Conditions (Including Laboratory Simulation)
Compiler: D. W. Jenkins, (S. Siegel) + (C. Zobell)
Suggested length 50 pages*

Monograph #4

Theoretical and Experimental Prerequisites of Exobiology
Compiler: G. C. Pimental
Suggested length 75 pages*

(Monograph #5) rejected

Search for and Investigation of Extraterrestrial Forms of Life
Compiler: ~~N. H. Horowitz~~
Suggested length 100 pages*

Monograph #6

Planetary Quarantine: Principles, Methods, and Problems
Compiler: L. B. Hall
Suggested length 150 pages*

Monograph #7

Biological Effects of Space Radiations
~~Effect on the Organism of Radiant Energy from Cosmic Space~~
Compiler: C. A. Tobias
Suggested length 200-250 pages*

Monograph #8

Effect on the Organism of the Artificial Gaseous Atmosphere of Spacecraft and Stations
Compiler: E. Roth
Suggested length 100 pages*

Monograph #9

Principles of Gravitational Biology
Compiler: A. H. Smith, T. Hoshizaki; I. H. Wagman; R. R. Burton
Suggested length 200 pages*

Monograph #10

Effect on the Organism of Dynamic Flight Factors (Chapters 2, 3, 4, 5 and 6)
Compiler: A. Graybiel
Suggested length 250-300 pages*

Monograph #11

Psychophysiological Problems Connected with Flight and Stays in Spacecraft or Space Stations
Compiler: H. Teuber
Suggested length 150-200 pages*

Monograph #12

Combined Effect of Various Flight Factors
Compiler: F. J. De Serres
Suggested length 100 pages*

Monograph #13

Methods of Investigation in Space Biology and Medicine
Compiler: W. R. Adey
Suggested length 100 pages*

Monograph #14

Biological Indicators for Space Flight Profiles
Compiler: F. P. Dixon
Suggested length 100 pages*

Monograph #15

Selection of Astronauts
Compiler: J. Bollerud
Suggested length 50 pages*
Training of Astronauts
Compiler: D. K. Slayton; W. J. North; C. H. Woodling; H. A. Kuehnel; S. Faber; J. J. Van Beckel; R. Zedeker
Suggested length 100 pages*

Monograph #16

Methods of Providing Life Support for Astronauts
Compiler: N. Pace
Suggested length 100 pages*

Monograph #17

I. Air Regenerating and Conditioning
II. Integrated Characteristics of Life - Support System Complex
Compiler: R. S. Johnston
Suggested length 350 pages*

Monograph #18

I. Astronauts' Clothing and Personal Hygiene
II. Isolation and Removal of Waste Products
III. Habitability of Spacecraft
IV. Individual Life-support Systems Outside a Spacecraft Cabin, Space Suits and Capsules
Compiler: G. Wells
Suggested length 300 pages*

Monograph #19

Protection of Man Against Adverse Flight Factors
Compiler: R. W. Lawton
Suggested length 150 pages*

*Based upon a double-spaced 8½ x 11 typewritten page.

BIOSCIENCE

"CAPSULE"

No. ~~12~~ 13 April, 1967
Biological Sciences Communication Project, 2000 "P" St., N. W., Washington, D. C. 20036

A REPORT ON THE NASA's BEHAVIORAL BIOLOGY PROGRAM

The Behavioral Biology Program of the National Aeronautics and Space Administration's Bioscience Programs Division is engaged in supporting research that has both immediate and long range implications for the exploration and utilization of space. There are two principal reasons for placing emphasis on the study of behavior within the context of space science. First, the extent to which human performance will be possible in space will set the limits of man as an astronaut, or as a scientist in space laboratories. The second reason stems from the position which the behavior of organisms occupies in biological theory. Behavioral biology is conceived within an evolutionary context; its objective is to seek fundamental knowledge concerning the behavior of organisms, their behavioral mechanisms of adaptation to artificial and extraterrestrial environments, and to determine the biological bases of such behavior, thus providing a broader understanding of the function of the total organism and its environmental interactions.

The program conducts both ground-based and in-flight studies designed to investigate the effects of weightlessness and other conditions peculiar to the space environment.

Emphasis is placed on intensive studies in the following four broad "task" areas, which appear to hold promise of the greatest return in terms of basic scientific information and future applicability to space research:

1) Effects of the space environment on behavior; 2) Experimental analysis of behavior; 3) Neurological and biochemical bases of behavior; and 4) Biological information and communication systems. The following project reports are grouped into these four large categories.

Effects of the space environment on behavior

1) Gravity level preference of animals, at the University of Kentucky (principal investigator: Dr. Karl O. Lange). The objectives of this research are twofold: (1) to determine the absolute threshold for linear acceleration (gravity) and to measure changes in sensation of gravity as a function of weightlessness; (2) to assess optimal G-level by allowing biological organisms to select their own gravity environment. Gravity appears to be an aversive stimulus; when allowed to select gravity levels between one G and any higher level, rats and monkeys consistently select the lowest level available.

2) In-flight gravity level preference, at NASA Wallops Station, Virginia (program scientist: Dr. Richard E. Belleville). A sub-orbital flight program to determine the preferred level of gravity of small animals. A spinning sounding rocket is used to generate an artificial gravity field between zero and one G. Animals vary the gravity level by changing the effective centrifuge radius.

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3) Behavioral effects of rotation and acceleration, at the NASA Ames Research Center, Mountain View, California (principal investigator: Dr. Norman Weissman). The purpose of this task is to explore the effects of prolonged high gravity environments on eating behavior, temporal discriminations, and motor responses of infra-human organisms. This includes studies using prolonged rotation with emphasis on determining behavioral thresholds of speed of rotation.

4) Behavioral regulation of gaseous environments, at Johns Hopkins University, Baltimore (principal investigator: Dr. Stephen A. Weinstein). A study designed to determine the ability of animals to regulate, by operant behavior, the composition of the respiratory environment. Its objective is to extend knowledge of the adaptive capacities of animals with respect to the respiratory environment and the chemical and physiological basis of such adaptation.

5) Circadian rhythms; an analysis under terrestrial conditions and in an orbiting biological satellite, at the Princeton University (principal investigator: Dr. Colin S. Pittendrigh). This program of ground based research on the mechanism of entrainment of circadian rhythms in mammals, and preparatory work for space experiments includes: (1) examination of the suitability of several biological systems for in-flight studies, and (2) carrying out (on the selected system) necessary ground based controls on the effects of all known conditions associated with space flight.

6) Circadian rhythms in man under controlled environmental conditions, at the Max-Planck Institute, Germany (principal investigator: Dr. Jurgen Aschoff). An investigation of the effects of noise level, light intensity and other variables on circadian rhythms. Comparisons are made between the rhythmic behavior of single isolated subjects with group isolated subjects.

7) Computer analysis of circadian rhythms, at the University of Minnesota (principal investigator: Dr. Franz Halberg with Dr. Otto Schmitt). A program of ground-based studies on internal and external synchronization or desynchronization of mammalian rhythms. The activity is directed toward the development of electronic computer techniques for the objective mapping of frequency, amplitude and phase of various rhythms with a view toward utilizing these techniques for analysis of rhythms data obtained during space flight.

8) Circadian rhythms in primates as influenced by latitude, longitude, gravity, and confinement, at the University of Kentucky (principal investigator: Dr. Robert E. Smith). An investigation of the effects of geophysical factors on circadian rhythms of primates intended to provide information useful in the interpretation of biosatellite data. The effects of gravitation, geomagnetism, and confinement upon primate circadian rhythms are considered.

9) Dissociation of certain physiological components of endodiurnal cycles, at the NASA Ames Research Center, Mountain View, California (principal investigator: Dr. C. M. Winget). Biochemical and biophysical mechanisms in circadian rhythm desynchronization are studied in rats and pigeons. Rhythm desynchronization is produced by certain light treatments and consequences such as efficiency loss and fatigue are assessed.

Experimental analysis of behavior

1) Behavior of organisms under conditions of space flight, at the University of Maryland (principal investigators: Dr. Joseph V. Brady and Dr. Joseph F. Dardano). The experimental approach places heavy emphasis on the use of operant conditioning techniques, and the study of the effects of procedural and environmental variables on behavior. Sensitive measurements of behavior are employed in assessing the relationship of behavioral indices to neurophysiological structure and function.

2) Effects of schedule and stimulus parameters on monitoring and observing behavior, at the Indiana State Hospital, Evansville (principal investigator: Dr. Fogle C. Clark). This research is designed to contribute to knowledge of monitoring behavior through the development of basic information on the nature and interactions of major independent variables. These include signal frequency, signal duration, and reinforcement schedule, which are known to control the probability of signal detection.

3) The establishment and maintenance of ongoing perceptual behavior, at the Institute for Behavioral Research, Silver Spring (principal investigators: Dr. Israel Goldiamond and Dr. Charles B. Ferster). The objective of this research is to develop and utilize operant conditioning procedures in studying a variety of complex perceptual phenomena. The problems explored include motion, size, form and color discrimination. Spacial and temporal discrimination are also analyzed experimentally.

4) Control and analysis of primate behavior by brain telestimulation and telemetry, at the Yerkes Regional Primate Research Center, Emory University (principal investigator: Dr. Bryan W. Robinson). The objectives of this task are to organize and pursue a long range program investigating the possibilities of controlling and analyzing primate behavior patterns in natural settings by means of telestimulation and telemetry, and to develop the instrumentation required for these investigations.

5) Development of an automated and continuous monitoring task for studying learning and memory in monkeys, at the Department of Neurology, Massachusetts General Hospital, Boston (principal investigator: Dr. Murray Sidman with Dr. Harry A. Mackay). A program of research for the development in monkeys, of an automated procedure for studying sequential learning and memory, utilizing two sensory modalities, singly and in combination. A primary objective is to follow changes in learning ability in an individual animal that is undergoing continually changing conditions of behavioral stress.

6) Research in psychobiology, at the Massachusetts Institute of Technology (principal investigator: Dr. Hans-Lukas Teuber with Dr. Richard Held and Dr. W. J. H. Nauta). This project encompasses broad programs of research covering three areas; neuropsychology, general experimental psychology, and comparative developmental psychology.

Neurological and biochemical bases of behavior

1) Phenomena underlying behavior of complex living systems in space, at the Brain Research Institute, University of California at Los Angeles (principal investigators: Dr. John D. French and Dr. W. Ross Adey). An interdisciplinary program encompassing life science disciplines such as neurophysiology, neurochemistry, cardiovascular physiology and endocrinology. Work is focused on the ability of the central nervous system to function under conditions of space environments.

2) Electrophysiological research of the brain, at Yale University (principal investigator: Dr. Robert Galambos). This research includes investigations of the participation of glial cells in brain activities, a series of immuno-electrophysiological experiments and the development of other techniques useful in studying brain function and behavior.

3) Neurobiological substrates of behavior, at the University of Maryland, Baltimore (principal investigator: Dr. Robert G. Grenell). The objective of this research is to define structural, chemical and physiological mechanisms in cells of the central nervous system which are correlated with or underlie behavior of the total organism. Studies are being made of changes found in the chemical composition of cells in the vestibular and related systems of the brain in animals exposed to increased gravity for varying periods of time.

4) Studies of the development of stress response mechanisms, at the University of Southern California at Los Angeles (principal investigators: Dr. James P. Henry and Dr. John P. Meehan). This research is aimed at assessing the role that early experience plays in the etiology of physiological and behavioral responsiveness to situational stress. Effects of environmental restriction, behavioral and social stress on the cardiovascular system are being assessed.

5) Electrophysiological correlates of perception and performance, at Baylor University (principal investigator: Dr. Peter Kellaway with Dr. Alfred C. Coats). These studies will attempt to determine the relationships between phenomenon of auditory masking as measured by electrophysiological and by psychophysical techniques and will attempt to establish a firmer physiological basis of psychological data. Other studies deal with neuroelectric phenomena such as the "contingent negative variation" and its behavioral correlates.

6) Analysis of brain activity in perception and performance, at the University of California at Los Angeles (principal investigator: Dr. Donald B. Lindsley). This research applies recent developments in computer methods to the study of electrophysiological correlates of perception and behavior, with a view toward further understanding of the role of specific and non-specific sensory systems in perception, and the interaction of autonomous and evoked potentials.

7) Vestibular brain mechanisms, at the NASA Ames Research Center, Mountain View, California (principal investigator: Dr. William R. Mehler). An experimental comparative neuroanatomical analysis of central nervous system pathways affecting the vestibular system and related brain mechanisms which effect neural control of inter-related somatic visceral and behavioral mechanisms.

8) Investigation of the neurological correlates of information reception, at the Indiana University Foundation, Bloomington (principal investigator: Dr. William D. Neff). This investigation is concerned with the nature of the code by which sensory information is transmitted within the nervous system. The goal is to gain information about patterns of neural activity which are equivalent to external sounds and to extend the amount of information that can be injected directly into the nervous system.

9) Electrophysiological and performance measures of visual excitability cycles in man, at the University of Arizona (principal investigator: Dr. Robert W. Lansing). Studies are directed toward determining changes in central nervous system excitability which accompany alterations in body chemistry and psychological states. An attempt is made to understand performance changes produced by physiological disturbances and provide specific neurophysiological predictors of such changes.

10) Psychocardiovascular reactions during conditions of weightlessness in an orbiting satellite, at the Pavlovian Laboratories, Johns Hopkins University, Baltimore (principal investigator: Dr. Jorge Perez-Cruet). A study utilizing both classically and instrumentally conditioned cardiovascular responses in monkeys and dogs to assess the effects of weightlessness on adaptive processes.

11) Neural control of hibernation in mammals, at the California Institute of Technology (principal investigator: Dr. Felix Strumwasser). This project is concerned with the role that neurophysiological mechanisms play in the origination and regulation of hibernation and the interrelationships between hibernation and biological clock problems. Research is concerned with the triggering of the entrance into hibernation and endogenous arousals from the low temperature state.

12) Methods of quantification with reinforcing brain stimulation, at the Fels Research Institute, Yellow Springs, Ohio (principal investigator: Dr. Elliot S. Valenstein).

A study of interrelated problems concerned with the reinforcing brain stimulation phenomenon, including: (1) investigation of convenient techniques for screening animals according to reinforcing consequences of brain stimulation; (2) methods of determining stimulus intensity thresholds, and (3) the feasibility of using reinforcing brain stimulation to maintain behavior for prolonged periods of time.

Biological information and communication systems

1) Neurosciences Research Program, at the Massachusetts Institute of Technology (principal investigator: Dr. Francis O. Schmitt). The purpose of this program is to foster fundamental and applied scientific research into the physical and chemical processes that underlie behavior by encouraging and fostering communication and interchange of ideas and carrying on educational activities intended to inform neuroscientists of developments in related disciplines.

2) Molecular organization and mental function, at the Michigan State University (principal investigator: Dr. Leroy G. Augenstein). This project is to carry out research on the nature of macromolecular organization and function and the molecular basis of nerve and brain function. The project includes studies on changes in biochemical (DNA and RNA) composition and electrical activity associated with learning in lower organisms and correlated studies on human information processing systems.

3) Mechanisms of integration and behavior in planaria, at the Colorado State University (principal investigator: Dr. Jay Boyd Best). Investigations are on the nature of the enhancement of performance transmitted via cannibalism in planaria, the nature of the substance transferred and its behavioral specificity. They also include determination of the cellular migrations and routes of transmission of information in regenerated tissue and determination of the physiological and biochemical mechanisms underlying rhythmic behavior in planaria.

4) Development of techniques for the use of rotifers, nematodes, and tardigrades in behavioral experiments, at the Yale University (principal investigator: Dr. Harold J. Morowitz). The general objective of this research is to determine whether information is stored in chemical structure. Three classes of metazoans have the ability to resist dehydration and freezing. An attempt will be made to condition organisms by classical and instrumental techniques to determine whether acquired information will survive desiccation and cold temperatures.

5) Research in the communication sciences, at the Massachusetts Institute of Technology (principal investigator: Dr. Henry J. Zimmermann). The program includes studies of communication processes in natural and man-made systems and of the interactions between them. The topics of major interest range from communication theory and engineering applications to neuroelectric phenomena in the sensory mechanisms.

Inquiries concerning the Behavioral Biology Program should be addressed to:

Dr. Richard E. Belleville
Chief, Behavioral Biology
Bioscience Programs
Office of Space Science and Applications
National Aeronautics and Space Administration
Washington, D. C. 20546

Editor's Note: This special printing of the BioScience Capsule is being sent to you because of your particular professional interest in the field of behavioral biology. Further information on the newsletter, which appears bi-monthly and carries information on the NASA bioscience program can be obtained by writing:

The Editor
BioScience Capsule
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BIOSCIENCE

"CAPSULE"

No. 14 May 24, 1967

Biological Sciences Communication Project, 2000 "P" St., N. W., Washington, D.C. 20036

PLANETARY QUARANTINE REPORT

The Planetary Quarantine Program of the National Aeronautics and Space Administration's Bioscience Programs Division has broad responsibilities relating to preventing lunar and planetary contamination from unmanned spacecraft designed to fly past, orbit, impact upon, or enter the atmosphere of the Moon or other planets. These responsibilities include defense of the scientific basis for sterilization, the development of techniques for sterilization, the definition of the reliability of these techniques, the conduct of bioassays, the monitoring of all phases of manufacture, assembly and launch, and approval of procedures proposed by the operating programs and certification of sterility to the Associate Administrator for the Office of Space Science and Applications. The Program is also involved in efforts to provide more persons trained in environmental microbiology.

In its efforts to prevent contamination of the Moon and other planets, the Program is guided by the recommendations of its Sterilization Advisory Committee, by various other NASA advisory committtees and by the scientific findings of various contractors. Defined standards are continually being developed and accepted as soon as they are established as feasible by laboratory experiments. An example of the standards for the sterilization of spacecraft is the following list of five requirements:

1. Heat up and cool down time will be integrated into the heat cycle. Total time of heating, compared to a constant temperature, will be reduced approximately 15%.
2. Sterilization cycle will be based on the number of viable heat resistant (spores?) organisms to be killed.
3. A flight acceptance cycle, equal to or in excess of the terminal cycle, will be required for engineering reasons; advantage will be taken of this to sterilize the interiors of subsystems.
4. The assembled capsule will be decontaminated with ETO before terminal sterilization. This will leave the major problem mating surfaces. Such surfaces can be sterlized with heat at D values only slightly above those for open surfaces.
5. Recent research suggests that heat resistance (D values) vary considerably with water activity (W_A).

Participation in international conferences, such as the invitational COSPAR symposium in July on sterilization techniques for instruments and materials, also contributes toward advancing the Program's efforts. (The July symposium, in London, will include topics on sterilization endpoints and protection mechanisms, combined sterilization techniques, supporting techniques (such as gnotobiotics), sampling and verification techniques. A report on the symposium will probably appear as a COSPAR information bulletin.)

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Generally, the needs of this phase of the national space program have stimulated impressive developments in microbiological technology. Many examples could be given, but a sample would include 1) Exploration of the effects of dry heat (105° to 160° C) on microorganisms and the determination of D and Z values; 2) Development of methods to quantitate viable organisms inside certain solids; 3) Determination of the quantities of microorganisms disseminated from humans; 4) Development of new techniques and equipment for reducing biological contamination of bioclean objects. Furthermore, automatic photoelectronic counting and identification of colonies is being developed and is very promising; stochastic methods of analyses predict microbial populations and point the way to systems procedures that simplify sterilization stresses and problems. As a result of this space-related technology, the entire field of applied microbiology has been broaded and has benefited.

Support of research is in three broad areas: 1) Sterilization techniques; 2) Microbiological analysis; and 3) Contamination control. The titles and a brief description of research under way in these three areas follow. (Within each category, reports are ordered alphabetically according to the name of the principal investigator.)

Sterilization techniques

1) Basic studies in environmental microbiology as related to planetary quarantine and training programs in environmental contamination and environmental health, at University of Minnesota (principal investigator: Prof. George S. Michaelsen).

2) Sterilization of spacecraft, at the U.S. Army Biological Laboratories (principal investigator: Dr. Charles R. Phillips). Emphasis is on microbial accumulation on surfaces, microbial die-off on surfaces, and low level dry heat sterilization cycles.

3) Investigation of a sono-chemical approach in sterilization problems, at St. John's University (principal investigator: Dr. Michael A. Pisano). Ultrasound is being combined with gaseous ethylene oxide for synergistic increases of microbial kill.

4) Study of analytical techniques in dry-heat sterilization of spacecraft, at Exotech, Inc. (principal investigator: Samuel Schalkowsky).

5) The resistivity of microorganisms to thermal inactivation by dry heat, at Massachusetts Institute of Technology (principal investigator: Dr. Gerald Silverman). Research is aimed at deriving precise mathematical equations to express the kinetics of dry heat sterilization.

6) Studies on trace elements in the sporulation of bacteria and the germination of bacterial spores, at Syracuse University (principal investigator: Dr. Ralph A. Slepecky). Research seeks to determine the requirements for metal cations in spore dormancy and germination.

Microbiological analysis

1) Biostatistics of space exploration: microbiology and sterilization, at Florida State University (principal investigator: Dr. Richard G. Cornell). Statistical techniques for microbial assay are being studied and developed, and probability models are being formed to determine decontamination levels for spacecraft in order to avoid contamination levels for spacecraft in order to avoid contamination of Mars.

2) An automatic microbiology laboratory, at University of California at Berkeley (principal investigator: Dr. Donald A. Glaser). Purpose of this study is to develop optical scanning system and densitometer for the automated detecting, counting, identifying, and characterizing of large numbers of microorganisms by rapidly and accurately observing their growth on solid media.

3) Life in extraterrestrial environments, at IIT Research Institute (principal investigator: Dr. E. J. Hawrylewicz). The effect of diurnal temperature cycling (-65°C to + 25° C) and of limiting concentration of moisture and oxygen is being studied on spore germination, vegetative cell growth and sporulation of *Bacillus cereus* and *B. subtilis*.

(continued on page 5)

MICELLANY

Implantable Temperature Telemeter

A note from the Franklin Institute Research Laboratories indicates successful development of a new model of their Mark IV implantable temperature telemeter, a model with a greatly increased operational lifetime. The Mark IV-LL has a computed lifetime of 18 months and a minimum operation lifetime of 12 months. Furthermore, by increasing the weight of the new model by about one gram and its volume by about 0.5 cubic centimeter, the computed lifetime of the telemeter becomes greater than 26 months (minimum of 18 months). This model is designated Mark IV-XLL. The Mark IV-LL model is said to be entirely compatible in all ways with the Mark IV-D unit now being used by researchers involved in Biosatellite programs.

R. M. Goodman, manager of the Bio-Instrumentation Laboratory, writes "We will be happy to supply experimental quantities of either of these devices to interested researchers". (Franklin Institute Research Laboratories, The Benjamin Franklin Parkway, Philadelphia, Pa. 19103.)

Spacecraft Sterilization Seminar

A seminar in system analysis for spacecraft sterilization is scheduled for June 5-9 in Washington, D.C. Sponsored by the Office of Space Science and Applications, the seminar is geared to engineers and scientists active in this area. (Additional seminars in other locations are planned.) The major objectives are to 1) Establish, through system analysis methods, a unified approach for the development of spacecraft sterilization technology, and 2) Disseminate system-oriented state-of-the-art information to active participants in this NASA program.

No tuition is charged but participants are responsible for their own travel and maintenance expenses. For information write to: Exotech, Inc., 525 School St., S. W., Washington, D.C. 20024, Attention: Samuel Schalkowsky.

Environmental Biology Course

The NASA-sponsored program of environmental biology courses for engineers has continued throughout 1966 and this Spring and has met with considerable success. The intensive, five-day course, designed for engineers actively engaged in NASA-oriented projects in spacecraft sterilization and planetary quarantine, includes laboratory, lectures, and seminar discussions, physical and chemical disinfection, sterilization, sampling and monitoring, contamination control, epidemiology and quarantine, an introduction to biology, and some taxonomy and nomenclature.

Approximately 100 persons have taken the course, which has been held at the University of Minnesota, the National Institutes of Health Service's Phoenix, Ariz., Communicable Disease Center, and at Cape Kennedy, Fla. No tuition is charged participants who are expected to provide their own maintenance and travel expenses. Thirty five students is the maximum that may register, with room for 10-15 auditors.

Requests for information should be directed to Donald E. Wright, Biological Sciences Communication Project, Suite 611, 2000 "P" St., N.W., Washington, D.C. 20036.

Ecology Proceedings Published

The proceedings of the first international interdiciplinary conference "Human Ecology in Space Flight", edited by Doris Howes Calloway, has been published (New York, N. Y. 10021; $7.00 a copy). Held in October 1963, the conference included discussions (leader's name appears in parentheses) on cabin atmosphere (A. DuBois), regenerative systems (A. H. Brown), gravity and acceleration (H. Bjurstedt), and radiation in space (W. F. Neuman). The 285-page book includes a bibliography with 174 entries and an introduction and summary by conference chairman Wallace O. Fenn of the University of Rochester School of Medicine and Dentistry.

Academy Satellite Study Underway

At the request of NASA, the National

Academy of Sciences will conduct a "Space Applications Summer Study" during the next two summers reviewing present and future plans for peaceful applications of earth satellites. About 60 to 70 scientists and engineers will participate in this summer's phase, which will be held at Woods Hole, Mass. For each potential application, the study group will make evaluations based on answers to three questions: Is it technically feasible? What are the benefits to be derived? Do space techniques have significant advantages over alternative methods of accomplishing the same objectives?

Chairman of the Central Review Committee which oversees the study is Dr. W. Deming Lewis, physicist and president of Lehigh University. Dr. Eugene T. Booth, most recently scientific director and vice president of Laser, Inc., Briarcliff Manor, N. Y., is executive director of the study which will be carried out within the Division of Engineering of the National Research Council.

Surplus for Science

Research scientists should familiarize themselves with the Federal Government's program of making surplus property available for education, public health and civil defense purposes. Property no longer needed by the government is available, through the Federal Property and Administrative Services Act of 1959, to state and local educational and public health agencies and civil defense organizations and to eligible nonprofit health and educational institutions exempt from Federal taxes. Virtually every kind of property imaginable -- including real estate -- ranging from motor vehicles to furniture, office supplies, hand and machine tools and laboratory equipment is available. An indication of the extent of the operation is obtained from the $27,000,000-plus "total acquisition value of property donated" reported for surplus personal property for March.

According to a spokesman from the Office of Surplus Property Utilization, often scientists and science teachers are not aware of the program while the maintenance staff and related personnel at their institutions make good use of the available surplus motors, lumber, electronic equipment, etc.

The program is administered through regional representatives and state agencies. For more detailed information we suggest you write to Director, Office of the Assistant Secretary for Administration, Office of Surplus Property Utilization, Department of Health, Education, and Welfare, Washington, D.C. 20201. The scope of the program is so broad that it is difficult to more than hint at it, even if space permitted! For information on a more local level, you may wish to write or telephone your regional representative. These names and addresses follow: after each name add "Regional Representative, Office of Surplus Property Utilization, Department of Health, Education, and Welfare." Region 1 (Maine, New Hampshire, Vermont, Connecticut, Massachusetts, and Rhode Island), Edward G. Bradley, John Fitzgerald Kennedy Federal Building, Boston, Mass. 02203 (telephone: 223-6837 a.c. 617); Region 2 (Delaware, New York, New Jersey, and Pennsylvania), Stephen L. Simonian, 42 Broadway, New York, N.Y. 10004 (telephone: 264-4031 a.c. 212); Region 3 (District of Columbia, Kentucky, Maryland, North Carolina, Puerto Rico, Virginia, Virgin Islands, and West Virginia), Eugene Link, 220 Seventh Street N.E., Charlottesville, Virginia 22901 (telephone: 296-1326 a.c. 703); Region 4 (Alabama, Florida, Georgia, Mississippi, South Carolina, and Tennessee) W. D. Musser, Room 404, Peachtree-Seventh Building, 50 Seventh Street N.E., Atlanta, Ga. 30323 (telephone: 526-5024 a.c. 404); Region 5 (Illinois, Indiana, Michigan, Ohio, and Wisconsin) R. Dale Wilson, 433 West Van Buren Street, Room 712, Chicago, Ill. 60607 (telephone: 353-5197 a.c. 312); Region 6 (Iowa, Missouri, North Dakota, South Dakota, Kansas, Minnesota, and Nebraska) E. Lyle Knight, 601 East 12th Street, Kansas City, Mo. 64106 (telephone: 374-3691 a.c. 816); Region 7 (Arkansas, Louisiana, New Mexico, Oklahoma, and Texas) Sam G. Wynn, 1114 Commerce Street, Dallas, Texas 75202 (telephone: 749-3385 a.c. 214); Region 8 (Colorado, Idaho, Montana, Utah, and Wyoming) Clayton S. Brown, Room 9017, Federal Office Building, 19th and Stout Streets, Denver, Colorado 80202 (telephone: 297-3719 a.c. 303); Region 9 (Arizona, California, Nevada, Oregon, Washington,

Alaska, and Hawaii) John P. Gifford, 50 Fulton Street, Room 558 (Mail to room 406), San Francisco, Calif. 94102 (telephone: 556-6651 a.c. 415).

(Cont')

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Contamination control

1) Ecology and thermal inactivation of microbes in and on interplanetary space vehicle components, at Robert A. Taft Sanitary Engineering Center (principal investigator: Drs. Keith H. Lewis and Robert Angelotti). This research is directed at improved methods of recovery and assay of microorganisms from the interior of solids; at establishing the hypothesis that the death rate of microbes by dry heat is independent of population density; and at developing curves for the heat kill kinetics for spores within solids at several temperatures below 135°C, determining D, F, and Z values in each case.

2) Services to be conducted by Communicable Disease Center in support of the planetary quarantine requirements of the National Aeronautics and Space Administration, at Communicable Disease Center (HEW/PHS) (principal investigator: Dr. Samuel W. Simmons).

3) Systems analysis and clean room monitoring for the planetary quarantine program, at Sandia Laboratories of the Atomic Energy Commission (principal investigators: H. D. Sivinsky and Dr. C. Trauth). Two immediate areas of effort in this study are a general system analysis to limit biological loading to 10^8 microorganisms on a planetary landing capsule before terminal sterilization and monitoring of several bioclean rooms.

Biological Sciences Communication Project
Suite 700
2000 "P" Street, N.W.
Washington, D.C. 20036

RETURN REQUESTED

O.K.

THE UNIVERSITY OF ROCHESTER
SCHOOL OF MEDICINE AND DENTISTRY
AND
STRONG MEMORIAL HOSPITAL
260 CRITTENDEN BOULEVARD
ROCHESTER, NEW YORK 14620

DEPARTMENT OF PSYCHIATRY
DIVISION OF PSYCHOLOGY

May 24, 1967

The Editor
BioScience Capsule
BSCP, Suite 700
2000 "P" Street, N. W.
Washington, D. C. 20036

Dear Sir:

I would appreciate your sending me future issues of BioScience Capsule.

Sincerely,

Robert Ader /scs

Robert Ader, Ph.D.
Research Associate Professor
of Psycniatry (Psychology)

RA/scs

April 10. O.K
1967

Please place my name on your mailing list.

Dr. Robert L. Campbell
Rehabilitation Institute
Southern Illinois Unviersity
Carbondale, Illinois 62901

Very truly yours,

R. L. Campbell

RLC/jlm

COLORADO STATE UNIVERSITY

FORT COLLINS, COLORADO 80521

DEPARTMENT OF PSYCHOLOGY

May 30, 1967

The Editor
BioScience Capsule
BSCP, Suite 700
2000 "P Street, N. W.
Washington, D. C. 20036

Dear Sir:

Please send me further information on the newsletter which appears bi-monthly and carries information on the NASA bioscience program.

Thank you.

Sincerely,

Charles W. Cole
Assistant Professor

jee

O.K

MEDICAL COLLEGE OF VIRGINIA
DEPARTMENT OF PSYCHIATRY
1200 EAST BROAD STREET
RICHMOND, VIRGINIA

DIVISION OF PSYCHIATRIC RESEARCH

May 18, 1967

The Editor
BioScience Capsule
BSCP, Suite 700
2000 P Street, N. W.
Washington, D. C. 2036

Dear Sir:

Thank you very much for the copy of your Newsletter. I would very much appreciate it if you would keep me on your mailing list.

Sincerely yours,

Karl C. Corley, Jr.

Karl C. Corley, Jr.
Assistant Professor

KCC/crf

0.K

THE FLORIDA STATE UNIVERSITY
TALLAHASSEE 32306

DEPARTMENT OF PSYCHOLOGY

May 25, 1967

The Editor
BioScience Capsule
BSCP, Suite 700
2000 "P" Street, N. W.
Washington, D. C. 20036

Dear Sir:

Thank you for your recent report on NASA's Behavioral Biology Program in your special printing of BioScience Capsule. I found it very informative and would like to request that I be placed on your newsletter mailing list.

Sincerely yours,

F. T. Crawford

F. T. Crawford

FTC:pji

307

O.K

THE UNIVERSITY OF MISSISSIPPI
Department of Psychology
UNIVERSITY, MISSISSIPPI
38677

May 30, 1967

The Editor
BioScience Capsule
BSCP, Suite 700
2000 "P" Street, N.W.
Washington, D. C. 20036

Dear Sir:

Please send information on the Bio-Sciences Capsule, specifically, how I can receive it.

Thank you very much.

Sincerely yours,

W. F. Crowder

W. F. Crowder, Ph.D.
Professor

WFC:kh

O.K.

Columbia University in the City of New York

DEPARTMENT OF PSYCHOLOGY

Schermerhorn Hall
New York, N.Y. 10027

May 19, 1967

The Editor
BioScience Capsule
BSCP, Suite 700
2000 "P Street, N.W.
Washington, D.C. 20036

Dear Sir:

Many thanks for the copy of BioScience Capsule. I would like information on what I can do to receive future issues of this newsletter.

Sincerely,

William W. Cumming

William W. Cumming

WWC:iis

O.K

VETERANS ADMINISTRATION
HOSPITAL
SEVENTH STREET AND INDIAN SCHOOL ROAD
PHOENIX, ARIZONA 85012

May 25, 1967

YOUR FILE REFERENCE:

IN REPLY REFER TO 5262-116

The Editor
BioScience Capsule
BSCP, Suite 700
2000 "P" St., N. W.
Washington, D. C. 20036

Dear Sir:

I would appreciate receiving further data on the BioScience Capsule which contains information on the NASA Bioscience program.

Yours very truly,

Donovan E. Fleming

DONOVAN E. FLEMING, Ph.D.
Chief, Psychology Research Laboratory

Show veteran's full name and VA file number on all correspondence. If VA number is unknown, show service number.

0.12

MERCK INSTITUTE
FOR
THERAPEUTIC RESEARCH
WEST POINT, PENNSYLVANIA 19486

May 25, 1967

The Editor
BioScience Capsule
BSCP, Suite 700
2000 "P" Street, N. W.
Washington, D. C.

Dear Sir:

I read the April number of the "Capsule" with great interest and would like to regularly receive future issues. Is there any way my name can be placed on the mailing list, subscription paid or arrangements made to accomplish this?

Sincerely,

Harley M. Hanson

Harley M. Hanson, Ph.D.
Senior Research Fellow
Physiology

HMH:mmh

WRIGHT STATE CAMPUS

MIAMI UNIVERSITY
THE OHIO STATE UNIVERSITY

COLONEL GLENN HIGHWAY
DAYTON, OHIO 45431
Phone 513-426-6650

May 23, 1967

The Editor
BioScience Capsule
BSCP, Suite 700
2000 "P" Street, N.W.
Washington, D.C. 20036

Dear Sir:

Thank you for the copy of BioScience Capsule which I received this morning. I would appreciate being kept on the mailing list for any future issues.

Very truly yours,

Sherwin J. Klein
Professor and Coordinator for Psychology
[The Ohio State University]

SJK/sl

O. K 5/24

WRIGHT STATE CAMPUS

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THE OHIO STATE UNIVERSITY

COLONEL GLENN HIGHWAY
DAYTON, OHIO 45431
Phone 513-426-6650

May 23, 1967

Dr. Richard E. Belleville
Bioscience Programs
Office of Space Science and Applications
National Aeronautics and Space Administration
Washington, D.C. 20546

Dear Dr. Belleville:

I just got a copy of the Bioscience "Capsule" and am very pleased that someone thought to put me on your mailing list. I would appreciate receiving them on a regular basis.

C/Y TO KULP G-W

About a year ago I sent an informal proposal on electrophysiological correlates of behavior to Dr. Walt Jones. I wonder if that ever got to him and, if so, whether it would be appropriate for NASA support.

C/Y TO W JONES

Cordially,

Sherwin J. Klein

Sherwin J. Klein
Professor and Coordinator for Psychology
[The Ohio State University]

SJK/sl

put on Capsule mailing list until 1968.
LAK

PARSONS RESEARCH CENTER

Studies in the experimental analyses of child behavior

A Cooperative Effort of

PARSONS STATE HOSPITAL
AND TRAINING CENTER
PARSONS, KANSAS 67357
Telephone 316 GA 1-6550

BUREAU OF CHILD RESEARCH
THE UNIVERSITY OF KANSAS
LAWRENCE, KANSAS 66045
Telephone 913 UN 4-3386

Refer Reply To: Parsons, Kansas

O.K

7 June, 1967

The Editor
BioScience Capsule
BSCP, Suite 700
2000 "P Street, N. W.
Washington, D. C. 20036

Gentlemen:

I recently received a special printing of the BioScience Capsule. I would appreciate further information on the newsletter and the possibility of receipt of such on a regular basis.

Very truly yours,

Bill Locke

Bill J. Locke, Ph.D.
Research Associate
BJL/mcb
cc: file

O.K.

BOSTON UNIVERSITY

DEPARTMENT OF PSYCHOLOGY
700 Commonwealth Avenue
BOSTON, MASSACHUSETTS 02215

June 2, 1967

Editor
Bio-Science Capsule
BSCP, Suite 700
2000 P Street, N. W.
Washington, D. C. 20036

Dear Sir:

I gratefully appreciate receiving the special printing of the Bio-Science Capsule which was sent to me a while back. I found the contents of that issue of extreme interest and I would greatly appreciate receiving the publication on a when-issued basis.

Any other information that your office might be able to supply relative to the activities of the Biological Sciences Communication Project would be greatly appreciated.

Very truly yours,

David Mostofsky

David Mostofsky
Associate Professor

DM:mm

O.K

CARNEGIE INSTITUTE OF TECHNOLOGY
SCHENLEY PARK
PITTSBURGH, PENNSYLVANIA 15213

DEPARTMENT OF PSYCHOLOGY

May 25, 1967

TELEPHONE: 621-2600
AREA CODE 412

The Editor
BioScience Capsule
BSCP, Suite 700
2000 "P" Street, N. W.
Washington, D. C. 20036

Dear Sir:

Thank you for the copy of the special printing of BioScience Capsule. I would appreciate receiving this publication on a regular basis. If there is a charge, please bill me and I shall remit the amount by return mail.

Sincerely,

K. E. Moyer

K. E. Moyer, Ph.D.

KEM/sjg

STATE OF CALIFORNIA—HEALTH AND WELFARE AGENCY

Ronald Reagan,
Governor

DEPARTMENT OF MENTAL HYGIENE

THE LANGLEY PORTER NEUROPSYCHIATRIC INSTITUTE

401 PARNASSUS AVENUE
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O.K.

May 24, 1967

The Editor
BioScience Capsule
BSCP, Suite 700
2000 "P" Street, N. W.
Washington, D. C. 20036

Dear Sir:

Please place my name on your list to receive the BioScience Capsule regularly, if that is possible.

Sincerely yours,

George C. Stone

George C. Stone, Ph.D.
Research Psychologist

GCS:mk

Letter sent June 12, 67 (see others)

HARVARD UNIVERSITY

OFFICE OF
PROGRAMMED INSTRUCTION

LARSEN HALL, APPIAN WAY
CAMBRIDGE, MASSACHUSETTS 02138

June 1, 1967

The Editor
BioScience Capsule
BSCP, Suite 700
2000 "P Street, N. W.
Washington, D. C. 20036

Dear Sir:

Thank you for sending us a copy of BioScience Capsule. We are interested in the field of behavioral biology and would therefore like to order a one-year trial subcription to this periodical.

Please bill the above address and send the bi-monthly issues to:

Librarian
Harvard Office of Programmed
Instruction
320 Larsen Hall
Appian Way
Cambridge, Massachusetts 02138

Sincerely,

Pamela Swart

Pamela Swart
Librarian

PS:va

THE UNIVERSITY OF ALABAMA
MEDICAL CENTER
BIRMINGHAM, ALABAMA, 35233

THE MEDICAL COLLEGE OF ALABAMA
DEPARTMENT OF PSYCHIATRY

O.K.

May 22, 1967

The Editor
BioScience Capsule
BSCP, Suite 700
2000 "P" Street, N.W.
Washington, D.C. 20036

Dear Sir,

I would like to receive the BioScience Capsule on a regular basis. Would you please add my name to your mailing list.

Sincerely yours,

Robert P. Travis

Robert P. Travis, Jr., Ph.D.
Chief, Psychophysiologic Section

RPTJr/rh

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file

NATIONAL AERONAUTICS AND SPACE ADMINISTRATION
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HUNTSVILLE, ALABAMA 35812

IN REPLY REFER TO: R-ME-MM

April 24, 1967

ETO BIBLIOGRAPHY

Dr. Lawrence B. Hall
National Aeronautics & Space Administration
Chief, Planetary Quarantine, SB
Bioscience Programs Division
Washington, D. C. 20546

Dear Larry:

I hope you had a successful review of the effort JPL is conducting for you.

Three thing I need from you when it is convenient. Do you have the wherewithal, with such people such as Don Wright or some of your other associates, to compile a state-of-the-art survey in the use of ethylene oxide for decontamination. We are interested in the present level of technology development, precautionary measures in the use of this material on metallic and non-metallic materials and on personnel, and we are interested in establishing a standard whereby all ethylene oxide used in sterilization and/or decontamination could be qualified as to quality.

We are finding our better conference rooms at MSFC in constant use and are having to schedule them two months in advance. When you determine some dates that you might conduct this briefing of Top Management on sterilization, please let me know and we can confirm when facilities and Top Management are available. Unless the people are available, the time spent would be ineffective.

Are you still considering MSFC to work with you as the field center for managing sterilization per our discussion when I was in your office. If so, would like to hear from you on this.

Sincerely,

W. A. Wilson

W. A. Wilson
Chief, Methods Development Branch
Manufacturing Engineering Laboratory

4 copies mailed June 14, 1967.

May 10, 1967

Miss Jean M. Swan
Supervisor, Acquisitions Department
Jet Propulsion Laboratory
4800 Oak Grove Drive,
Pasadena, California 91103

Dear Miss Swan:

In reply to your letter of May 4 concerning our bibliographic file on spacecraft sterilization I would like to propose two alternates:

1) Our office can provide the Jet Propulsion Laboratory with microfilm containing 400 reprints of pertinent articles (this project is continually expanded).

2) We plan to incorporate all of our citations into a bound bibliography covering NASA policy, microbiological techniques and engineering developments which will become available in the last quarter of this year.

The above items will be made available free of charge to Planetary Quarantine contractors and NASA centers. In the past our attempts to reproduce our card file by xerography have proven prohibitively expensive and the file did not lend itself to useful cataloging or retrieval.

Unless you have a request for a specific subject (which we could meet immediately), I would recommend waiting for the bound bibliography which will contain both author and subject indexes.

Sincerely,

RONALD E. WRIGHT
Sr. Scientist

DEW:lmk

JET PROPULSION LABORATORY California Institute of Technology • 4800 Oak Grove Drive, Pasadena, California 91103

May 4, 1967
Request No.: 015

George Washington University
Biological Sciences Communications Project
of the Airlie Center
Suite 700
2000 P. Street, N.W.
Washington, D.C., 20036

Attention: Donald E. Wright, Senior Scientist

Dear Mr. Wright:

This library is interested in obtaining a reproduced copy of your card file on spacecraft sterilization, which we understand contains 440 abstracts and 510 literature citations.

If there is a charge for this reproduction, please let us know what it is before proceeding with the work.

Very truly yours,
JET PROPULSION LIBRARY

Joan M Swan

(Miss) Joan M. Swan
Supervisor, Acquisitions
Department

JMS:bb

Telephone 354-4321

Twx 213-449-2151

April 14, 1967

Mr. Richard Stedman
American Electronics Laboratory
6629 IRON Place
Springfield, Virginia 22150

Dear Mr. Stedman:

In regards to your telephoned inquiry concerning information on the automatic microanalytic apparatus for primate urine specimens on the Biosatellite, the National Aeronautics and Space Administration has informed us that such information should be derived from the principal investigator himself - Dr. Gerald Soffen. You can communicate with him at the address cited below.*

We are sorry we could not be of more help to you on this matter, however, in the future we would be glad to be of any assistance in providing you with information concerning space biology. This service is rendered on a contractual basis with our organization and the Bioscience Programs Division at NASA.

Sincerely,

Leslie A. Kulp, Ph.D.
Senior Research Scientist

LAK:sr

*Dr. Gerald A. Soffen
Space Science Division
Jet Propulsion Laboratory
Pasadena, California

cc:Mrs. V. M. Bolton
Lt. N. M. Voorhies

June 26, 1967

Mr. Donald C. Sandt, Biologist
University of Maryland
Natural Resources Institute
Chesapeake Biological Laboratory
P.O. Box 38
Solomons, Maryland

Dear Mr. Sandt:

Your letter of May 22 to the National Aeronautics and Space Administration requesting information on microbial ecology of closed aquatic systems in relation to water reuse has been referred to me for a reply. This service is provided by a contractual arrangement between the Bioscience Programs Division of NASA and the BSCP of The George Washington University.

Enclosed is a directory of sixteen scientists active in this area of pursuit together with a list of some of their publications. The number to the left of their citations refers merely to our citation accession number and the number to the right, of course, is the date of publication. By writing to these scientists, you will most likely be able to acquire copies of these publications as well as other valuable information which will assist your organization in establishing a research project in this field. We are also enclosing a brochure on NASA's policies and procedures for grants and research projects.

Your interest in this field is greatly appreciated and if we can be of any further assistance to you, please do not hesitate to call on us.

Sincerely,

Leslie A. Kulp, Ph.D.
Senior Research Scientist

LAK:sr

Enclosures (2)

FE/ ①

SB/ Dr. Jenkins

Received by BSCP

June 23, 1967

UNIVERSITY OF MARYLAND
NATURAL RESOURCES INSTITUTE

CHESAPEAKE BIOLOGICAL LABORATORY
BOX 38
SOLOMONS, MARYLAND

May 22, 1967

Research Director
National Aeronautics and Space Administration
300 7th St. N.W.
Washington, D.C.

Dear Sir:

a Cyan + hydro

I have become interested in the microbial ecology of closed aquatic systems in relation to water reuse and would like to develop a graduate program for myself concerning this project. It occurred to me that your organization might be sponsoring such research and that perhaps you would direct me to an investigator in this field.

I would appreciate any attention this letter may receive. Thank you.

Sincerely yours,

Donald G. Sandt

Donald G. Sandt
Biologist

JUN 1 1967

SC 9521-A (5-66)

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BOX 5800
ALBUQUERQUE, N.M. 87115

The George Washington University
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Gentlemen:

The logistics of finance are so overwhelming that we are dispensing with charges for the microfilm magazines and will provide them at no cost to Planetary Quarantine contractors. We will process the first three rolls next week but I am unable to give you a delivery date.

Sincerely,

DONALD E. WRIGHT
Sr. Scientist

Dr. Ralph A. Slepecky
Associate Professor of Microbiology
Syracuse University

Dr. Joseph Stern
Jet Propulsion Laboratory

Mr. Jack Banks
Langley Research Center

Sincerely,

DONALD E. WRIGHT
Sr. Scientist

DEW:lah

March 29, 1967

[illegible]
[illegible] Corp.
Albuquerque, New Mexico 87115

Gentlemen:

In line with our policy of communicating information relative to [illegible] Quarantine program, we request permission to distribute copies of Sandia Report SC-[illegible]-67-114, "A New Approach to the Microbiological Sampling of Surfaces: the Vacuum Probe Sampler."

Individuals who would benefit from information contained in this report are as follows:

Dr. Wm. C. Cooley
[illegible], Inc.

Mr. [illegible]
George C. Marshall Space Flt Ctr

Dr. [illegible] H. Goodwin
[illegible] Director
[illegible] Field Station

Dr. E.J. Nowrylowicz
Asst. Director for Life Sciences Research
[illegible]

Mr. Alexander Irons
JPL

Prof. George S. Michaelson
Director, Division of Environmental Health and Safety
University of Minnesota

Dr. Keith H. Lewis,
Chief, Milk & Food Research
USDHS National Center for Urban and Industrial Health

Mr. George Mallison
CDC - Atlanta

Mr. William W. Paik
Research Engineer, JPL

Dr. G. Briggs Phillips
[illegible] - Houston

Dr. Charles R. Phillips
Chief, Physical Defense Division
U.S. Army Biological Labs.

Dr. Michael A. Pisano
St. John's University

Dr. Samuel W. Simmons
CDC - Atlanta

See reply apr 18th

SANDIA CORPORATION
SANDIA BASE, ALBUQUERQUE, N. M.

APR 1 8 1967

Mr. Donald E. Wright, Senior Scientist
The George Washington University
200 P Street NW, Suite 700
Washington, D. C., 20036

Dear Mr. Wright:

Re: Sandia Report SC-RR-67-114

In reply to your letter of March 29, 1967, the Patent Attorney, AEC/Albuquerque Operations Office, has informed us that there is no objection from a patent standpoint to the distribution of the above report. This report carries a patent caution stamp on the outside cover and on the inside front cover. This patent caution stamp no longer applies and may be removed prior to distribution.

Very truly yours,

G. C. Newlin
Invention Reports Division

GCN:6011:jg

address labels
& mail

Copies forwarded Apr 21, 1967 to SAC &
contractors on mailing list with exception of
those already listed in the Report from Sandia

May 4, 1967

Dr. Gregg Mamikunian
2775 N. Mivalle Mile
Tucson, Arizona 85705

Dear Dr. Mamikunian:

Under separate cover we are sending you all the material we have concerning NASA's Exobiology Program. These include the contractor and grantee progress reports and some publications supported by this office. Also enclosed is a copy of our most recent bibliography in this category.

It has been a pleasure meeting and talking with you and we are looking forward to the first issue of "Space Life Sciences". If we can be of any additional assistance regarding the preparation of the manuscripts for "Significant Achievements", please do not hesitate to call us.

Sincerely,

Leslie A. Kulp, Ph.D.
Senior Research Scientist

LAK:sr

cc:Mrs. Bolton

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C/O U. S. ATOMIC ENERGY COMMISSION
SAVANNAH RIVER OPERATIONS OFFICE
P. O. BOX A
AIKEN, NORTH CAROLINA 29801

TELEPHONE NORTH AUGUSTA, 824-6331
EXT. 2752, 3175

received by [illegible] June 23, 1967

April 27, 1967

Mr. [illegible] Jenkins, Chief
[illegible] Biology Program
National Aeronautics and
Space Administration
Washington, D. C.

Dear Jenkins:

I am an research associate in the Institute of Ecology in the University of Georgia and am doing the research work related to micro-ecosystem.

Recently I had an opportunity to read your paper entitled "Electrolysis-Hydrogenomonas Bacterial Bioregenerative Life Support System" and felt it very interesting.

Could you send the reprints concerning to the field of life support system and hydrogenomonas biology? Additionally, if you teach me address and name of persons who are doing on the hydrogenomonas system, I would appreciate it very much.

Yours sincerely,

Y. Kurihara

Y. Kurihara
Bldg. 772-G, SROO
P. O. Box A
Aiken, S. C. 29801

1. bibliog. on Environ.

2.

June 23, 1967

Mr. Y. Kurihara
Institute of Ecology
Savannah River Operations Office
P. O. Box A
Aiken, South Carolina 29801

Dear Mr. Kurihara:

Your letter of April 27 to Dr. Dale Jenkins, Assistant Director of the Bioscience Programs Division of the National Aeronautics and Space Administration requesting information on life support systems and hydrogenomonas biology together with a directory of scientists involved in these fields has been referred to me for reply. This service is rendered on a contractual basis between the Bioscience Programs Division of NASA and the BSCP of The George Washington University.

Enclosed is a directory of nineteen scientists working on various aspects of Hydrogenomonas biology as it relates to life support systems in space. The title of their research project is also included. In addition, we are sending you a bibliographic record of all the publications supported by the Environmental Biology Program at NASA which includes a number of citations as well as scientists and laboratories active in this pursuit.

Your interest in this subject is appreciated and if we can be of further assistance, please do not hesitate to call on us.

Sincerely,

Leslie A. Kulp, Ph.D.
Senior Research Scientist

LAK:sr

Enclosures listed on
separate page

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NUTRITIONAL PROPERTIES OF HYDROGENOMONAS EUTROPHA

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GENETIC STUDIES OF HYDROGEN BACTERIA AND THEIR APPLICATION TO BIOLOGICAL LIFE SUPPORT SYSTEMS

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RESEARCH ON TECHNIQUES AND PROCEDURES FOR CULTIVATION OF HYDROGEN-FIXING BACTERIA

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APPLICATION OF HYDROGEN BACTERIA TO BIOREGENERATIVE LIFE SUPPORT SYSTEMS

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USE OF IMMOBILIZED ENZYMES IN CLOSED ECOLOGICAL SYSTEMS

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STUDIES ON THE BIOLOGY OF HALOPHILIC BACTERIA

HOMANN, DR. H.R.
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MOORHEAD, MINNESOTA 56560

PENTOSE AND PENTITOL METABOLISM IN HYDROGENOMONAS SPECIES

JORDAN, DR. JOHN PATRICK
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INTERDISCIPLINARY STUDIES OF THE EFFECTS OF SPACE ENVIRONMENTS ON BIOLOGIC SYSTEMS

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CARBON DIOXIDE METABOLISM OF HYDROGENOMONAS

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INSTITUTE OF ECOLOGY
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ATHENS, GEORGIA 30601

RELATIONSHIPS BETWEEN SIZE, DIVERSITY, AND STABILITY OF SEMI-ENCLOSED ECOSYSTEMS

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PHYSIOLOGY AND METABOLISM OF HYDROGENOMONAS EUTROPHA

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EL MONTE, CALIFORNIA

STUDIES OF PHOTOSYNTHETIC HALOPHILES FROM OWENS LAKE

TISCHER, DR. ROBERT G.
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DEPARTMENT OF MICROBIOLOGY
STATE COLLEGE, MISSISSIPPI 39762

INFLUENCE OF METABOLIC ACCUMULATION OF PRODUCTS OF HYDROGENOMONAS CELLS AND THEIR CONTINUED GROWTH

TSUCHIYA, DR. H.M.
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INSTITUTE OF TECHNOLOGY
DEPARTMENT OF CHEMICAL ENGINEERING
MINNEAPOLIS, MINNESOTA 55455

DEVELOPMENTAL PROGRAM ON CONTINUOUS PROPAGATION OF HYDROGEN-FIXING ORGANISM

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WIXOM, DR. R.L.
UNIVERSITY OF MISSOURI
COLUMBIA, MISSOURI 65201

NITROGEN METABOLISM IN AUTOTROPHIC BACTERIA

ENCLOSURES

1. "Significant Achievements in Space Bioscience 1958-1964"

2. "Electrolysis-Hydrogenomonas Bacterial Microgenerative Life Support System"

3. "A Continuous Culture Apparatus for the Microbial Utilization of Hydrogen Produced by Electrolysis of Water in Closed-Cycle Space Systems"

4. "The Effects of Controlled Environment on the Growth of Hydrogenomonas Bacteria in Continuous Cultures"

5. "Photosynthetic Halophiles from Owens Lake"

6. "Scientific Publications of the Bioscience Programs Division - National Aeronautics and Space Administration, Volume II. Environmental Biology"

7. Directory of Nineteen Scientists Working on This Particular Subject

14 Samoset Road
Cranford, N. J. 07016
February 5th, 1967

Dr. Orr E. Reynolds, Director
Bioscience Programs
Route #1, Box 58, Green Valley Rd.
Clarksburg, Md.

Dear Dr. Reynolds,

I am a senior at Cranford High School and very much interested in the space program. Last year at this time I requested information to help prepare a term paper. I am doing another similar project and will need material published during the past year. I would be deeply gratified for your help.

Sincerely,

Lance Koved

Lance Koved

April 10, 1967

Mr. James Koved
14 Samoset Road
Cranford, New Jersey 07016

Dear Mr. Koved:

Your letter of February 5 to the National Aeronautics and Space Administration was referred to me for a reply. We regret to inform you that the correspondence held with you last year has apparently been misplaced and we are not aware of the type of information you would like to have. If you would write directly to me stating precisely the information you need for your term paper, we would be happy to be of service to you.

Sincerely,

Leslie A. Kulp, Ph.D.
Senior Research Scientist

LAK:sr

received by PSCW
June 23, 1967

3731 Harrison St. N.W.
Washington, D.C.
May 10, 1967

(3)

Office of Public Information
N.A.S.A. Headquarters
Washington, D.C., 20546

Dear Sir -

I am a Senior at Woodrow Wilson High School and I am currently working on a chemistry project entitled "Photosynthesis in Outer Space Travel".

Any information you can give me in this regard will be much appreciated.

Sincerely,

Nina L. Kilian

June 26, 1967

Miss Nina L. Kilian
3751 Harrison Street, N. W.
Washington, D. C. 20015

Dear Miss Kilian:

Your letter of May 10 to the National Aeronautics and Space Administration requesting information on photosynthesis in outer space travel has been referred to me for a reply. This service is provided by a contractural arrangement between the Bioscience Programs Division of NASA and BSCP of The George Washington University.

I am enclosing a bibliography entitled "Scientific Publications of the Bioscience Programs Division, Vol. II. Environmental Biology" which includes a number of citations which should be of some help to you. Additional references are also enclosed.

We appreciate your interest in this field and if we can be of any further assistance to you please do not hesitate to call on us.

Sincerely,

Leslie A. Kulp, Ph.D.
Senior Research Scientist

(SR)

Enclosures listed
on separate page

ENCLOSURES

1. "Significant Achievements in Space Bioscience 1958-1964"
2. "Life Support Systems and Outer Space"
3. "Closed Ecological Systems"
4. "Life Support Systems - Photosynthesis"
5. "An Attempt at Quantitation of the Sharp Light-Induced Electron Paramagnetic Resonance Signal in Photosynthetic Materials"
6. "Reducing Power Generated in the Second Photoact of Photosynthesis"
7. "Scientific Publications of the Bioscience Programs Division - National Aeronautics and Space Administration, Vol. II. Environmental Biology"

June 14, 1967

Donald Howard
Code RRM
NASA Headquarters
Washington, D.C. 20546

Dear Sir:

Enclosed are reprints from our film file which should be of value in the area of environmental control.

Two of the most important documents, which are too large to reproduce, can be obtained from the NASA Scientific and Technical Information Division. They are:

1) Determination of indigenous microflora of men in controlled environments by Phyllis Riely, et al. Republic Aviation/Wright Patterson AFB, AMRL-TR-65-33.

2) A report of the physiological, psychological and bacteriological aspects of 20 days in full pressure suits by Kenneth R. Coburn. Parts I, II, III. NASA CR-708 Feb. 1967.

Sincerely,

DONALD E. WRIGHT
Sr. Scientist

DEW:lmh
Enclosure

Factors affecting the rapid disappearance of bacteria placed on the normal skin by Rebell, G. J. Invest. Derm. 14: 247-264, 1950

Liberation of organisms from contaminated textiles by Rabbo, S.M. J. of Hygiene 61: 507-513, 1964

Infection from bacteria: the environment by Shooter, R.A., McGraw-Hill, New York, p. 277-289, 1965. In: Symposium on skin bacteria.

Preliminary study on the microbial flora of normal human subjects in a restricted environment by Rack, J.V. USAF, AMRL-TDR 66-59 1965.

2nd Int'l. Conf. on Aerobiology by Wolfe, E.K., Jr. Bact. Rev. 30(3): 1-698. Sept. 1966.

Skin carriage of bacteria in the human by Ulrich, J.A. Spacecraft Sterilization Technology, NASA SP-108, p. 87-95, 1966.

Microbiological constraints on long-term manned space mission by Thomas, F.W. and Bergson, M.H. ASM Ann. Mtg., Los Angeles 1966.

Quantitative aspects of shedding of microorganisms by humans. By Riemensnider, R.K. Spacecraft Sterilization Technology, NASA SP-108, p. 97-103, 1966.

Microbial interaction between man and their environment in simulated space chambers by Gall, L.S. ASM Annual Mtg., Los Angeles, 1966.

Microbial bionomics in space systems by Prince, A.E. ASM Annual Mtg., Los Angeles, 1966.

Contaminants from Manned Spacecraft Simulations by Mason, E.E. and Wilson, C.H. NASA Langley. Selected papers on environ. and attitude control of manned spacecraft. Dec. 1966.

An integrated program approach to the control of space cabin atmospheres by Cotton, J.E., et al. Atmospherein space cabin and closed environments. Appleton-Century-Crofts. New York 1966.

6/8/67 6/12

Saybrook Rd.
Middletown, Ct.
06457

Gentlemen:

I am presently a senior at Central Connecticut State College, majoring in biology and will be graduating next fall. I have become interested in the fascinating field of space biology and would appreciate very much any information pertaining to post graduate program in this area of study. Thank you,

Ronald Gordon.

June 15, 1967

Mr. Ronald Gordon
Saybrook Road
Middletown, Connecticut

Dear Mr. Gordon:

Your letter of June 8 to NASA requesting information on post graduate training in the field of space biology has been referred to me for a reply. This service is provided by a contractual arrangement between the Bioscience Program Division of NASA and the BSCP of The George Washington University.

Thank you for your interest in this field of pursuit although it should be pointed out that the fundamental scientific aspects of space biology are essentially those of biology in general but with added emphasis on space flight factors and exobiology. NASA does not provide a list of institutions offering degrees by subject categories but has announced a total of 152 colleges and universities participating and their graduate training program. By writing to the institution of your interest, you can acquire from them information on this matter. This list is being enclosed for your use.

As you stated in your letter, the field of space biology is a fascinating and rewarding one and I wish you success in this pursuit. If we can be of any further assistance to you, please do not hesitate to call on us.

Sincerely,

Leslie A. Kulp, Ph.D.
Senior Research Scientist

cc:Mrs. V. M. Bolton
Mr. Michael Voorhies

Enclosure

7/20/67

630 Cabell Avenue
Charlottesville,
Virginia
March 28, 1967

Dr Leslie A. Kulp
Suite 700
2000 P Street
Washington, D.C.

Dear Dr. Kulp:

Thank you very much for your letter of March 13, and the citations enclosed. They were a great help. I found by using the medical problems, I was able to imagine some of the things nurses might contribute to the space medical effort. Perhaps soon the nurse will have a role in the space effort.

Thanking you again -

Sincerely,

Susan Donckers

Barbara J. Biwer
92 University Circle
Ormond Beach
Florida 32074

4/12/67

March 23, 1967

Dear Dr. Jenkins,

I am in the ninth grade and take biology. Each student is doing a yearly project on some branch of biology. My project is on the effects of gravity on plants.

I am experimenting with plants by rotating them with the stems horizontal. This will give me data on how the plants grow compared to my control plants. I have been looking in the libraries for reports that I can study but have been unable to find any.

I read that NASA has done some research on this subject and that there are some reports that I might be able to obtain. Would you

April 14, 1967

Miss Barbara J. Biwer
92 University Circle
Ormond Beach, Florida 32074

Dear Miss Biwer:

Your letter of March 23, to the National Aeronautics and Space Administration, requesting information on the effects of gravity on plants has been referred to me for a reply. This service is provided by a contractural arrangement between the Bioscience Programs Division of NASA and Biological Sciences Communication Project of The George Washington University.

I am enclosing a bibliography on geotropisms and a number of references, which I believe, will be of help to you in your science project. Additional references will be sent under separate cover. If we can be of additional service to you, please do not hesitate to call upon us.

Sincerely,

Leslie A. Kulp, Ph.D.
Senior Research Scientist

(SR)

Enclosures

cc:Mrs. V. M. Bolton

April 25, 1967

Miss Barbara J. Biwer
92 University Circle
Ormond Beach, Florida 32074

Dear Miss Biwer:

Enclosed are additional references which should be of some help to you in your science project.

If we can be of further assistance, please do not hesitate to call upon us.

Sincerely,

Leslie A. Kulp, Ph.D.
Senior Research Scientist

(SR)

Enclosures

cc:Mrs. Virginia M. Bolton

April 20, 1967

Dr. D. E. Deischer
U.S. Naval School of Aviation
Medicine Center
Pensacola, Florida 32512

Dear Dr. Deischer:

Your request to Lt. N. Michael Voorhies of the National Aeronautics and Space Administration concerning information on biomagnetism was referred to me for a reply. This service is rendered on a contractual basis with our organization and the Bioscience Programs Division of NASA.

Enclosed are a number of references; others will be sent under separate cover. If we can be of additional service to you, please do not hesitate to call on us.

Sincerely,

Leslie A. Kulp, Ph.D.
Senior Research Scientist

(SR)

Enclosures

cc: Lt. N. Michael Voorhies

Mrs. Virginia M. Bolton

May 5, 1967

Dr. D. E. Beischer
U.S. Naval School of Aviation
Medicine Center
Pensacola, Florida 32512

Dear Dr. Beischer:

Enclosed are some additional references on biomagnetism. If we can be of further assistance to you, please do not hesitate to call upon us.

Sincerely,

Leslie A. Kulp, Ph.D.
Senior Research Scientist

(SR)

Enclosures

cc:Lt. N. Michael Voorhies

III PERSONNEL

The following individuals have spent a considerable amount of time on the forestated activities under this contract during this quarterly period.

A. BSCP Regular Staff Members

1. Dr. C. W. Shilling, M.D., Director
2. Mr. Irvin Mohler, M.S., Assistant to the Director (new)
3. Dr. L. A. Kulp, Ph.D., Senior Research Scientist - Bioscience Communications
4. Mr. D. E. Wright, M.P.H., Senior Research Scientist - Planetary Quarantine
5. Miss Frances Hong, B.S., Research Assistant
6. Miss Ann Serrell, B.A., Research Assistant (resigned)
7. Mr. Bruce Berman, M.A., Science and Technical Writer (new)
8. Mr. Morton Werber, M.S., Science and Technical Writer
9. Miss Benita Tall, B.A., Science Writer
10. Miss Helen Selvig, A.B., Science and Technology Librarian
11. Miss Sheila Rollins, Assistant
12. Mrs. Michele Griggs, Secretary
13. Miss Patricia Fox, Secretary (NASA Liaison)
14. Miss Jacqueline Gainesford, Secretary (new)
15. Mrs. Rebecca Vaz, Secretary (resigned)
16. Miss Betti Markowski, B.S., Secretary (resigned)
17. Miss Marlene Mostowy, Secretary
18. Mrs. Mary Hourican, Secretary (new)
19. Miss Lydia Homann, Secretary

20. Mrs. Marilyn Whitehead, Administrative Associate

21. Mrs. Wilma Gardner, Administrative Assistant (resigned)

22. Mrs. Barbara Caldwell, Secretary (new)

B. BSCP Part-Time Staff

23. Mr. Robert Griggs, Clerk

24. Mr. Bert Kenyon, Clerk

25. Miss Ann Walker, B.S., Clerk

C. Non BSCP Staff Support (consultants, special services, etc.)

26. Dr. Mary Danielli, State University of New York at Buffalo

27. Dr. G. Levin, President, Biospherics, Inc.

28. Dr. G. Mammikunian, Biometrics Research Institute

29. Dr. Norman Scotch, Associate Professor in Social Anthropology, The Johns Hopkins University

30. Dr. J. Stammler, Executive Director, Chicago Health Research Foundation

31. Dr. G. W. Wythe, Professor of Economics, Emeritus, The George Washington University