SIGNIFICANT ACHIEVEMENTS DURING SIX YEARS OF

SPACE BIOSCIENCE RESEARCH AND APPLICATIONS

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ABSTRACT

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The research progress in life sciences in NASA is summarized for the period 1958 to 1964, and especially the Bioscience Programs since 1962. The major goals of this Program are: (1) the determination of the origin, nature, and level of development of extraterrestrial life; (2) the determination of the effects of the space environment on Earth organisms including man; and (3) support of long duration manned space flight.

A broad research and flight mission program in space biology has been developed and supported by NASA. This includes (a) the development of life detection experiments and a concept for an automated biological laboratory for planetary exploration for life; (b) the development of a Biosatellite Program including scientific experiments to study the biological effects of weightlessness, radiation combined with weightlessness, and removal from the Earth's rotation; (c) the development of a recoverable Biosatellite spacecraft system; and (d) all of the supporting research and technology required for these flight missions as well as biological support for manned space flight.

Some biological flight programs have been carried out by NASA in flying various primates to test systems and to demonstrate the safety before flight of astronauts. Both monkeys and chimpanzees were flown in sub-orbital and orbital flights. Biosatellites will be placed in orbit during the 1966 and 1967 time period.

In the Exobiology field, progress has been made in biosynthesis of organic life-related materials and lifelike objects and the possible origin of life, study of organic constituents of meteorites, development of life detection instruments, and a new concept of integrated life detection for unmanned spacecraft for Martian exploration. New methods and procedures have been developed for spacecraft sterilization.

Various Earth organisms have been subjected to simulated planetary environmental conditions and the growth or survival established. A detailed program on the exact definition of the physical and chemical environmental extremes for growth and reproduction of Earth organisms have helped establish extreme environmental parameters of life which can indicate the potential existence of life on other planets. author

A new bioregenerative life support system has been developed which appears to be a technological breakthrough. Water is split into hydrogen and oxygen by electrolysis, and Hydrogenomonas bacteria use the hydrogen and waste products from the astronaut. A broad research and development program of NASA indicates a very efficient system as compared with other regenerative systems.

The effects of bed rest, simulating weightlessness, have been studied and calcium loss using X-ray bone densitometry and cardiovascular studies have been completed. These techniques are being used with astronauts and orbiting primates. Studies are being supported on reduced metabolism and hibernation related to prolonged manned missions. Promising results have been obtained using a chemically defined liquid diet with human volunteers.

PREFACE

This is a summary of certain aspects of the space biology program of NASA and brings together some of the results of research in which NASA is interested and has sponsored under grants and contracts during the period 1958 through 1964.

The space biology program has had a slow start in comparison with the space physics program and there was only a token program before 1962. Much of the present research involves preparation of space flight experiments and obtaining adequate baseline information. Perhaps half of the research results reported are derived from the NASA program. Additional information is included from many other sources, especially the United States Air Force with its long history of aviation and aerospace medicine.

Relatively few biological space flight experiments have been undertaken. These have been to test life support systems and to demonstrate, prior to manned space flight, the capability of an animal to survive. Few critical scientific biological experiments have been placed in orbit by NASA, but a Biosatellite program will soon make a detailed study of the fundamental biological effects due to weightlessness, biorhythms, and radiation.

The search for extraterrestrial life has been limited to ground-based research and planning for planetary and lunar landings. Life detection experiments have been developed and tested; and an important and exciting program is in the making to detect and study extraterrestrial life, if it exists.

Interest in space biology has been slow in developing, and there has been some caution and controversy in the scientific community. However, a strong wave of interest is starting to push forward the frontier of this new and important scientific field and the future appears to be optimistic.

This report has been written by the members of the Bioscience Programs Division of the Office of Space Science and Applications; the Life Sciences Division of the Ames Research Center; and the Biological Sciences Communication Project, George Washington University.

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SIGNIFICANT ACHIEVEMENTS DURING SIX YEARS OF SPACE BIOSCIENCE RESEARCH AND APPLICATIONS

INTRODUCTION

NASA's biological program had a slow start. A small Life Sciences group, organized in 1958, was concerned with life support and use of primates for system and vehicle testing for the Mercury Program. Three small sub-orbital flights of biological materials were flown in space.

The Bioscience Program Office of the Office of Space Science and Applications was organized in 1962, with the overall objective of determining the presence, origin, nature, and level of development of extraterrestrial life, and determining the effects on Earth organisms of space and planetary environments, in order to provide support for long duration manned space flight. The Biosatellite Program, started in 1963, has the first of six flights scheduled for 1966. Considerable effort has been and is continuing to be expended on research and development of life detection experiments and instruments for future flights to Mars for searching for extraterrestrial life. A modest program of biological research has been supported primarily for development of flight mission environmental, behavioral, and exobiological experiments, and support of longer-term manned flights.

Space exploration has demanded a rigorous conceptual and methodological development, especially in the biosciences area. Investigation of the solar system for exotic life forms, the environmental extremes to which Earth organisms (including man) are being exposed, the possibilities for modification of planetary environments by biological techniques yet to be developed, and the problems of communication in biosystems through new dimensions of space and time are areas which have required refinement of the theoretical framework of biology before progress could be made rapidly enough to keep pace with technological advances in transportation.

Of all the sciences, biology alone has not yet benefited from comparisons with the universe beyond Earth. It is reasonable to suppose that breakthroughs might be made in biology on the basis of comparisons with life from other worlds. Organisms elsewhere may have found alternative solutions to processes we think of as basic scientific principles.

In contrast, physical science has advanced sufficiently to provide a great body of unalterable laws which may be expressed in mathematical terms, and by which phenomena may be predicted with complete accuracy.

A well-known characteristic of biological phenomena is variability. The Darwinian concept of evolution is perhaps the only pervading generalization in biology. This concept has been supported by evidence of a hereditary mechanism in the discovery of genes and gene mutations.

Space bioscience represents the convergence of many disciplines with a single orientation, an orientation whose direction is determined by the problems which have made possible manned space travel and which have, in turn, created a host of bioengineering problems concerned with supporting man in space.

Of far greater interest to the scientist, however, are those questions which man has pondered long before the advent of rocketry, but which the tools of rocketry may enable him to answer.

Foremost among these questions is the possibility of the existence of extraterrestrial life. The field which is concerned with the search for extraterrestrial life has come to be called "exobiology." In addition to the challenge of great technological problems which must be solved, exobiology is so closely related to the central scientific questions in biological science that it is considered by some to be the most significant pursuit in all of science.

One of the major opportunities already presented by the advances in propulsion systems is the ability to escape from the influence of the Earth, which has made possible the study of organism-environment relationships, particularly the role that environmental stimuli play in the establishment and maintenance of normal organization in living systems.

Transcending even these formidable objectives of space bioscience is an objective shared by all life sciences, the discovery of nature's scheme for coding the messages contained in biological molecules. Extraterrestrial biology seeks to find not only evidence of life now present, but the vestigial chemicals of its previous existence. The ways and means have already been made available to study molecules on whose long, recorded messages is written the autobiography of evolution--the history of living organisms extending back to the beginnings of life. On this same basis, it is now within the realm of science to foresee the means of predicting the development of life from primordial, nonliving chemical systems. Closely allied to the search for extraterrestrial life is research which seeks to identify the materials and the conditions which are the prerequisites of life.

Space bioscience research is now extending human knowledge of fundamental biological phenomena, both in space and on Earth, just as the physical sciences explore other aspects of the universe. The accomplishment of bioscience objectives is totally dependent upon advances in the technology of space flight. A highly developed launch vehicle capability is essential to accomplish the long duration missions required in the search for extraterrestrial life.

Life on other planets in the solar system (with emphasis on Mars) will be investigated by full exploitation of space technology which will allow both remote (orbiter) and direct (lander) observations of the planetary atmosphere, surface, and sub-surface. Certain characteristics of terrestrial life, such as growth and reproduction, provide a basis for relatively simple experiments which may be used on early missions to detect the existence of such activity on Mars. Later missions will provide extensive automatic laboratory capabilities for analyzing many large samples taken from various depths and locations. Because of the hypothetical nature of current experiment design concepts, it is likely that visual observations of the planet will be required--inspite of the technological problems involved in storing and transmitting the large amounts of data over planetary distances. Such visual observations might very well be crucial in interpreting results from other experiments. critical to all exploration of the moon and planets are the requirements to: (1) prevent contamination of the Martian environment with Earth organisms and preserve the existing conditions of the planet for biological exploration, (2) minimize contamination of the Moon, and (3) provide strict quarantine procedures for all objects returned to Earth from the Moon and planets.

The biological exploration of Mars is a scientific undertaking of the greatest significance. Its realization will be a major milestone in the history of human achievement. The characterization of life, if present, and the development of understanding concerning the evolutionary processes involved and their relationship to the evolution of terrestrial life would have a great scientific and philosophical impact on the world. What is at stake is nothing less than knowledge of our place in nature.

Extended Earth orbital flights with sub-human specimens will be used to determine the effects on Earth organisms of prolonged weightlessness, radiation, and removal from the influence of the Earth's rotation. Such flights on the Biosatellite and other suitable spacecraft are expected to: (1) establish biological specifications for extending the duration of manned space flight; (2) provide a flexible means of testing unforeseen contingencies, thus providing an effective biological back-up for manned missions; (3) yield experimental data more rapidly by virtue of the greater number and expendability of subjects; (4) through use of animal subjects with more rapid development and aging, anticipate possible delayed effects appearing in later life or in subsequent generations; (5) develop and test new physiological instrumentation techniques, surgical preparations, prophylactic techniques, and therapeutic procedures which are not possible on human subjects; and (6) provide a broad background of experience and data which will permit more accurate interpretations of observed effects of space flight on living organisms, including man.

II. EXOBIOLOGY

The discovery of an independent origin of a "life form" on a planetary surface other than Earth presents an unequalled challenge in man's history of scientific search. Therefore, the discovery or detection of an alien life within the solar system is a major objective of space research in the foreseeable future.

The scientific data presently available concerning the possible existence of a "life form" and the nature of the chemical constitution of the surface of Mars are disappointingly few. In fact, it is impossible to make a definite statement about any of the many surface features, other than the polar caps, with any degree of certainty. Many hypotheses have been advanced to account for the observational results. It seems imperative that new evidence soon be forthcoming that will decide among the present conflicting views.

The observational arguments (Rea, 1963) for the existence of Martian life are based on:

1. The various colors, including green, exhibited by the dark areas.
2. The seasonal changes in the visual albedo and polarization of the dark areas.
3. The ability of the dark areas to regenerate after an extensive "dust storm" and
4. The presence of 2,700 to 3,000 cm^{-1} "absorption" bands, attributed to organic molecules.

Conflicting interpretations have been advanced for the above observations. The argument on the basis of the colors is inconclusive. It has been suggested by several workers that the color is a contrast effect with the bright reddish continents. The meagre quantitative data have been discussed by Öpik (1958), who has reduced Kozyrev's photometric observations of the very dark area of Syrtis Major to intrinsic reflectivities by factoring the estimated atmospheric attenuation and reflectivity. Kuiper (1957) similarly demonstrated the absence of the near-infrared reflection maximum, which is characteristic of most green plants, indicating that chlorophyll was not responsible for the color.

The second and third arguments remain the most cogent. However, serious limitations are imposed on the second if one considers the severity of the Martian climate. Föcas (1962) has measured photometrically the seasonal evolution of the fine structure of the dark areas of Mars. He concludes that:

1. The dark areas of Mars show periodic variation of intensity, following the cycle of the darkening element.
2. The average intensity of the dark area, not including the action of the darkening waves, increases from the poles toward the equator.
3. The action of each of the darkening waves decreases from the poles toward the equator. This decrease is balanced in the equatorial zone by the combined action of the two darkening waves alternately originating at the two poles. The action of the darkness-generating element seems to be constant for all latitudes during the Martian year.

The above phenomenon has been explained recently by non-life mechanisms for Depressio Hellespontica (an area showing one of the greatest seasonal changes) (Öpik, 1958). Similar non-life mechanisms may be applicable to the other dark regions; and, thus, the "darkening" can be used only as circumstantial evidence in support of a Martian life.

If arguments on the seasonal albedo variation are accepted, then an inorganic interpretation must also be advanced for the polarization variation. Two possibilities can be suggested:

1. A change in texture of the surface material, where it becomes more absorbing in the spring, causing both the albedo and polarization to change in the manner observed.
2. A change in surface texture, in which the surface material becomes rougher, which will also explain the observed polarization data (Dollfus, 1951).

The third argument against the regenerative feature of the dark areas being a life process has been advanced by Kuiper (1955). It is based on atmospheric circulation causing dust, presumably lava, to be blown on the dark areas of Mars during the late summer, autumn, and winter, and then removed during the spring. A similar explanation has been advanced recently by Mamikunian and Moore (1965) that carbonaceous chondrites or astroidal matter may induce the observed phenomenon if they are abundant on the planet's surface. The pulverized chondritic material will exhibit a high degree of opacity due to localization and, hence, a change in polarization characteristics and a decrease in polarization following a process of mixing with the indigenous surface minerals and soil.

It has been shown recently that the last observational argument--namely, the Sinton "bands" (Sinton, 1957)--is at least doubtful. Rea, Belsky, and Calvin (1963) recorded the infrared reflection spectra for a large number of inorganic and organic samples, including minerals and biological specimens, for the purpose of interpreting the 3 to 4 μ spectrum of Mars. These authors state that a previous suggestion that the Martian "bands" be attributed solely to carbohydrates is not a required conclusion. At the same time, they fail to present an alternate satisfactory explanation, and the problem remains unsolved. More recently Rea, O'Leary and Sinton (1965) noted the similarity of the 3.58 and 3.69 micron minima in the Martian infrared spectra and those of D_2O-HDO-H_2O mixtures and, particularly, of HDO.

With the foregoing marked disagreement in interpreting the observational data concerning Mars, it becomes clearly evident that the current experimental approach to the detection of "life" on Mars should provide the maximum positive information possible. Some "life detection" experiments which have been developed with NASA support have been summarized by Quimby (1964).

The purpose of the biological exploration of a planet other than Earth is to conduct a series of complementary experiments proceeding from the general to the very specific. The general experiments will emphasize gross characterization of the planet's environment and surface to determine the probability for the presence of an active biota (life). Data from the general experiments will be significant in:

1. Defining the nature of specific experiments in which life detection is the major objective and
2. Providing a high degree of confidence in undertaking specific experiments, since indications from the gross characterization of the planet in question strongly support the objectives of the specific experiments.

<u>The biological exploration of the planets</u> is then to be defined as <u>the approach or the search for those parameters that are relevant to the origin, development, sustenance, and degradation of life in a planetary environment</u>. This definition will give rise to a <u>critical question</u> for each progressively specific and complex experiment to determine:

1. <u>Whether there is life on Mars</u>;
2. <u>The degree of similarity or dissimilarity</u> (structure and function) <u>with respect to terrestrial life</u>;
3. <u>The origin of this planetary life</u>.

The immediate objective of the biological explorations of the planet is to define the state of the planetary surface, which may exhibit the following properties:

1. A prebiota (defined by the absence of life);
2. An active biota (defined by the presence of life);
3. An extinct biota (defined by evidence of former life).

The identification and the detailed characterization of each of the above stages of planetary development constitute the subject matter of the biological exploration of the planets and, specifically, Mars.

The Experimental Investigation of Chemical Evolution

Attempts have been made to simulate and approximate models of primitive Earth conditions for abiogenic synthesis, and successful synthesis of essential biochemical constituents necessary for maintaining life has been partly accomplished. The concept of "chemical evolution" seems plausible. The available evidence (Urey, 1959) supports the theories on the origin of the elements and, in particular, the nucleosynthesis of carbon.

H, C, O, N and, possibly, P and S are particularly significant in their abundance and organization with respect to life and its ecological environment. In Table 1, the abundance of the ten most common elements are listed. Only He, H, C, Fe, and Si are important in the initial stage of planetary formation preceding the organic evolution.

Table 1. Initial cloud fall out characteristics

Element	Ionization potentials	Atomic weights	Relative abundance	Cloud	Mass %
He Ne	24.5 21.5	4.0 20.2	3.5×10^7 50,000	A	28
N O H C	14.5 13.5 13.5 11.2	14.0 16.0 1.0 12.0	160,000 220,000 3.5×10^8 80,000	B	71
S Si	10.3 8.1	32.1 28.1	3,500 10,000	C	0.22
Fe Mg	7.8 7.6	55.8 24.3	18,300 8,870	D	0.31

The chemical constitution of the preceding elements will vary in the following ways:

A-Cloud

First helium (and neon) will fall out of the cloud toward the central body. This is a slow process, and only a fairly small amount of helium is separated.

B-Cloud

The second cloud consists of hydrogen together with oxygen and nitrogen and what is left of helium and neon. The falling out is rapid and, under favorable conditions, a mass comparable to the solar mass may be separated.

C-Cloud

From the small fraction of the cloud now left, carbon and sulfur fall out rapidly.

D-Cloud

Further cooling de-ionizes iron, magnesium, and silicon and makes them fall in rapidly toward the central body.

In reality, the clouds are certainly not so chemically pure as in the simplified scheme presented. There are probably many secondary processes which introduce other elements as impurities, resulting in density differences among the planets. This is the case especially when the temperature decreases so rapidly that one process has not been completed before the next starts.

Knowledge gained through laboratory studies of the abundance, distribution, and interaction of these elements is essential in determining the geochemical, the atmospheric, and the prebiota parameters that will eventually determine the character of the biota on a planetary surface.

Miller (1955) has shown that four of the most abundant elements--H, C, O, and N--are the major constituents in the organic compounds whose formation is a prerequisite for establishing the prebiota. The participation of P and S in the formation of organic moieties at the early stages of organic synthesis is perhaps less common because of their low abundance and increases the instability and reactivity of their organochemical bonds.

Several geochemical and atmospheric characteristics common to all the planets (especially the terrestrial planets) can be expected according to the cosmic abundance of the elements and from the fallout pattern of clouds B, C, and D. Since the abundance of hydrogen in this mixture is in excess of O, N, and C by a factor of more than 10^2, the common molecules in the cold cosmic "ices" will be the hydrides (Table 2).

Accordingly, the primary atmosphere of the planets will be reducing due to the thermodynamic properties of these gases with respect to cosmic distribution (Urey, 1959). The equilibrium constants at 25°C for the reactions of the gaseous species are given in Table 2 (Miller, 1955);

Table 2. Equilibrium Constants of Gaseous Species at 25°C

Reaction of H_2 with the abundant elements	K_{25}
$CO_2 + 4H_2 \longrightarrow CH_4 + 2H_2O$	7×10^{22}
$CO + 3H_2 \longrightarrow CH_4 + H_2O$	3×10^{26}
$C + 2H_2 \longrightarrow CH_4$	8×10^{8}
$N_2 + 3H_2 \longrightarrow 2NH_3$	7×10^{5}
$\frac{1}{2}O_2 + H_2 \longrightarrow H_2O$	4×10^{41}
$S + H_2 \longrightarrow H_2S$	6×10^{5}

they indicate that, in the presence of hydrogen, the carbon will be present as methane, the nitrogen as ammonia, and the oxygen as water. Urey (1952) was first to point out the possible role of a reducing atmosphere in the synthesis of prebiological organic molecules. Miller (1957) synthesized a variety of amino acids in a reducing atmosphere by means of an electrical discharge. A variety of organic compounds have been synthesized by the action of various energy sources upon reducing atmospheres and several investigators have extended the Urey-Miller-type reactions to synthesize nucleic acids (Oró, 1960), adenosine triphosphate (Ponnamperuma, et al. 1963), and a host of biologically essential organic compounds.

It is likely that in the synthesis of organic moieties--the formation of C-H, C-N, C-O bonds--simple and specific molecules were produced in the reducing atmosphere of the planets. Further complexity or degradation of the organic compounds produced varied, depending on the geochemical changes of the planet's surface, the atmospheric constituents, the degree of interaction between surface and environment, and the rate of the organic synthesis. Oparin (1938) presented the most detailed mechanisms for the spontaneous generation of the first living organism arising from a sea of organic compounds synthesized in a reducing atmosphere of the Earth.

It is generally accepted that, under "favorable conditions", based on the above evidence that life can be induced by spontaneous generation. A primary requirement for this initiation is that there be abundant organic compounds concentrated in one or more specific zones. These simple organic molecules would undergo modification to develop a greater structural complexity and specificity, finally giving rise to a "living" organism. Therefore, because of the ease with which organic compounds can be synthesized under reducing conditions, planetary surfaces may contain an abundant source of similar organic matter. However, difficulties arise in postulating steps for further organization or modification of the above synthesized organic matter into a living state on a particular planet. Most of the original organic matter produced in the primary reducing atmospheres of the planets may have been quite similar. However, major bifurcations in chemical composition and specificity beyond the prebiotic stage must have been the rule rather than the exception.

The primary interest in this area of research has been the realization of the possible existence of organic moleculesc on planetary surfaces and, particularly, Mars. The mechanism of such a synthesis may be either due to biological activity or produced abiologically. The research conducted in the simulation of cosmo-chemical synthesis has utilized most of the available solar energy. Simulation experiments devised to study the effects of these energies on the assumed early atmosphere of the Earth have yielded products that play a dominant role in molecular and biochemical organization of the cell.

Calvin (1951) was the first to irradiate water and carbon dioxide in a cyclotron, obtaining formaldehyde and formic acid. Miller (1953) found that when methane, ammonia, water and hydrogen were subjected to a high-frequency electrical discharge several amino acids were produced along with a variety of organic products.

Corroborating experiments established that the synthesis of amino acids occurred readily. The apparent mechanism for the production of amino acids is as follows: Aldehydes and hydrogen cyanide are synthesized in the gas phase by the electrical discharge. These substances react in the water phase of the system to give amino and hydroxy nitrides, which are then hydrolyzed to aminohydroxy acids. Among the major constituents were aspartic acid, glutamic acid, glycine, α-alanine, and B-alanine.

The so-termed "Miller-Urey" reaction mixture has been extended and several modifications introduced. Oró (1963) introduced hydrogen cyanide into the system as the primary gas component. Adenine was obtained when Oró heated a concentrated solution of hydrogen cyanide in aqueous ammonia for several days at temperatures up to 100°C. Adenine, an essential component of nucleic acids and an important coenzyme, was synthesized in a non-biological system. Oró further obtained guanine and uracil as products of nonenzymatic reactions by using certain purine intermediates as starting materials.

Ponnamperuma (1964) also obtained adenine upon irradiation of methane, ammonia, hydrogen, and water, using a high-energy electron beam as the source of energy of irradiation. This indicates that adenine is most readily synthesized under pre-biotic conditions. Adenine, among the biologically important purines and pyrimidines, has the greatest resonance energy, thus making its synthesis more likely and imparting greater radiation stability to the molecule. Guanine and urea were the two other products identified in the hydrogen cyanide reaction.

The formation of adenine and guanine, the only purines in RNA and DNA, by a relatively simple abiological process lends further support to the hypothesis that essential biochemical constituents of life may have originated on Earth by a gradual chemical evolution and selection. It is in this respect that the search and examination of planetary surfaces--specifically Mars--presents practical implication of the current research on the problem of chemical evolution or cosmo-chemical synthesis.

Significant results were obtained by Ponnamperuma et al. (1963) when he exposed adenine and ribose to ultraviolet light in the presence of a phosphate; adenosine was produced. When the adenine and ribose were similarly exposed in the presence of the ethyl ester of polyphosphoric acid, adenosine diphosphate (ADP) and adenosine triphosphate (ATP) were produced. The abiological formation of ATP was a major stride in the path of chemical evolution, since ATP is the principal free energy source of living organisms.

If protein is absolutely essential for all living things, the very beginning of life must have been a spontaneous formation of protein at a certain age of the Earth.

Oparin (1938) postulated that α-amino acids could have been formed non-biologically from hydrocarbons, ammonia and hydrogen cyanide at the age of the Earth when the atmosphere contained these substances in high concentrations. Bernal (1951) has emphasized the role played by ultraviolet light in the formation of organic compounds at a certain stage of the evolution of the Earth. Oparin's hypothesis has received strong experimental support, as evidenced by the work of Miller (1957).

Generally it has been believed that the first proteins or fore-protein were non-biologically formed by the polycondensation of preformed free amino acids (Fox, 1954). This belief is based solely on the fact that proteins in present-day organisms are synthesized via free amino acids.

Akabori (1955) proposed a hypothesis concerning the origin of the fore-protein and speculated that it must have been produced through reactions consisting of the following three steps.

The first step in the formation of aminoacetonitride from formaldehyde, ammonia and hydrogen cyanide.

$$CH_2O + NH_3 + HCN \longrightarrow H_2N - CH_2 - CN$$

The second is the polymerization of aminoacetonitride on a solid surface, probably in the state absorbed on clay, followed by the hydrolysis of the polymer to polyglycine and ammonia.

$$x\ H_2N - CH_2 - CN \longrightarrow (-NH - CH_2 - \underset{\underset{NH}{|}}{C} -)_x$$

$$\Big\downarrow + x\ H_2O$$

$$(-NH - CH_2 - CO -)_x + x\ NH_3$$

The third step is the introduction of side chains into polyglycine by the reaction with aldehydes or with unsaturated hydrocarbons. Akabori has demonstrated experimentally the formation of cystinyl and cysteinyl residue in his above-postulated mechanism.

Further organization of the individually synthesized amino acids into higher molecular weight units has been achieved by Fox (1965). This area of research represents the formation of the first protein molecule(s) and the origin of somewhat organized structure--possible models for the pre-cell in the scheme of chemical evolution.

Fox's theory of thermal copolymerization (Harada and Fox, 1965) suggests that proteins or like molecular units could have formed in the Earth's crust, whereby geothermal activity prevailed. The accumulated amino acids were heat polymerized and transported into the primary oceans for further modifications and specificity. Fox has obtained anhydropolymers, consisting of all eighteen amino acids that are usually present in proteins. The polymerization is generally done at 180° to 200°C, although in the presence of polyphosphoric acid it can be accomplished at temperatures below 100°C. Molecular weights increased from 3600 in a proteinoid made at 160°C to 8600 in one made at 190°C.

Fox showed that when hot saturated solutions of thermal copolymers containing the eighteen common amino acids were allowed to cool, large numbers of uniform, relatively firm and elastic spherules separate. These range from 1.5 μ to 3 μ in diameter. Various chemical observations suggest the presence of peptide bonds in the structural organization of these microspheres.

Continuing observations of these microspheres have established further characteristics that point to the possibility of their interpretation as a kind of primitive protein macromolecule with self-organizing properties such that a primitive form of cell, with boundary and other contemporary properties, might form.

In laboratory experiments the behavior of Gram-negative and Gram-positive microspheres in dilute alkali parallels that of Gram-negative and Gram-positive bacteria (Harada and Fox, 1965). Furthermore, time lapse studies indicate that the proteinoid microspheres undergo a septate kind of fission in what can be described as mimicking cell division as shown in Fig. 1. These same time lapse studies provide evidence that the microsphere's boundary is membrane-like in having a primitive selectivity. Electron micrographs of sections of stained microspheres also indicate the presence of a boundary.

Oparin (1938) states that the type of organization peculiar to life could originate only as the result of the evolution of a multi-molecular organic system separated from its environment by a distinct boundary but constantly interacting with this environment in the manner of open systems. Oparin (1959) has presented the concept of coacervates as pre-cell models. He indicates that present day protoplasm possesses a number of features similar to coacervate structure. Therefore, these coacervates represent the starting point for the evolution leading to the origin of life. Moreover, in the course of their evolution the initial systems must gradually become more complex and elaborate both in space and in time. Oparin (1938) showed that mixing solutions of different proteins and other substances of high molecular weight produced these coacervate droplets. These are characterized by a molecular migration and the formation of a surface layer with altered structure and mechanical properties, thus providing a somewhat selective barrier in which to house a molecular system capable of replication. However, these coacervates are unstable structurally and their acceptance as pre-cell models leaves unanswered the origin of the high molecular weight molecules, including proteins, that are needed for their formation.

Thus it can be seen from this brief summary that much has been accomplished in the abiogenic synthesis of organic molecules relating to pre-biotic state of a planetary surface and its implications for the origin of life.

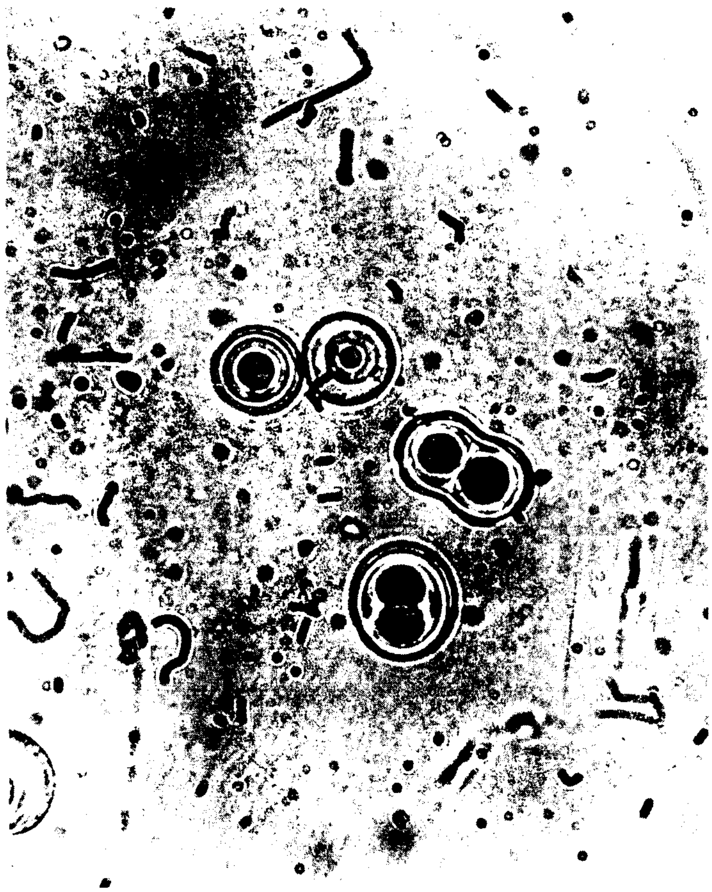

Figure 1.--Proteinoid microspheres undergoing septate fission. [From Fox, S. W.; 1965]

The NASA program has further provided considerable impetus for continuing research with respect to the chemical evolution of life, since its life detection experiments may encounter prebiological molecules in their search for extraterrestrial life on other planetary surfaces.

In the area of exobiological research, the significant accomplishments to date have been:

1. The reconstruction of some of the pathways which may have led to the origin of life, by means of laboratory simulation of prebiological organic molecules.
2. The developments in experimental biology, specifically the role of nucleic acid-protein interactions in storage and transmission of information both within living cells and between generations of cells.

3. The suspected role of ribonucleic acid in information storage and the development of new concepts of the coding mechanism in deoxyribonucleic acid that unveils frontiers for the development of a universal biological theory embracing evolutionary as well as homostatic adaption to environment and learned behavioral systems.

With the essential biochemical constituents of life and the mechanism of replication understood, the challenge for the synthesis of "living" matter by abiogenic experimental techniques has become to many scientists the ultimate goal of the scientific era.

NASA has established an Exobiology Laboratory at Ames Research Center in addition to the sizable support of research at various academic centers of excellence for the continuation of abiogenic synthesis.

The program of organochemical evolution is in its infancy. The resultant data of the few experiments have already created an immense enthusiasm to gain knowledge of the biochemical pathways of evolution. It is not only the terrestrial evolution of life that this kind of research will ultimately elucidate but, perhaps, the nature of life on other planetary bodies and distribution of life in our galaxy.

This program, with its vast demands on the scientific community at large, is coordinated with related endeavors of a number of Federal agencies. It is allied with certain biochemical studies of the National Institutes of Health for the eventual solution of both the dynamic and the static pathways in cosmochemical synthesis of life's essential biochemical constituents.

Meteorites and Organic Geochemistry

Meteorites

A significant area of exobiological research is the investigation of stony meteorites known as "carbonaceous chondrites." It is increasingly apparent that almost all life detection concepts rely on the eventual analysis of "solid materials" that may be available on Mars and other planetary surfaces. Cosmic dust and meteorites are two classes of material bodies that reach the Earth from outer space. The carbonaceous chondrites--a special class of stony meteorites--are the only extraterrestrial materials that contain "organic" carbon.

The study of meteorites has generated an astonishing diversity of hypotheses. There is agreement at only one point: that meteorites are preserved chunks of very ancient, perhaps primordial, planetary matter and that when we are able to understand the curious structures and chemical and isotopic variations in the meteorites, we shall also know a great deal about early planetary and perhaps even preplanetary history.

Meteorites provide a more representative sample of average planetary matter than the highly differentiated crust of the earth. Although it is known that the meteorite parent bodies ceased to be geochemically active very shortly after their formation, some 4.5×10^9 years ago, there is no consensus on the nature of the meteorite parent bodies, not even on such basic properties as size, location, and multiplicity. This is not surprising because the meteorite samples commonly available for study represent only about 10^{-23} to 10^{-26} of the parent body.

Carbonaceous Meteorites

The classification of carbonaceous chondrites has been divided into two research areas:

a. Analysis and characterization of the chemical constituents (organic), including the possible mode and mechanism of their formation; and

b. Enhancement of both the research and methodology of analyzing samples from the moon and planets and interpreting remote automated biological analyses on the planets' surfaces.

Carbon has been detected in all meteorites analyzed. However, both the amount and forms present vary considerably. Among the forms of meteorite carbon are diamond, graphite, cohenite $(Fe, Ni, Co)_3C$, moissanite SiC, Calcite $CaCO_3$,.dolomite $CaCO_3$, bruennerite $(Mg, Fe)CO_3$.

A summary of the results of carbon analyses on large numbers of meteorites is given in Table 3 (Briggs and Mamikunian, 1963).

Table 3. Meteorite carbon

Meteorite Group	No. Analyzed	Mean Carbon Content (Percentage by weight)
1. Pallasites	10	0.08
2 Ureilites	2	0.69
3. Bronzite chondrites	12	0.05
4. Hypersthene chondrites	8	0.04
5. Enstatite chondrites	8	0.29
6. Carbonaceous chondrites	16	2.04

Aside from the carbonaceous chondrites, most meteorites possess only traces of carbon and studies of this carbon indicate that it is composed largely of graphite, cohenite, and moissanite, with some diamond. However, studies of the carbon in the carbonaceous chondrites have failed to detect any of these forms. Some carbonates are present in a minority of the carbonaceous group, but account for only a very small percentage of the total carbon (perhaps about 10% of the total C in type I only).

In the absence of any evidence of inorganic carbon, a reasonable working hypothesis is that the carbonaceous chondrites contain organic carbon. The word "organic" is not used in any biological sense, merely as a chemical term to describe compounds of carbon other than carbonates bicarbonates and carbides. This hypothesis has been confirmed by all experimental studies conducted on these meteorites. To date there is no evidence of any form of carbon other than organic, except for traces of carbonates.

Various studies have demonstrated possible methods of estimating the total amount of organic matter present in the meteorites.

Wiik (1956) has suggested that organics can be estimated by measurements of loss of weight on ignition. Unfortunately, this method has several objections and gives very low values. One must consider the weight changes due to oxidation of reduced constituents, such as FeO, Fe, Ni, Co, etc., and also to correct for weight losses due to H_2O, S, etc.

The water-loss is exceedingly difficult to estimate as part comes from the combustion of organic hydrogen, and part comes from the loss of mineral-bound water. The carbon also proves difficult to totally combust and very high temperatures (over 1000°C) are required for efficient conversion to CO_2.

In the following account the major fraction of organic matter removed proves to have a carbon content of about 47 per cent (Briggs, 1963). Hence, if all the meteorite carbon is present as organic matter of approximately this composition, total organics must be approximately double the carbon content, i.e., 2.0% by weight carbon indicates 4.0% by weight organic matter. This estimate may be too low, for Mueller (1962) has extracted a major organic fraction containing only 24% carbon, but this work has not been confirmed for other meteorites.

Briggs and Mamikunian (1963) have pointed out that only 25% of the organic matter has been extracted and only about 5% of this has been chemically characterized. Most of this 5% is a complex mixture of hydroxylated aromatic acids together with various hydrocarbons of the paraffin, naphthene and aromatic series. Small amounts of amino acids, sugars and fatty acids are also present.

Thus far, these chemical analyses point to an abiogenic origin for the organic matter; and there is no conclusive evidence of biological activity on the meteorite parent body.

Microbiological investigations of samples of the carbonaceous chondrites have yielded only inconclusive evidence on the problem of "organized elements".

Several of these microstructures from different carbonaceous chondrites are illustrated in a paper by Mamikunian and Briggs (1963). While it has been difficult to identify the organized structures, and most do not have morphologies identical to known terrestrial microorganisms, they may prove to be a variety of mineral grains, droplets of organic matter and sulphur, as well as a small amount of contaminating terrestrial debris.

A comparison between photographs of the organized elements observed in the Orgueil and Ivuna meteorites and the synthetic proteinoid microspheres observed by Fox (1965) point to similarities between the two. One inference from this finding is that the organized elements in carbonaceous chondrites were never alive but, rather, should be considered as natural experiments in molecular evolution. Also, these similarities strengthen the belief that the laboratory experiments are similar to the natural experiments in space.

In cooperation with the Smithsonian Astrophysical Observatory, NASA has a network to track meteors in the mid-western United States (South Dakota, Nebraska, Kansas, Oklahoma, Iowa, Missouri, and Illinois). Photographs of meteor trails are used for scientific study and attempts are made to track and recover meteorites which will be examined for traces of organic material of extraterrestrial origin.

Fundamental research in terrestrial organic geochemistry has shown that ancient sediments and drill core samples subjected to organic analysis have indicated the nature and the stability of certain biochemical components of past life. This preserved record is significant not only in studies of early life chemical pathways, but the interaction of organic matter with the geological factors. Since life on any planetary body will still maintain its relationship with the soil (or surface material), it is of timely interest to understand the interaction of these two vital forces.

Concepts for Detection of Extraterrestrial Life

It is not possible to present completely convincing evidence for the existence of extraterrestrial life. The problem often reduces to probable considerations and to estimates of observational reliability. In almost all cases there is an optimistic view that the evidence is strongly suggestive of--or, at the worst, not inconsistent with--the existence of extraterrestrial life. Alternatively, there is a pessimistic view that the evidence adduced in favor of extraterrestrial life is unconvincing, irrelevant, or has another, non-biological explanation.

In studies of the laboratory synthesis of life-related compounds and its significance with respect to the origin of life, several of these results seem to suggest that organo-chemical synthesis is a general process, occurring perhaps on all planets which retain a reducing atmosphere. The temperature ranges must be such that precursors and reaction products are not thermally dissociated. The rate of chemical reaction for the construction of more complex organic molecules diminishes to a negligible value when the temperature range is below 100°C. Within these limits it appears that life must be a pervasive component of the solar system.

In addition to the planetary parameter of temperature, there is an even more fundamental necessity for a living state--a liquid solvent system. For terrestrial life forms, water serves this purpose, functioning as an interaction medium and as a thermal reservoir. Water has these and other properties of biological significance because of hydrogen-bonding between adjacent molecules in the liquid state.

With respect to an energy source, needed for replication and metabolism, ultraviolet radiation could serve as an extraterrestrial energy source. Research shows that, while an atmosphere is important, there is evidence that living systems can survive a wide range of ambient pressures; and that living systems are little affected by a wide range of magnetic field strengths.

There is evidence that oxygen is not a prerequisite for all living systems. An oxygen atmosphere offers an energy advantage in that it is more efficient in the extraction of free energy from food sources such as glucose. While it is sometimes concluded that free oxygen is needed for all but the simplest organisms, it appears that less complete metabolic oxidation coupled with higher food collection efficiency--or a more sluggish metabolism--would do just as well. Earth is the only planet in the solar system on which molecular oxygen is known to be present in large amounts. Since plant photosynthesis is the primary source of atmospheric oxygen, it seems safe to infer that no other planet has large-scale plant photosynthesis accompanied by the production of oxygen.

The possibility of there being extraterrestrial life raises the important question of our being able to detect it and thus knowing if it exists. Research on extraterrestrial life detection is predicated on our being able to develop ways to detect it even when the living systems are based on principles entirely different from those on Earth. Thus, some metabolic activity must be expected.

The substitution of various molecules for those of known biological significance to living organisms as we know them has been investigated; the substitution of NH_2 for OH in ammonia-rich environments leads to a diverse and biologically very promising chemistry. The hypothesis that silicon may replace carbon does not support the construction of extraterrestrial genetics based on silicon compounds. (Silicon compounds participate in redistribution reactions which tend to maximize the randomness of silicon bonding, and the stable retention of genetic information over long time periods is thus very improbable. Furthermore, silicon compounds never have conjugated double bonds.)

Evidence relevant to life on Mars has been summarized by Sagan (Chapter I, Quimby, 1964):

"<u>The Origin of Life</u>

> In the past decade, considerable advances have been made in our knowledge of the probable processes leading to the origin of life on Earth. A succession of laboratory experiments has shown that essentially all the organic building blocks of contemporary terrestrial organisms can be synthesized by supplying energy to a mixture of the hydrogen-rich gases of the primitive terrestrial atmosphere. It now seems likely that the laboratory synthesis of a self-replicating molecular system is only a short time away from realization. The syntheses of similar systems in the primitive terrestrial oceans must have occurred--collections of molecules which were so constructed that, by the laws of physics and chemistry, they forced the production of identical copies of themselves out of the building blocks in the surrounding medium. Such a system satisfies many of the criteria for Darwinian natural selection, and the long evolutionary path from molecule to advanced organism can then be understood. Since nothing except very general primitive atmospheric conditions and energy sources are required for such syntheses, it is possible that similar events occurred in the early history of Mars and that life may have come into being on that planet several billions of years ago. Its subsequent evolution, in response to the changing Martian environment, would have produced organisms quite different from those which now inhabit Earth.

"Simulation Experiments

Experiments have been performed in which terrestrial microorganisms have been introduced into simulated Martian environments, with atmospheres composed of nitrogen and carbon dioxide, no oxygen, very little water, a daily temperature variation from +20° to -60°C, and high ultraviolet fluxes. It was found that in every sample of terrestrial soil used there were a few varieties of microorganisms which easily survived on "Mars." When the local abundance of water was increased, terrestrial microorganisms were able to grow. Indigenous Martian organisms may be even more efficient in coping with the apparent rigors of their environment. These findings underscore the necessity for sterilizing Mars entry vehicles so as not to perform accidental biological contamination of that planet and obscure the subsequent search for extraterrestrial life.

"Direct Searches for Life on Mars

The early evidence for life on Mars--namely, reports of vivid green coloration and the so-called "canals"--are now known to be largely illusory. There are three major areas of contemporary investigation: visual, polarimetric, and spectrographic.

"As the Martian polar ice cap recedes each spring, a wave of darkening propagates through the Martian dark areas, sharpening their outlines and increasing their contrast with the surrounding deserts. These changes occur during periods of relatively high humidity and relatively high daytime temperatures. A related dark collar, not due to simple dampening of the soil, follows the edge of the polar cap in its regression. Occasional nonseasonal changes in the form of the Martian dark regions have been observed and sometimes cover vast areas of surface.

"Observations of the polarization of sunlight reflected from the Martian dark areas indicate that the small particles covering the dark areas change their size distribution in the spring, while the particles covering the bright areas do not show any analogous changes.

"Finally, infrared spectroscopic observations of the Martian dark areas show three spectral features which, to date, seem to be interpretable only in terms of organic matter, the particular molecules giving rise to the absorptions being hydrocarbons and aldehydes.

"Taken together, these observations suggest, but do not conclusively prove, that the Martian dark areas are covered with small organisms composed of familiar types of organic matter, which change their size and darkness in response to the moisture and heat of the Martian spring. We have no evidence either for or against the existence of more advanced life forms. There is much more information which can be garnered from the ground, balloons, Earth satellites, Mars flybys, and Mars orbiters, but the critical tests for life on Mars can only be made from landing vehicles equipped with experimental packages...."

Results of Kaplan et al. (1964) indicate that Mars has no detectable oxygen but does contain small amounts of water vapor, more abundant carbon dioxide, possibly a large surface flux of solar ultraviolet radiation, and estimated daily temperature variations of 100°C at many latitudes. Studies have shown that terrestrial microorganisms can survive these Martian, extremely harsh, environments. Furthermore, a variety of physiological and ecological adaptations might even enable the biota to survive the low night time temperatures and the attendant problem of ice crystallization.

We have less evidence to support the possibility of extraterrestrial life on other planets. The Moon has no atmosphere, and various temperature extremes characterize its surface. However, it has been suggested that the Moon has a layer of subsurface permafrost beneath which liquid water may be trapped. The temperatures of these regions may be biologically moderate.

Microwave radio emissions from Venus, arising from its surface, support the observation that the planet has a high average temperature even in its dark hemisphere. However, these findings are disputed. The possibility of life as an indigenous biological aerosol in Venusian clouds can be envisioned.

Studies by Davis and Libby (1964) on the atmosphere of Jupiter support the possibility of the production of organic matter--molecules based on carbon--in its atmosphere in a manner analogous to the processes which may have led to the synthesis of organic molecules in the Earth's early history. It is difficult to assess the possibility that life has evolved on Jupiter during the four- or five-billion-year period in which the planet has retained a reducing atmosphere.

The question of extraterrestrial life and the question of the origin of life are interwoven. Discovery of the first may very well elucidate the second.

The oldest form of fossil known today is that of a microscopic plant similar in form to common algae found in ponds and lakes. Scientists know that similar organisms flourished in the ancient seas over two billion years ago. However, since algae are a relatively complex form of life, it is possible that life in some simpler form originated much earlier. Organic material similar to that found in modern organisms can be detected in these ancient deposits as well as in much older Precambrian rocks.

By studying the radioactive decay of minerals, scientists have determined that the surface of the Earth hardened into its present form about 4.5 to 5 billion years ago. Life itself probably arose during the first billion years of the Earth's history.

Although the planets now have differing atmospheres, it is believed that in their early stages the atmospheres of all the planets may have been essentially the same. The most widely held theory of the origin of the solar system states that the planets were formed from vast clouds of material containing the elements in their "cosmic" distribution.

The Earth's present atmosphere consists of nitrogen and oxygen in addition to relatively small amounts of other gases; most of the oxygen is of biological origin. Some of the atmospheric gases, in spite of their low amounts, are crucial for life. The ultraviolet absorbing ozone in the upper atmosphere and carbon dioxide are examples of such gases.

Scientists believe that the synthesis of organic compounds preceding the origin of life on Earth occurred before its atmosphere was transformed from hydrogen and hydrides to oxygen and nitrogen, supporting their theory by laboratory experiments of Calvin (1951), Miller (1955) and Oró (1965).

Spacecraft have flown past, and crashed on, the Moon. Mariner II, launched from Cape Kennedy on August 27, 1962, flew past Venus on December 14, 1962, taking readings and transmitting data (e.g., planet's temperature) which are significant in the search for extraterrestrial life. Mariner II's measurements showed temperatures on the surface of Venus of the order of 800°F, too hot for life as known on Earth.

The question, "Is life limited to this planet?", can be considered on a statistical basis. Although the size of the sample (one planet) is small, the statistical argument for life elsewhere is believed by many to be very strong. While Mars is generally considered to be the only other likely habitat of life in our solar system, Shapley (1958) has calculated that there are more than one hundred million stars which have planets sufficiently similar in composition and environment to Earth to support life. Of course, yet unknown factors may reduce significantly or even eliminate this probability.

Spacecraft Sterilization

The search for extraterrestrial life with unmanned space probes requires the total (external and internal) sterilization of the landing capsule and its contents. Scientists agree that terrestrial organisms released on other planets would interfere with exobiological explorations (Lederberg and Cowie, 1958; CETEX, 1958, 1959; Sagan, 1960; Davies and Communtzis, 1960; Lederberg, 1960; Brown, 1962; Imshenetskii, 1963). Any flight that infects a planet with terrestrial life will compromise a scientific opportunity of almost unequaled proportions. Studies on microbiological survival in simulated deep space conditions (low temperature, high ultra-violet flux, and low dose levels of ionizing radiation) indicate that these conditions will not sterilize contaminated spacecraft (Portner et al., 1961; Morelli et al., 1962; Davis et al., 1963; Silverman et al., 1964; Imshenetskii et al., 1964). Furthermore, many terrestrial sporeformers and some vegetive bacteria, preferably those with anaerobic growth capabilities, readily survive in simulated Martian environments (Hawrylewicz et al., 1962; Packer et al., 1963; Scher et al., 1963; Young et al., 1963; Hagen et al., 1964; Hawrylewicz et al., 1965). It has been estimated that a single microorganism with a replication time of 30 days could, in eight years of existence on Mars, equal in number the bacterial population of the Earth. This would result not only in competition with any Martian life, but in drastic changes in the geochemical and atmospheric characteristics of the planet. To avoid such a disaster, certainly the first, and probably many succeeding landers on Mars, must be sterile--devoid of terrestrial life (Nicks and Reynolds, 1963). Since the space environment will not in itself kill all life aboard, the lander must leave the Earth in a sterile condition.

The sterility of any object is a concept that implies the complete absence of life. The presence of life or the lack of sterility may be proven, but the absence of life or sterility cannot be proven for the one viable organism that negates sterility may remain undetected. Many industrial products which must be guaranteed as sterile cannot be tested for sterility in a non-destructive manner. A similar situation exists in determining the sterility of a spacecraft. Certification of sterility, based on experience with the sterilizing process used, knowledge of the kinetics of the death of microorganisms, and computation of the probability of a survivor from assays for sterility, is the only accurate approach to defining the sterility of such treated items.

Macroscopic life can be readily detected and kept from or removed from the spacecraft, but the detection and removal of microscopic and submicroscopic life is an extremely difficult task. The destruction of microorganisms can be achieved by various chemical and physical procedures. Sterilizing agents have been evaluated not only for their ability to kill microbial life on surfaces and sealed inside components, but also for the agents' effects on spacecraft reliability as well (Phillips and Hoffman, 1960; Hobby, 1962; Bruch et al., 1963; Koesterer, 1964). Of the available agents, only heat and radiation will penetrate solid materials. Radiation is expensive, hazardous, difficult to control, and appears to damage more materials than does heat. Heat, therefore, has been selected as the primary method of spacecraft sterilization and will be used, except in specific instances where radiation may prove to be less detrimental to the reliability of critical parts (Hall and Bruch, 1965).

The sterilization of spacecraft is a difficult problem if flight reliability is not to be impaired. The development of heat-resistant parts will enable the design and manufacture of a heat-sterilizable spacecraft. Without careful microbiological monitoring of manufacture and assembly procedures, much bacterial contamination could be entrapped in parts and subassemblies. To permit the sterilization at the lowest temperature-time regimen that will ensure kill of all organisms, the microbiological load inside all parts and of all subassemblies must be held to a minimum.

The role of industrial clean rooms in reducing the biological load on spacecraft is currently being defined. NASA-supported studies indicate that biological contamination in industrial clean rooms for extended time periods is about one logarithm less (10-fold reduction) compared with conditions in a well-operated microbiological laboratory (Portner et al., 1964). With the use of clean room techniques and periodic decontamination by low heat cycles or ethelyne oxide treatment, it should be possible to bring a spacecraft to the point of sterilization with about 10^6 organisms on board (Hall and Bruch, 1965).

The sterilization goal established for Mars landers is a probability of less than one in 10,000 (10^{-4}) that a single organism will be present on the spacecraft. Laboratory studies of the kinetics of dry heat kill of resistant organisms show that at 135°C the number of bacterial spores can be reduced one logarithm (or 90%) for every two hours of exposure (Bruch et al., 1963; Bruch, 1964). The reduction in microbial count needed is the logarithm of the maximum numbers on the spacecraft (10^6) plus the logarithm of the reciprocal of the probability of a survivor (10^{-4}) or a total of ten logarithms of reduction in microbial count. Thus, with an additional two logarithms added as a safety factor, a total of 12 logarithms of reduction in count has been accepted as a safe value which can be achieved by a dry-heat treatment of 135°C for 24 hours. This is the heat cycle that is currently under study and being developed for use in spacecraft sterilization (Hall and Bruch, 1965).

However, other heat treatments are under study at temperatures as low as 105°C for periods of time of 300 hours or longer (Bruch, 1965).

Based on results to date, it is reasonable to believe that a full complement of heat-sterilizable hardware will be available when needed for planetary exploration. Every effort is being made to improve the state of the art to a point where spacecraft can not only withstand sterilization temperatures, but will be even more reliable than the present state-of-the-art hardware that is not heated.

III. ENVIRONMENTAL BIOLOGY

Weightlessness and Zero Gravity

High priority has been given to weightlessness and gravity studies. Gravity is one of the most fundamental forces that acts on living organisms, and all life on Earth appears to be oriented with respect to gravity although certain organisms are more responsive to it than others. The gravity force on Earth is one g, but this force may be experimentally varied from zero g, or weightlessness, to many thousands of g's.

Zero gravity or decreased gravity occurs during free fall, in Keplerian trajectory, or during orbit around the Earth. Gravitational force decreases by the square of the distance away from the Earth's surface. It is reduced about 5 percent at about 200 nautical miles altitude. Greater gravitational force than one g can be obtained by acceleration, deceleration, or impact. It also can be increased by using a centrifuge which adds radial acceleration to the one g of Earth.

On the ground, the biological effects of gravity have been studied at one g, with normal gravity coming from the center of the Earth. However, experimentally, many g forces have been produced, varying up or down from the norm. In addition, modifications of the effects of the one g force have been induced by suspension of the organism in water, or by horizontal immobilization of an erect animal such as man. The biological effects of such modification have been of significant value in understanding some of the possible consequences of human exposure to the zero g environment of space.

Weightlessness in an Earth-orbiting satellite occurs when the continuous acceleration of Earth's gravity is exactly counterbalanced by the continuous radial acceleration of the satellite in its high velocity path around the Earth. In such a weightless state, organisms are liberated from their natural and continuous exertion against one g, but this liberation may carry with it certain serious physical penalties.

Cellular Effects

The effects of gravity and zero g on living cellular organisms must have their ultimate explanation in the physical behavior of matter.

Some of the physical processes which appear to have the greatest probable biological effects are: (a) consecutive flow of fluid, e.g., protoplasmic streaming, transport of nutrient materials, oxygen and waste products and CO_2 from the immediate environment of the cell; and (b)

sedimentation occurring within cells; substances of higher density sediment in a gravitational field, and those of lighter density rise. A separation of molecules of different densities probably occurs. The removal of gravity would change a distribution of particles like mitochondria by 10 percent (Pollard, 1962).

Gravity has effects on the physical processes involved in mitosis and meiosis. Study under weightlessness might contribute to our understanding of the general cellular information relay process.

A gravitational effect is known in the embryonic development of the frog Rana sylvatica. The eggs after fertilization subsequently rotate in the gravitational field so that the black animal hemisphere is uppermost. Development becomes abnormal if this position is disturbed. If the egg is inverted following the first cleavage and held in this position, two abnormal animals result, united like Siamese twins. This phenomenon appears to be related to the gravitational separation of low and high density components of the egg cell. The size of the egg is about 1-2 mm and is suspended in water of about the same density. This system is very sensitive to gravity; and, under weightlessness, the separation of different density components might be irregular, leading to aberrant development. When certain aquatic insect eggs are inverted, subsequent development results in shortened abnormal larvae.

The directional growth of plants and plant roots is probably due to this sedimentation phenomenon, particularly the effect on movement of auxins (Lyon, 1963).

Free convection flow is a major transport process, and under its influence the mixing of substances is much more effective than when diffusion operates alone. Free convection flow is a macroscopic phenomenon which increases not only with g, but varies also approximately with the 5/4 power of the bulk concentration involved. Whether or not convection is important at the microscopic level remains an experimentally unsolved question. The Grashoff number limits free convection to the macroscopic domain. It would appear in weightlessness, and when only a single force field is present, that the contribution of free convection flow would be small and that only diffusion should occur. This phenomenon would cause equilibration to occur much more slowly than that occurring with free convection alone. In the absence of convective transfer, this raises a problem as to how nutrients may be obtained and waste products removed in living cells during weightlessness. In a liquid substrate, nutrients and oxygen would be utilized and waste products would accumulate around the cell.

Absence of gravity may have far reaching consequences in the homeostatic aspects of cell physiology. The outstanding characteristics of living cells which are most likely to be influenced by the absence of gravity are: the capability of the cell to maintain its cytoplasmic membrane in a functional state, the capacity of the cell to perform its normal functions during the mitotic cycle, and the capacity of the cytoplasm to maintain the constant reversibility of its sol-gel system (McKinney et al., 1963).

Two phase systems, i.e., air-in-water and air-in-oil, possess entirely different characteristics at zero g than at one g. These physical differences in phase interaction could well be suspected of interfering with the orientation and flow pattern of cell constituents, thus hindering the cellular processes involved in the movement, metabolism, and storage of nutrients and waste.

On the basis of theoretical calculations, weightlessness can be expected to have some effect even on one individual cell if its size exceeds 10 microns in diameter (Pollard, 1962). Cell colonies might be affected. In larger cells there may be a redistribution of enzyme-forming systems, which would give rise to polarization. The low surface tension of the cell membrane lends itself to hydrostatic stress distortion, implying an alteration in permeability and hence an almost certain alteration of cell properties under low gravity conditions.

Another aspect of gravity, which affects the growth and development of living organisms, is the directionality of the gravitational field. In fact, some plants are so sensitive that they are able to direct their growth with as little stimulus as 1×10^{-6} gravitational field. This effect has no counterpart in a zero g environment. Investigations of plant growth in altered gravitational fields are under way at Argonne National Laboratories and Dartmouth College.

The clinostat makes possible randomization of the directionality of the gravitational field. Using the clinostat with an omnidirectional 4 pi rotator developed by Dr. Solon Gordon at the Argonne Laboratory, which distributes the pull of gravity in all directions, the effects of gravity have been altered to simulate the randomization of a weightless environment. A number of gravitational force effects have been discovered by observing the growth of plants within the influence of this apparatus, thus refining the threshold of gravitational stimulus to plant growth parameters.

By continually compensating the gravitational force by rotating an organism, it is possible to study simulated weightlessness effects in plants. It was shown that certain plants grew more slowly and had fewer and smaller leaves, while others had about 25 percent greater replication of fronds and had greater elongation of certain plant parts.

It will be extremely interesting to compare these effects under zero g conditions in orbiting spacecraft.

The effect of gravity in transporting growth hormones in plants has been demonstrated at Dartmouth College using radioactive carbon-labelled growth hormones. The effect of gravity on plant geotropisms and growth movements has been studied and Biosatellite experiments have been developed.

Anatomy is considered to be a derivative adaptation to gravity (Space Science Board, 1964). There is a large background of plant research on the effect of reorientation on plant responses. Information from clinostat experiments is considered susceptible of extrapolation to low gravity conditions because the threshold period for gravitational triggering is relatively long.

Once over critical minimum dimensions, the major effects of low gravity would be assumed to occur in those heterocellular organisms that develop in more or less fixed orientation with respect to terrestrial gravity and which respond to changes in orientation with relatively long induction periods; these are the higher plant orders. On the other extreme are the complex primates which respond rapidly, but whose multiplicity of organs and correlative mechanisms make the occurrence of malfunction and disorganization probable, but not certain. It may be suggested that the heterocellular lower plants and invertebrates will be less affected. Perturbations of the environment to which the experimental organism is exposed must be limited or controlled in order to reduce uncertainties in interpretation of the results. At the same time, the introduction of known perturbations may assist in isolating the effects due solely to gravity. Study of *de novo* differentiation and other phenomena immediately after syngamy may be of particular importance. Study of anatomical changes after exposure of the organism to low gravity is important.

The effects of weightlessness on organisms is discussed further in the section on Biosatellites and the effects on man in the section on Physiological Problems of Manned Space Flight.

Biological Effects of Space Radiation [1]

Radiation sources in the space environment are of three types: galactic cosmic radiation, Van Allen Belts, and solar flares with an

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[1] This includes part of the Summary of the Panel on Radiation Biology of the Environmental Biology Committee (1963), Space Science Board, NAS/NRC; and the results of research by the Bioscience Programs, NASA.

intense proton flux. In space, cosmic radiation has energy levels of significantly greater magnitude than radiation produced by man-made accelerators.

The Panel on Radiation Biology, while recognizing the need for radiobiological studies of an applied nature with reference to manned flight programs, stated that it would be short-sighted for the United States to confine its efforts only to the solution of immediate problems, since in the long run successful exploration of space will be aided by the contributions of basic research. Both the immediate biological research program and the continuing program for basic studies should be built upon the large body of existing knowledge of radiation effects. The attitude that all radiobiological experiments need be repeated in the space environment should be resolutely rejected. Since fundamental radiobiology cannot be performed easily in space; it has been recommended that wherever possible these investigations be carried out in ground laboratories in preference to flying laboratories.

Space environment does vary from the terrestrial environment, but the variations are not so unique as to lead to the expectation of strikingly different biological effects of radiation in space. It is, however, conceivable that radiations whose effects are well known under terrestrial conditions may have some unsuspected biological effects when combined with unusual features of the space environment, e.g., zero gravity. Prior space radiobiological studies have depended solely on very low and inaccurately measured doses of ambient space radiation. It has been difficult to distinguish between the observed response levels and the random noise; thus, experiments have been inconclusive.

Biological Effects of Heavy Ions and Mesons

The biological effects of heavy ions (especially $Z > 2$), mesons, and the long-range effects of low levels of radiation are of specific interest to space radiobiology.

Controlled Radiobiological Experiments in Space

There is the remote possibility that the radiobiological response may be modified by factors as yet unknown and perhaps not susceptible to study terrestrially. Experiments have been designed to settle this matter including the exposure of biological materials during space flight which meet the following criteria of reliability: 1) the use of well known biological systems, e.g., mutation induction or chromosome breakage; 2) the use of a sufficient number of individuals in the experiment to guarantee statistical precision on the results; 3) the exposure of the system to known quantities and qualities of radiation; 4) the use of adequate controls.

High altitude balloon ascents of the 1930's initiated study of the biological effects of cosmic rays. They were limited to exploration of secondary cosmic radiation effects. After World War II, the research extended to the use of V-2 rockets fired from the White Sands Proving Ground. Interest returned to balloons and a significant program was underway by 1950, first using mice then hamsters, fruit flies, cats, and dogs. These flights gave no evidence of radiation damage. However, the scientists realized that the flights were too far south to obtain a significant exposure, and more northerly flifhts began in 1953. Mice and guinea pigs were flown on these later flights. Chase (1954) showed the most unequivocal results to that time, a statistically significant increase in light hairs on black animals and the streaks of white hair up to 10 times wider then expected. Brain lesions were detected in the guinea pigs flown on Man High in 1957. Many other types of biological material were sent aloft in an effort to further corroborate existing information and to investigate genetic and developmental effects of cosmic radiation.

From the earlier V-2 rocket flights to the Jupiter Missile launchings of the monkeys Able and Baker, cosmic ray research was continued, but the short flight durations of these vehicles did not provide substantial information. The USAF Discoverer Satellite program has given impetus to the cosmic ray research and provided for longer "stay-times."

It has been difficult to separate radiation effects from other space flight factors; hence, some of the alterations observed are still subject to debate. Vibration, acceleration, and weightlessness appear to be the three most important additional parameters. Measurements of radiation dosage have been made by chemical and photographic dosimetry, ion chambers, and biological dosimetry. All evidence to date indicates that radiation exposure levels are not hazardous to man at present orbital altitudes up to 200 n.m. The greatest preponderance of biological materials flown so far has been for the express purpose of investigating space radiation levels and effects. The biological materials have ranged from tissue cultures to entire organisms and from phage and bacterial cells to man. The studies have required much of the space and weight resources allotted biology by the U.S.S.R. and the U.S.A. They have been accompanied by ground-based studies which also served as "controls."

The Vostok series provided the following data:

A small, but statistically significant increase was observed in the percentage of chromosome aberrations in the rootlet cells of air-dried wheat and pea seeds after germination and that, in this case only, the increase did not depend on flight duration.

Lysogenic bacteria exhibited an increase of genetic alterations and increased phage production. Length of flight was associated with intensified bacteriophage production by the lysogenic bacteria. There was an increase of recessive lethals coupled with non-covergence of chromosomes (sex-linked) in the fruit fly. A "stimulation" of cell-division in wheat and pea seeds was observed. Cultures of human cells exposed to space flight factors did not differ significantly from terrestrial controls with respect to such indicators as proliferation rate, percentage of lethality, and morphological, antigenic, and cultural properties. Repeated flights of the identical HeLa cells revealed that there was a longer latent period for restoration of growth capacity than in cells carried into space once or not flown at all.

The most definite radiation effects observed were only revealed in genetic tests. There has been no discoverable harmful influence on those characteristics affecting the viability of the organism.

The Air Force Discoverer series launched from the West Coast had a few successful flights incorporating organisms. With severe environmental stress and long recovery times, data on radiation exposure was equivocal up to Discoverer XVII and XVIII when human tissue cultures were flown, recovered, and assessed for radiation exposure effects. Comparison with ground-based controls revealed no measurable differences.

Radiation dosimetry from the Mercury series established that minimal exposures were encountered at those orbital altitudes, a typical example is the MA-8 flight of W. M. Schirra, Jr. during which the body surface dosage was less than 30 millirads.

NASA has supported fundamental radiation studies at the Oak Ridge National Laboratories and the Lawrence Radiation Laboratories. Emphasis has been placed on the biological effects of high energy proton radiation and particulate radiation from accelerators.

At the NASA Ames Research Center extensive fundamental studies are being carried out on the effects of radiation, especially in the nervous system. It has been demonstrated that deposits accumulate in the brain following exposure to large doses of ionizing particle radiation as well as after X-irradiation. These deposits, referred to as a chemical lesion, result from an accumulation of a complex sugar known as glycogen. The formation of these deposits during exposure to large doses of X-irradiation was not increased in environments of 99.5 percent oxygen and increased atmospheric pressure.

Simulation of Planetary (Martian) Environments

Attempts have been made to "simulate" to some degree the various parameters of the Martian environment, such as atmospheric composition, pressure, radiation flux, temperatures, and the day-night as well as seasonal cycles. One cannot yet simulate certain factors for Mars, such as soil composition, gravitational field, magnetic field, electrical field, etc.

Caution is required in interpreting all simulation experiments. How Earth organisms respond to simulated Martian environments probably has nothing to do with life on Mars, but these experiments may show whether or not there is anything in the environment of Mars which makes life as we know it impossible. We must expect that on Mars, life will have evolved and have adapted over long periods of time under conditions which are quite different from conditions on Earth. The simulation experiments also provide some information about the possibility of contaminating the planet Mars, or any planet, with organisms from Earth. In addition, they give us some clues about the possibilities of adaptation and evolution of life under these conditions.

From an evolutionary point of view, if life has evolved on Mars, we expect it to have evolved at least to a microbial stage. On Earth, microorganisms are found everywhere and are the most ubiquitous organisms. One would expect this to be true of any extraterrestrial body.

Microorganisms have been selected as the best test organisms; and bacteria and fungi have been used because they are durable and easy to grow. Also, because of their rapid growth, many generations can be studied in a relatively short period of time. The organisms include chemoautotrophic bacteria, which are able to synthesize their cell constituents from carbon dioxide by energy derived from inorganic reactions; anaerobic bacteria, which grow only in the absence of molecular oxygen; photoautotrophic plants such as algae, lichens, and more complex seed plants; and small terrestrial animals. Some experiments have been done using higher plants, such as lichens and seed plants.

Organisms have been collected from the tundra, desert, alpine, hot springs, and saline habitats to obtain species with specialized capabilities to conserve water, balance osmotic discrepancies, store gases, accommodate to temperature extremes, and otherwise meet stresses. An attempt is made in these simulation experiments to extend these processes across the possible overlapping microenvironments which Earth and Mars may share.

Scientists have developed various special environmental simulators, including "Mars jars" and "Marsariums." These have made possible controlled temperatures, simulated atmospheres at desired pressures, water contents, and soil conditions for duplicating assumed Martian surface. A complex simulator, developed by Young et al. (1963), simulates the formation of a permafrost layer with the presence of some water tied up in the form of ice beneath the soil surface. This simulator serves as a model to study the wave of darkening, supporting the hypothesis that the pole-to-equator wave of darkening is correlated with the availability of subsurface water. The simulator is a heavily insulated 2 cu. ft. capacity chamber with an internal pressure of 0.1 atmosphere. The chamber contains a soil mixture of limonite and sand, and an atmosphere of carbon dioxide and nitrogen. With the use of a liquid nitrogen heat exchanger at one end and an external battery of infrared lamps at the other end, the temperature simulates that of Mars from pole to equator. Thermocouples throughout the soil monitor the temperatures in the chamber.

Zhukova and Kondratyev (1965) designed a structure measuring 100 x 150 x 180 cm. Microorganisms were placed at the surface of a bar made of electrolytic copper into a special groove isolated by glass cloth. Copper was selected as one of the best heat-conducting materials permitting a rapid change of temperature. The lower end of the bar was immersed into a mixture of dry ice and ethyl alcohol, which made it possible to create a temperature of -60°C. Heating was performed by means of an incandescent spiral mounted in the bar.

As the knowledge concerning the Martian environment becomes more refined, scientists can more accurately simulate this environment under controlled conditions in the laboratory. Determination of the effects of the Martian environment on Earth organisms will permit better theorization on the forms of life we might find on Mars, and will permit us to estimate the potential survival of Earth contaminants on Mars.

However, until the environmental conditions of Mars are defined more accurately, the experiments must be changed continually to fit newly determined conditions. This also makes existing simulation data less valid for comparison. The data resulting from the simulation experiments for Mars have been compiled in Table 4, and the experiments have been summarized below.

The earliest simulation studies were carried out by the Air Force, and the studies during the past six years have been supported by NASA. Recently, these studies have received less support or have been terminated in favor of critical studies on the effects of biologically important environmental extreme factors on Earth organisms. These critical studies permit the establishment of the extreme environmental factor parameters in which Earth life can grow or survive. These data will have valuable application with reference to the consideration of life on any planet; to design of life detection instruments; to sterilization of space vehicles; and to the problem of contamination of planets.

Some exploratory experimental studies are in progress to study the capabilities of organisms to grow under the assumed conditions on Jupiter. These include studies at high atmospheric pressure with liquid ammonia, methane, and other reducing compounds.

TABLE 4. SURVIVAL AND GROWTH OF ORGANISMS IN SIMULATED PLANETARY (MARTIAN) ENVIRONMENTS

SPECIES	SURVIVAL (MONTHS)	MOISTURE (%)	TEMPERATURE °C	ATM. PRESS. (in mm Hg)	N_2 (%)	CO_2 (%)	SUBSTRATE
(Conditions on Mars)	--	14±7u in air	-70 to +30	85, 25±15, 11	? prob. high	3 to 30	--
Anaerobic spore formers *Clostridia*, *Bacillis*, *Planosarcina*	6	low ($CaCO_3$)	-60 to +20	76	95	5	air dried soil
Anaerobic non-spore formers *Pseudomonas*, *Rhodopseudomonas*	"	" "	" "	"	"	"	" " "
Anaerobes *Aerobacter aerogenes* *Pseudomonas sp.*	growth	very wet	-75 to +25	760	100	?	Difco Infusion Broth
Clostridium, *Corynebacteria*, "thin short rod"	10	1.0 or less	-25 to +25	65	"	"	soil
Bacillus cereus	2	0.5 soil	-25 to +25	65	94	2.21	sandstone soil
Clostridium sporogenes	1 (growth)	8.4	-25 to +23	"	"	2.0	enriched soil
Clostridium botulinum *Klebsiella pneumoniae*	10 6	lyophilized in vacuum and air	-25 to +25	"	95	0 to 0.5	lava soil
Bacillus subtilis var. *globigii*	4	2.0	-25 to +25	85	"	0.3	media
Sarcina aurentiaca	4	0.5	-25 to +25	"	"	"	desert soil
Clostridium tetani	2 or less	1.0	-60 to +25	"	"	"	soil
Pea, bean, onion, tomato, rye, sorghum, rice	0.3	moist	+25	75	100	0	filter paper
winter rye	0.6	"	-10 to +23	76	98	0.24	soil
Aspergillus niger *Aspergillus oryzae* *Mucor plumbeus* *Rhodotorula rubra*	over 6 hours	very dry	-60 to +25	"	95.5	0.25	glass cloth on copper bar

Early experiments simulating Martian conditions using soil bacteria were carried out by Davis and Fulton (1959) at the Air Force School of Aviation Medicine, San Antonio, Texas. Mixed populations of soil bacteria were put in "Mars jars" with the following conditions: 65 mm Hg pressure, 1 percent water or less, nitrogen atmosphere, sandstone-lava soil, and a temperature day-night cycle of +25° to -25°C. The moisture was controlled by desiccating the soil and adding a given amount of water. Experiments, conducted up to 10 months, demonstrated that obligate aerobes died quickly. The anaerobes and sporeformers survived. Although a small increase in total number of organisms indicated growth, the increases in the number of bacteria may have been due to breaking up clumps of dirt.

Roberts and Irvine (1963) reported that, in a simulated Martian environment, colony counts of a sporeforming bacterium, Bacillus cereus, increased when 8 percent moisture was added. Moisture was considered more important than temperature or atmospheric gases inasmuch as a simulated Martian microenvironment containing 8 percent moisture permitted germination and growth of endospores of Clostridium sporogenes. Increases in colony counts of Bacillus cereus appeared to be due to temperature cycling (Roberts and Wynn, 1962).

Studies of the effects of simulated environments on sporeforming anaerobic bacteria were carried out by Hawrylewicz et al. (1962). They showed that the encapsulated facultative anaerobe, Klebsiellapneumoniae, survived under simulated Martian atmosphere for 6 to 8 months but were less virulent than the freshly isolated organisms. Spores of the anaerobe Clostridium botulinum survived 10 months in the simulator. Hagen et al. (1964) found that the addition of moisture into dry simulated Martian soil did not improve the survival of Bacillus subtilis of Pseudomonas aeruginosa. Bacillus cereus spores survived, with added organic medium plus moisture, but there was no germination of the spores.

Hawrylewicz et al.(1962) put rocks from Antarctica bearing various lichens in simulated Martian conditions in a large desiccator. They found the algal portion of a lichen, T. erici, showed only slight resistance to the Martian environment. They also pointed out the effect moisture had on the physical condition of lichen. The undersurface of a lichen has great water absorbing capability; and the slightest amount of moisture on a rock surface is absorbed by the lichen which can turn green in 15 minutes.

Scher et al. (1963) exposed desert soils to simulated environmental conditions and diurnal cycles of Mars. The atmosphere was 95 percent nitrogen, 5 percent carbon dioxide, (no oxygen) and was dried, using calcium sulphate as a desiccant. The total atmospheric pressure was 0.1 atmosphere. The temperature ranged from -60° to +20°C in 24-hour cycles. One hour was spent at the maximum and at the minimum temperatures. The chambers were irradiated with ultraviolet, 2537 Å, with a dose of 10^9 ergs/cm^2, which is comparable to a daily dose found on Mars, and easily exceeds the mean lethal dose for unprotected bacteria. Soil aliquots were removed weekly and incubated at 30°C. The scoring was done both aerobically and anaerobically. Both obligate and facultative sporeforming anaerobes, including *Clostridium*, *Bacillus*, and *Planosarcina*, and nonsporeforming facultative anaerobes, including *Pseudomonas* and *Rhodopseudomonas*, were found. The experimental chambers were frozen and thawed cyclically up to six months. Organisms that were able to survive the first freeze-thaw cycle were able to survive the entire experiment. The ultraviolet irradiation did not kill subsurface organisms, and very little soil served as an ultraviolet shield. All of the samples showed survivors.

Young et al. (1963) assumed that there is water present on Mars, at least in microenvironments, and that nutrients would be available. The primary objective of their experiments was to determine the likelihood of contaminating Mars with Earth organisms should a space probe from Earth encounter an optimum microenvironment in terms of water and nutrients. The experiments used bacteria in liquid nutrient media. The environment consisted of a carbon dioxide-nitrogen atmosphere; and the temperature cycling was -70° to +25°C, with a maximum time above freezing of 4-1/2 hours. *Aerobacter aerogenes* and *Pseudomonas sp.* grew in nutrient medium under Martian freezing and thawing cycles. Atmospheric pressure was not a significant factor in the growth of bacteria under these conditions.

Silverman et al. (1964) studied bacteria under extreme — but not "Martian" — conditions. Spores of five test organisms (*B. subtilis var. niger*, *B. megaterium*, *B. stearothermophilus*, *Clostridium sporogenes* and *Aspergillus niger*) and soils were exposed while under ultrahigh vacuum to temperatures of from -190° to +170°C for four to five days. Up to 25°C there was no loss in viability; at higher temperatures, differences in resistivity were observed. At 88°C only *B. subtilis* and *A. niger* survived in appreciable numbers; at 107°C only *A. niger* spores survived; and none were recoverable after exposure to 120°C. *B. subtilis* survived at atmospheric pressure and 90°C for five days, but none of the other spores were viable after two days. Four groups of soil organisms (mesophilic aerobes and anaerobes, molds, and actinomycetes) were similarly tested in the vacuum chamber. From one sample only actinomycetes survived 120°C, while one other soil sample yielded viable bacteria after exposure to 170°C. Several organisms resisted 120°C in ultrahigh vacuum for four to five days. When irradiated with gamma rays from a cobalt-60 source, differences were observed between vacuum dried spores irradiated while under vacuum and those exposed to air immediately before irradiation. A reduction of from 1/3 to 1/9 of the viability of spores irradiated in vacuum occurred with vacuum treated spores irradiated in air.

Siegel et al.(1964), in approximate simulations of Martian environments, studied tolerances of certain seed plants, such as cucumbers, corn, winter rye, etc., to low temperatures and lowered oxygen tensions. Lowered oxygen tensions enhanced the resistance of seedlings, particularly cucumber and rye, to freezing and lowered the minimum temperature required for germination. Germination of seeds in the absence of liquid water has also been studied. In this case, seeds of xerophytes have been suspended in air at 75 mm Hg pressure above water. The air is thus saturated. Germination is slow but does occur.

Siegel et al. (1963, 1964) found that the growth rate of several higher plants was enhanced by certain gases usually thought to be toxic, such as N_2O. This is significant inasmuch as the presence of nitrogen oxides in the Martian atmosphere has been cited as evidence for the nonexistence of plants on that planet by Keiss et al. (1960). Exploratory survival tests showed various mature plants as well as the larvae, pupae and adult specimens of a coleopteran were undamaged when exposed to at least 40 hours of an atmosphere containing 96.5 percent N_2O, 0.7 percent O_2 and 2.8 percent N_2.

Detection of biological activity of slow growing lichens was detected by metabolic gas exchange. Lichens are of interest because of their ability to survive and thrive under extreme environmental conditions on Earth. Carbon dioxide production is especially good as a detector. Siegel points out that this is a sensitive and nondestructive method, to be preferred to staining techniques, which at present are limited because they are only semi-quantitative, subjective, and destructive of the lichen.

A Russian study of simulated planetary environments has been performed with good simulation but for periods of only two to six hours. Comments on simulation experiments made by Zhukova and Kondratyev (1965) are presented as follows:

> "On the basis of modern conceptions on Martian conditions it is difficult to imagine that higher forms of animals or plants exist on the planet. A Martian change of seasons similar to that of our planet empowers us to think that there is a circulation of an organic substance on Mars, which cannot exist without participation of microbic forms of life. Microorganisms are the most probable inhabitants of Mars although the possibility is not excluded that their physiological features will be very specific. That is why the solution of the problem concerning the character of life on Mars is of exceptional interest. But still the answer to this question can be verified only by simulating Martian conditions, taking into account the information obtained from astrophysicists.

"Experiments aimed at creating artificial Martian climatic conditions have been started quite recently, their number is not large since they cannot be combined with the results of numerous experiments investigating the effect of extreme factors on microorganisms. The result of the effect of such physico-chemical parameters of the medium as pressure, sharp temperature changes, the absence of oxygen and insolation, depends on their combination and simultaneity. These examples convincingly show that while simulating Martian conditions one should strive to the most comprehensive complex of simultaneously acting factors. The creation of individual climatic parameters acting successively leads to absolutely different, often opposite results. It should be mentioned also that refusal to imitate insolation and the performance of experiments with specimens of soil which itself has protective effect on cells of microorganisms, but not with pure culture of bacteria, are usual shortcomings in the bulk of studies on this problem." (Davis and Fulton, 1959; Scher, Packer and Sagan, 1963.)

It appears that organisms from Earth, introduced to Martian environment, might survive in large numbers. Whether these organisms will be capable of growth and explosive contamination of the planet in a biological sense or not is highly questionable. The likelihood of an organism from Earth finding ideal conditions for growth on Mars seems extremely low. However, the likelihood of an organism from Earth serving as a contaminant for any life detection device flown to Mars for the purpose of searching out carbon-based life is considerably higher. The chance that life has originated and evolved on Mars is a completely separate question and much more difficult to answer.

It would be interesting to attempt to determine possible evolutionary trends which might occur on a planet by means of artificial selection of organisms in a planetary simulated environment. Rapid genetic selection combined with radiation and chemicals to speed up mutation rate under these conditions should reveal potential evolutionary trends under the planetary environmental conditions. This could be attempted after the planetary environments are more accurately defined.

Extreme and Limiting Environmental Parameters of Life

The question of the existence of extraterrestrial life is one of the most important and interesting biological questions facing mankind and has been the subject of much controversial discussion and conjecture. Many of the quantitative and even qualitative environmental constituents of the planets also are still subjects of controversy and speculation. Best guesses about a relatively unknown planetary environment, combined with even greater lack of information about the complete capabilities of Earth life to grow in extreme environments, do not provide the basis for very strong pronouncements.

Life on Earth is usually considered to be relatively limited with regard to its ability to grow, reproduce, or survive in extreme environmental conditions. While it is true that many common plants and animals (including man) are quite sensitive or incapable of surviving severe chemical and physical changes or extremes of environment, a large number of microorganisms are highly adapted and flourish in environments usually considered lethal. Certain chemoautotrophic bacteria require high concentrations of ammonia, methane, or other "lethal" chemicals to grow. Anaerobic bacteria grow only in the absence of oxygen.

In addition to adaptation to the extremes of environments on Earth, life is also capable of growing and reproducing under extreme environmental conditions not normally encountered, e.g., from a few rad of radiation in normal habitats to 10^6 or more rad from artificial sources, from 0.5 oersted (gauss) of Earth magnetism to 167,000 oersted in man-made fields, and from 1 g force of gravity to 110,000 g. The ranges of physical and chemical environmental factors for growth, reproduction, and survival for living Earth organisms are phenomenally large.

Life is ubiquitous on Earth and is found in almost every possible environment, including the most severe habitats, from the bottom of the ocean to the highest mountain tops and from cold arctic habitats to tropical jungle and hot springs, as well as in volcanic craters, deep wells, salt flats, and mountain snow fields. Certain microorganisms have also been obtained at 40,000 - 60,000 ft. altitudes during balloon flights. Earth life has become adapted to, and has invaded, nearly every habitat, no matter how severe. The physiological and morphological adaptations of life are exceedingly diverse and complex.

Surprisingly, the extreme parameters or ranges of the physical and chemical environmental factors permitting growth, reproduction, and other physiological processes of Earth organisms have not been critically compiled. A partial compilation of certain selected environmental factors has been made by Vallentyne (1963). A compilation of available published data on a few environmental extremes, particularly from recent NASA-supported research, is presented in Tables 5-8. These tables indicate the types of physical and chemical factors involved as well as the ranges for each factor. These data can serve as a starting point for more intensive literature review by specialists, critical evaluation, standardization of end points, and especially to point out areas where critical experimentation is urgently needed. The literature citations in the tables can be consulted in a more detailed report on this subject (Jenkins, 1965).

TABLE 5. EXTREME PHYSICAL ENVIRONMENTAL FACTORS PERMITTING GROWTH

PHYSICAL FACTORS	MINIMUM	ORGANISM	MAXIMUM	ORGANISM	ACTIVITY
Temperature	-30° C	algae (photosynthesis) pink yeast (growth)	104° C (1,000 atm.)	Desulfovibrio desulfuricans	growth and reduces sulfate
Magnetism	0-50 gamma (=x 10^{-5} oersted)	human	167,000 oersted (= gauss)	Neurospora, Arbacia, Drosophila	1 hr. - no effect Arbacia development delayed
Gravity	0 G	human, plants, animals	400,000 G 110,000 G	Ascaris eggs Eschericia coli	1 hr. - eggs hatch 40 days growth
Pressure	10^{-9} mm Hg. (5 days)	Mycobacterium smegmatis	1,400 atm.	marine organisms	growth
Electricity			10,000 volts, 10,000 amp.		
Infrared	0		2,450 mc, 0.3-1w/cm^2, 12.24 cm, 68 hours	Drosophila	growth not affected
Visible	0 foot candles	animals, fungi, bacteria	50,000 foot candles 17,000 " "	Chlorella higher plants	seconds, recurrently continuous
Ultraviolet	0 ergs per cm^2		10^8 ergs/cm^2, 2537°A	bean embryos	suppressed growth
X-ray	0 rad		2×10^6 rad		
Gamma ray	" "		2.45×10^6 rad	Microcoleus, Phormidium, Synechococcus	continued growth
Beta	" "		10^5 rad		growth
Sonic			1,000 kc	Elodea, yeast, rotifers	slow damage (in water)
Acoustic			140db or 6,500 dynes/cm^2 at .02 to 4.8 kc/sec	man	threshold of pain

TABLE 6. EXTREME TEMPERATURE LIMITS OF SURVIVAL

°C	ORGANISM	TIME OF EXPOSURE	°C	ORGANISM	TIME OF EXPOSURE
-190	yeast 10 sp. bacteria		140	bacterial spores	5 hrs. immersion
-197	Trebouxa from lichens				
-197	protozoa, Anguillula		170-200	desiccated rotifers	5 minutes
-252	yeasts, molds 10 sp. bacteria		151	" "	35 minutes
-253	black current, birch				
-273	bacteria, many species		150	Clostridium tetani	180 minutes
-273	" "		170	aerobic bacteria, molds, actinomycetes	5 days at 6×10^{-9} mm Hg.
-272	desiccated rotifers	8 hours	260 (dry)	bacteria (in activated charcoal)	60 minutes
-269	human spermatozoa		230 (wet)	Bacillus subtilis var niger	400 minutes
			248	" "	" "
			284	" "	70 minutes
			320	" "	15 minutes
			356	" "	2 minutes
			370	" "	1 minute
			248 (dry)	Bacillus stearothermophilis	100 minutes
			284	" "	12 minutes
			320	" "	2 minutes
			330	" "	1 minute
			248 (wet)	" "	25 minutes

TABLE 7. <u>EXTREME LOW AND HIGH TEMPERATURE EFFECTS PERMITTING LIFE PROCESSES</u>

TEMPERATURE °C	ORGANISM	ACTIVITY	TEMPERATURE °C	ORGANISM	ACTIVITY
-11	bacteria	growth (on fish)	73	thermophyllic organisms	growth (p^{32} metabolism)
			73	*Phormidium* (alga)	"acclimatized"
-12	bacteria	growth	70-73	*Bacillus calidus*	growth and spore germination
-12	molds	growth			
-15	*Pyramidomas*	swimming	70-74	*Bacillus cylindricus*	growth and spore germination
-15	*Dunaliella salina*	swimming	70-75	*Bacillus tostatus*	growth and spore germination
-17.8	mold	growth	80	*Bacillus stearothermophilus*	cultured in laboratory
-17.8	yeast	growth	83	sulfate-reducing bacteria	found in a well
-18	*Aspergillus glaucus*	growth (in glycerol)			
-18-20	mold	growth (in fruit juice)	89	" "	found in oil waters
-18-20	pseudomonads	" "	65-85	" "	cultured in laboratory
-20	bacteria	growth	89	microorganisms	found in hot springs
-20	"	"			
-20	"	luminescence	95	*Bacillus coagulans*	in 80 min. - sporulation activation
-20-24	insect eggs (diapause)	development accelerated			
-30	algae	photosynthesis			
-30 (?-34)	pink yeast	growth (on oysters)	110	" "	in 6 min. - sporulation activation
-30	lichens	photosynthesis	104	*Desulfovibrio desulfuricans*	grow and reduce sulfate at 1000 atm.
-20-40	lichens and conifers	"			
-44	mold spores	sporulation and germination			

TABLE 8. EXTREME CHEMICAL ENVIRONMENTAL FACTORS PERMITTING GROWTH

CHEMICAL FACTOR	MINIMUM	ORGANISM	MAXIMUM	PRESSURE (ATM.)	TIME (DAYS)	ORGANISM	ACTIVITY
O_2	0%	Hela cells, *Cephalobus*, anaerobic bacteria	100%	1		plants animals	growth
O_3 (ozone)	0%		100 ppm 500 ppm		5 "	*Armillaria mellea*	growth light emission
H_2	0%		100%			various plants	germination
H_2O	Aw 0.48 Aw 0.5	*Pleurococcus vulgaris* *Xenopsylla cheopis* (pre-pupae)	Aw 1.0	1		various aquatic organisms	growth
H_2O_2	0%		0.1 M/liter			rye	germination enhanced
He	"		100%			wheat, rye, rice	germination
CO	"		" 80%	 1.1	 4	rye *Hydrogenomonas*	" growth
CO_2	"		100%	"	"	rye "	growth and germination
CH_4	"		"	"	"	"	germination
CH_2O	"		50%			"	"
CH_3OH	"		"			"	"
N_2	"		100%	0.1	10	various plants	germination and root growth
NO	"		18%	0.018	10	sorghum, rice	" "
NO_2	"		"	"	"	rye, rice	" "
N_2O	"		100% 96.5%	1.2	4 1.7	rye " *Tenebrio molitor*	germination germination maintained
NH_3	"		95%				
Ar	"		100%	1.2	2	rye	germination
NaCl, Na_2SO_4, $NaHCO_3$			67%			Photosynthetic bacteria	growth

TABLE 8. EXTREME CHEMICAL ENVIRONMENTAL FACTORS PERMITTING GROWTH (CONT'D.)

CHEMICAL FACTOR	MINIMUM	ORGANISM	MAXIMUM	PRESSURE (ATM.)	TIME (DAYS)	ORGANISM	ACTIVITY
H_2S	0%		.96 g/L			Desulfovibrio desulfuricans	growth
H_2SO_4	"		7%, 2.5N			Acontium velatum Thiobacilli	" " reproduction
$CuSO_4$	"		1N sat. solu. 6% solution			Thiobacillus ferroxidans	growth
Cu^{++}	"		12 g/liter			" "	"
Zn^{++}	"		17 g/liter			" "	"
pH	0	Acontium velatum Thiobacillus thioxidans	13			Plectonema nostocorum Nitrobacter, Nitrosomas	" "
Eh	-450mv at pH9.5	sulfate-reducing bacteria	850 mv at pH3			iron bacteria	"

This critical compilation involves a review of a very broad and complex range of subjects involved in many different disciplines with widely scattered literature. Since the effects of many of the specific environmental factors are harmful, it is difficult to select a point on a scale from no effect to death, and use some criteria to say that normal or even minimal growth and reproduction are occurring. The effects of environmental factors are dependent on (a) the specific factor, times (b) the concentration or energy, times (c) the time of exposure or application of the factor. Many reports, especially older ones, do not give all of the necessary data to permit proper evaluation. A complicating factor is that the effect of each environmental factor also depends on the other environmental factors before, during, and after the application of a single stress factor. The condition of the organism itself is a great variable. Proper evaluation requires the critical review by a variety of biological specialists in the various disciplines as well as various types of physicists and chemists.

In order to determine the potentiality of Earth organisms to survive or grow under other planetary environmental conditions, a number of experiments have been carried out attempting to simulate the complex of planetary environments, especially of Mars, as reviewed in the previous chapter. While the results are of real interest, they do not provide much basic information. Further, as the Martian environment is more accurately defined, the experimental conditions are changed. In addition, some experimenters have altered certain factors, such as water content, to allow for potential microhabitats or for areas which might contain more water at certain times.

IV. BEHAVIORAL BIOLOGY

Effects of the Space Environment on Behavior

NASA was established in 1958, shortly after the Russian launching of the second Earth satellite, Sputnik II, which was the first vehicle to carry life into orbit around the Earth. This accomplishment was preceded by the pioneering work of Henry et al. (1952), in which animals were exposed briefly to sub-gravity states in Aerobee rockets. A motion picture camera photographed the behavior of two white mice in rotating drums during this series of flights, which marked the first time that simple psychological tests were made on rats in the weightless condition. While this behavioral experiment was relatively simple, it provided the basic concepts for recent studies which involved rotation of animals during the weightless state. Subsequent flights such as project MIA (Mouse in Able) reflected a preoccupation with physiologic measures (van der Wal and Young, 1958, 1959), although the flights of Baker and Able included pre- and post-flight performance studies (Graybiel et al., 1959). Able's behavior was recorded in detail on in-flight film, but none of the behavior was programmed nor was it under experimental control.

The first flights in which behavior or performance was explicitly programmed were those of Sam and Miss Sam in flights of the Little Joe Rocket with the Mercury capsule, launched from Wallops Island in 1959 and 1960 (Green et al., 1961). The first major achievement in the behavioral sciences was the successful in-flight measurement of behavior during the flight of the chimpanzee Ham in early 1961, in which the pre-trained animal performed throughout the flight. The second achievement along these lines was accomplished in 1962 when the chimpanzee Enos made several orbits around the Earth and performed continuously on a complex behavioral task. The behavioral tasks which the animals performed during both of these flights have been described in detail by Belleville et al. (1963) and the results of the in-flight performance have been presented by Henry and Mosely (1963). These were the first and last flights in which behavior was of central concern. These early flights provided much of the technological framework of knowledge on which current scientific experiments on biological organisms exposed to flights of extended durations are based. Due largely to the efforts of Grunzke (1961a and b), the apparatus needed to sustain animals during space flight, such as zero gravity watering and feeding devices, are now commonplace (Gilbert, 1964). Advanced systems of programming stimulus presentations and response recording, developed for Project Mercury, may now be seen in many basic research laboratories throughout the country.

Several other noteworthy advances have been made as an outgrowth of the Mercury animal flights. The orbital flight, MA-5, in which the chimpanzee Enos was employed, disclosed at flight time the unexpected fact that this five year old animal was hypertensive. Subsequent centrifuge studies showed that its vascular responses exceeded those of a control group. Consideration of the animal's pre-flight experience led to speculation concerning the mechanism of origin of this hypertension. An explanation of the high blood pressure responses detected in Enos has been pursued by Meehan, et al. (1964). Persistent hypertension has been produced in other laboratory chimpanzees restrained in the same manner as those participating in space flight and exposed to demanding performance tasks, a demonstration which has important implications for prolonged manned space flight and for cardiovascular medicine in general.

Studies more directly concerned with behavior and performance have been extended from those of Project Mercury. These extensions have been in the following directions: (a) In the establishment and maintenance of complex behavioral repertoires under conditions of full environmental control, (b) In the refinement of behavioral techniques for assessing sensory and motor processes, and (c) In problems surrounding the maintenance of sustained performance under conditions of long-term isolation and confinement, and preliminary extension of such experimental analysis to man (Findley and Migler, 1963).

Numerous studies with primate subjects, including several at Ames Research Center, have been devoted to developing the technology for maintaining optimum performance in environments with limited sources of stimulation. Monkeys, baboons, and chimpanzees, for example, have been isolated for periods of longer than two years with no decrement in performance on complicated behavioral tasks (Findley and Weissman, 1961). The behavioral technology employed in these studies is closely related to that employed on human subjects under NASA sponsorship at the University of Maryland (Findley and Brady, 1963). The essence of this technology is in the proper programming of environmental stimuli (Findley, 1962). Performance appears to be as much a function of the contingencies which produce the environmental stimuli as it is of the environmental stimuli produced. Thus, it is not sufficient to provide the subject with his physiological requirements for survival, but he must be given the psychological motivation for using these provisions. This, of course, is an oversimplification of the problem, but it serves to illustrate the essence of these experimental programs. If by programming the environment, one can establish a firm interrelationship between the less rewarding (aversive) and more rewarding (positive reinforcing) behaviors and stimuli, he will greatly reduce the problems of a restricted environment.

It has long been known that gravity is one of the major factors influencing various life processes as well as the orientation of both plants and animals. One of the most challenging problems of space research has been to define more precisely the nature of this influence. In the study of the role of gravity on living processes is an implicit concern for the related problem of the effects of weightlessness. Of particular interest to psychologists are possible modifications an altered gravitational environment might produce in behavioral patterns basic to the animal's maintenance and survival, such as eating, sensory and discriminative processes, development and maturation, and learning capacity, to name a few (Beasley and Seldeen, 1964).

One very prominent method of studying gravitational effects is to simulate a change in gravity by centrifugation. Smith, et al. (1959) and Winget, et al. (1962) have investigated the effects of long-term acceleration on birds, primarily chickens, while Wunder (1961, 1962) and his co-workers (1963) have been engaged in research on fruit flies, mice, hamsters, and turtles (Dodge and Wunder, 1962; Oyama and Platt, 1964; Steel, 1962; and Mathews, 1953). The general findings are that when animals are subjected to a prolonged period of acceleration of moderate intensity they exhibit a suppression of growth, delayed maturation, and an increase in the size of certain muscles and organs, dependent on the species. With regard to the suppression of growth effect, the data of these investigators show some exceptions. When the gravitational increase is kept below a certain limit, growth was seen to be enhanced over that of control subjects in the fruit fly, turtle, mouse and chicken. The limit below which enhancement of growth was observed varied with the species studied.

The data with regard to food intake do not present a consistent picture. Wunder (1961) found that food intake in accelerated mice was markedly reduced from the intake of non-accelerated control animals. Smith, however, found that in chickens food intake increased up to 36% over intake of control animals, and has derived an exponential function between food intake and acceleration intensity. It is interesting that food retention of the centrifuged chickens was not altered noticeably from that of control chickens. After six generations of selective breeding, Smith has produced a strain of chickens which has shown better adapability to prolonged exposure to high g environments.

A very relevant finding of their research with birds was that exposure to chronic acceleration in some way appears to interfere with habituation to rotatory stimulation. Chickens who were being subjected to chronic acceleration were given repeated rotatory stimulation tests to estimate their labyrinthine sensitivity. This study revealed that centrifuged animals showed a marked reduction in labyrinthine sensitivity as exhibited by their failure to habituate to the stimulus. This effect appeared to persist after the acceleration was terminated. In animals who developed gait or postural difficulties as a result of acceleration, there was no evidence of an after-nystagmus in response to the rotatory stimulation test, which, the investigators point out, may be evidence of a lesion in the labyrinth or its neural pathways.

Smith has implicated social factors as playing a strong role with regard to acceleration effects. His subjects were typically accelerated 4 or 6 to a cage. When the groups are intermixed midway through the experiment they exhibit a higher mortality rate and incidence of acceleration symptoms than do groups whose constituency has remained unchanged.

At the U.S. Naval School of Aerospace Medicine, numerous studies have been conducted concerning the effects of slow rotation on the behavior and physiology of humans and animals (Riccio, 1965). The behavioral findings, thus far, have been conclusive but non-specific. Rotation initially produces decrements in performance, but adaptation to a rotating environment ensues quite rapidly (Guedrey et al., 1957, 1958, 1962). Perceptual distortion, nausea, nystagmus, and other behavioral evidences of discomfort are prevalent responses to slow rotation. These symptoms are generally reduced with continued exposure (adaptation). Interestingly, however, adaptation is delayed when the subjects are exposed to a fixed reference outside of their rotating environment.

At NASA-Ames rodents have been used in experiments to further delimit the stimulus effects of rotation (Weissman and Seldeen, 1965). In these experiments the subjects must discriminate between different speeds of rotation in order to obtain food reinforcement. The results thus far provide evidence that these animals are capable of discriminating between the different speeds at which they are being rotated. The range of speeds studied was 0-25 rpm with tests of discriminability being made at intervals of less than 5 rpm. Experiments such as these will lead to the development of techniques for measuring behavioral sensitivity in many species of subjects including man.

The optimum type configuration of future manned spacecraft will depend, in part, upon biomedical considerations. Voluminous literature now exists on the possible hazards to man of prolonged exposure to zero gravity conditions. Should prolonged weightlessness prove to be a serious detriment to health, careful consideration must be given to design concepts which provide artificial gravity.

No data exist on the minimal gravity requirements necessary to sustain basic biological functions for extended periods of time. A limit of 0.2 g has been given as the lower level at which man can walk unaided (Loret, 1961). It has also been recommended that angular velocity be maintained at the lowest possible level in order to minimize the occurrence of vestibular disturbances.

These recommendations are based on human factors requirements, rather than upon biological considerations, which may significantly modify these values. In our studies, a technique has been devised which promises to provide reliable criteria for biological acceptability, since it is based on fundamental biological and behavioral principles.

In the progression of organisms up the phylogenetic (or evolutionary) scale, survival depends less and less upon stereotyped physiological reactions which occur in reflex fashion, in response to environmental stimulation. In higher organisms, survival depends more upon the capacity of organisms to modify their behavior. At the highest levels of functional efficiency, the ultimate form of adaptation is seen--the manipulation of the environment by the organism. This type of behavior is termed "instrumental" by psychologists, in that responses of the organism are instrumental in producing an environmental change.

Developments in behavioral science now permit us to utilize the adaptive behavior of animals to investigate many problems of biological interest. Recent studies on the self-selection of gravity levels represent a further attempt to exploit the adaptive capacities of animals, in order to provide information relevant to problems of space exploration.

One such project allows animals to select their own gravity environment in an apparatus designed to create g-forces through centrifugal action by rotating around its central shaft at 60 rpm (Belleville et al., 1964). The surface of this centrifuge is parabolic, so that the resultant force of the centrifugal g and the Earth's gravity of 1g are always normal to the surface.

When the animal moves away from the center, increasing the radius of rotation, it is exposed to increasing gravity. Motion toward the center reduces the gravity level. By this means, an animal is free to select its own gravity environment. These procedures, if conducted in a weightless environment, would permit selection of levels down to the zero g level.

When the animal moves either toward or away from the center, he is moving from one tangential velocity to another. He is therefore acted upon by a third force--due to Coriolis' acceleration. The effects of such Coriolis forces are a major problem which is difficult to extricate in studies such as these, but which must be taken into account in the design of spacecraft which produce artificial gravity by centrifugation. Motion of the head in any direction not parallel to the centrifugal force vector would result in bizarre stimulation of the semicircular canals and consequent motion sickness. This effect is likely to become even more pronounced if the threshold of responsiveness of these organs is lowered by prolonged exposure to reduced gravity. Methods such as these are currently being developed for conducting a refined psychophysical analysis of gravity, including studies on the perception of angular, linear and Coriolis' acceleration (Lange and Broderson, 1965).

The results of animal studies such as these, which combine the skills of mechanical engineering and experimental psychology will be of great value in arriving at a decisive judgment concerning the need for artificial gravity in a manned orbiting space station, or other vehicles designed for long-term occupancy.

To aid in the interpretation of in-flight data, other studies are under way to determine the functions of the vestibular system, as a principal brain center related to orientation in space and to the physiology of posture and movement, as well as with the influences of acceleration, rotation, and weightlessness. Experiments are presently being conducted on monkeys and cats in order to trace these complex neurological connections and to determine their functional organization.

Biological Information Systems

The nature of "memory" has been the subject of considerable speculation in the past. It has long been felt intuitively that retention of information in the central nervous system involves either an alteration of pre-existing material or structure, or, alternatively, synthesis of materials not present previously. The cellular site of operational alternation was unknown but, again intuitively, was felt to be closely associated with the synapses. The problems faced by early investigators were great; but, nevertheless, much data of pertinence to the question of biological information storage were obtained. With the relatively recent advent of more refined tools and methodologies, there has been rapid progress.

A significant amount of the work which has been conducted in the area of biological information and communication systems is easily defined as "basic research" (Hebb, 1949; Jeffress, 1951; Konorsky, 1950; Eccles, 1953; Young, 1954; Elliot et al. 1955).

This discussion will be limited to those aspects closely related to the fields of molecular biology and experimental psychology, which seem to have universal application to all animal life forms (as we know life). Studies elaborating the basic principles of acquisition, processing, storage, and retrieval of information in living systems are emphasized.

Early Work

Early speculations on the operational nature of memory have been based upon relatively little experimental evidence. Charles Darwin observed that domestic rabbits had smaller brains than their wild counterparts, and attributed this to lack of exercise of their intellect, senses, and voluntary movements. Unfortunately, subsequent studies of the brains of man with greatly differing intellectual capability did not substantiate the hypothesis. Idiots sometimes had larger brains than geniuses.

Thus, an idea proposed by Ramon y Cajal came into favor. Since neural cells did not increase in number after birth, he proposed that memory involved the establishment of new and more extended intercortical connections. Unfortunately, methods were not available to test this hypothesis adequately and it has remained, until quite recently, in the realm of conjecture.

Another major hypothesis was that there were two or more stages in the information storage process. The final form the information took in the brain was called a brain engram or memory trace. However, prior to the formation of the engram, a transitory, purely electrical process denoted as "reverberational memory" was postulated to exist for a relatively short time (minutes to hours) (Hebb, 1949; Jeffress, 1951). This hypothesis was used by Pauling to explain why an elderly chairman of a board could brilliantly summarize a complex eight-hour meeting and yet, after its conclusion and his return to his office, not even remember having attended the meeting. Thus, this individual's reverberational memory functioned well, but advanced years had seriously impaired his brain's ability to form a permanent engram. Similar, although less dramatic, observations in other situations are not uncommon. A wide variety of experiments have been conducted to study this aspect of memory and to relate it to the process whereby the information is transformed to a more stable form (Thorpe, 1956; Sperry, 1955; Burns, 1958).

More recently, the concept of a specific biochemical activity during the process of long-term storage of information has gained considerable favor. Initially, neither the site nor the nature of the change was well defined. Quite recent studies by Krech et al. (1956, 1959), Bennett et al. (1958a), Rosenzweig et al. (1956, 1960) support the view that alteration of the levels of acetyl-cholenesterase at cortical synapses play an important role in information storage. These studies will be discussed in a later section. However, these authors do not claim that the changes observed are unambiguously related to the storage of memory. It may well be that the alterations observed are in some way related to this process but are still secondary to some other, more basic, process.

An alternative hypothesis is that the information resides in its ultimate form in some more central structure of the neurone than the synapse. (It has even been postulated that the basic information is stored in non-neurono-cortical material.) Perhaps Halstead was the first to postulate the involvement of nucleoprotein in this process (Jeffress, 1951). From the biochemist's point of view, this is an extremely attractive hypothesis. Both proteins and nucleic acids possess sufficient possible permutations of structure to permit storage of a lifetime's accumulation of bits of information in an organ the size of the brain. From the previously known ability of the nucleic acids to code information (of a genetic nature), they are the prime suspects. However, from the known regulatory ability of nucleic acids to mediate specific protein synthesis, it is possible that the final repository is protein.

Recent Biochemical Studies

Among the foremost investigators of the chemistry and biochemistry of the central nervous system is Holger Hyden at the University of Gottberg, Sweden. He (1962) and others (Brucke, 1959; Brachet and Mirsky, 1960) have for many years developed and performed elegant microanalytical studies of single nerve cells. The evidence which he has obtained is consistent with the hypothesis that the initial electrical reverberations in the brain induce a change in the molecular structure of the ribonucleic acid (RNA) of the neurones which, in turn, leads to a subsequent deposition of specific proteins. It is well known from other investigations that a major role of RNA in any type of cell is to specify and mediate synthesis of the protein enzymes of the cells. Thus, in this hypothesis, it is only necessary to postulate the modification of brain RNA by the activities associated with reverberational memory. Particularly pertinent to this hypothesis are observations that:

1. Large nerve cells have a very high rate of metabolism of RNA and proteins, and, of the somatic cells, are the largest producers of RNA.

2. Vestibular stimulation by passive means leads to an increase in the RNA content of the Deiters' nerve cells of rabbits (Tower and Schade, 1960). The protein content of these cells is also increased.

3. Changes in the RNA composition of neurones and glia of the brain stem occur during a learning situation.

The animals were trained over a period of four to five days to climb a steeply inclined wire to obtain food. The big nerve cells and their glia of the lateral vestibular apparatus were analyzed, since the Deiter's neurones present in this structure are directly connected to the middle ear. The amount of RNA was found to be increased in the nerve cells; and, more significantly, the adenine to uracil ratio of both the nuclear RNA of nerve cells and glia cells became significantly increased (Hyden, 1962).

A variety of control experiments were conducted. Although there was an increase in RNA content of these cells in animals exposed to passive stimulation, there was no change in the ratio of adenine to uracil. Nerve cells from the reticular formation, another portion of the brain, only had an increased content of RNA.

Animals subjected to a stress experiment involving the vestibular nucleus showed only an increase in content of RNA. Litter mates living in cages on the same diet as learning animals showed no change in content of RNA. Thus, it would appear that the change in the base ratio of the RNA synthesized is not due to increased neurone function *per se*, but is more directly related to the learning process. The fact that this was nuclear RNA infers that it was immediately related to chromosomal DNA.

4. Neuronal RNA with changed cytidine-guanine values synthesized during a short period of induced protein synthesis could be blocked by actinomycin D. It was concluded, therefore, that the RNA was immediately DNA-dependent and directly related to the genetic apparatus.

5. There is an increase in content of cortical neurones during a learning situation.

Rats which were normally right-handed were forced to modify their handedness in order to obtain food. The RNA of nerve cells in that part of the cortex, whose destruction destroys the ability to transfer handedness, was analyzed. A significant increase in RNA of nerve cells of the fifth to sixth cortical layers on the right side of the brain was observed. The corresponding nerve cells on the opposite side of the same brain served as controls. There was an increase in RNA and an alteration in the base ratios leading to a significant increase in the purine bases relative to the pyrimidine bases in the learning side of the cortex. When the animals were not forced to learn a new procedure, only an increase of RNA was observed, with no change in base ratio.

Dr. Frank Morrell, Head of the Neurology Department at the Stanford Medical School, has also been active in this field during the past six years. He has found that if a primary epileptic lesion is induced on one side of the cortex, a secondary mirror lesion eventually develops in the mirror focus of the contralateral homologous cortex. This secondary lesion, which showed epileptiform discharge which was self-sustaining, could be isolated, whereupon the epileptiform discharge disappeared. This was interpreted as learned behavior of the secondary lesion. Based upon changes in the staining properties of the secondary lesion, Morrell concluded that changes in RNA had occurred in the cell. Changes in the composition of the RNA could not be shown by these techniques.

At the University of California at Berkeley, Drs. Rosenzweig, Bennett, and Krech have conducted extensive studies related to this topic. These investigators have directed their efforts toward demonstrating alterations in the cerebral cortex of animals exposed to continuing learning situations or continuously deprived of sensory stimulation. In a recent publication (Rosenzweig et al., 1956), which also summarizes a considerable amount of previous work, they report studies which demonstrate the following:

1. Rats given enriched experience develop, in comparison with their restricted litter mates, greater weight and thickness of cortical tissue and an associated proportional increase in total acetyl-cholenesterase activity of the cortex.

2. The gain in weight of cortical tissue is relatively larger than the increase in enzymatic activity. Acetyl-cholenesterase activity increases in other portions of the brain even though tissue weight decreases.

3. The changes appear in a variety of lines of rats, although differing in amount and between strains.

4. The changes are observed in both the young and adult animals.

The previous studies were comparisons between enhanced experienced animals and animals maintained in isolated condition. Animals which were housed in colonies, but given no special treatment, showed intermediary effects in those situations studied.

The Berkeley group emphasized that the findings of changes in the brain subsequent to experience does not prove that the findings or the changes have anything to do with memory storage; rather, the findings establish the fact that the brain can respond to environmental pressure. However, the results are compatible with the hypothesis that long-term memory storage involves the formation of new somatic connections among neurones. Calculations of the amount of additional material required to permit this to exist are compatible with the increases observed.

A number of investigators have studied the effects of antimetabolites and drugs on the learning process. Since their specific metabolic effects are known in other tissues, the rationale is that if these materials do interfere with memory then specific types of metabolic activities may be implicated in the deposition of the engram.

One of the initial studies of this type was conducted by Dingman and Sporn (1961), presently at the National Institute of Mental Health. They showed that 8-azaguanine, a purine antagonist of the nucleic acids, injected intracisternally was incorporated into the RNA of the brain of rats. Associated with this incorporation was an impairment of the maze-learning ability of the animals. These findings have been confirmed.

Flexner and his associates injected puromycin, an inhibitor of protein synthesis, into the brains of mice, which were then trained to perform in a maze. Losses of short-term or long-term memory were obtained, depending upon the site of the injection. The results indicate that the hippocampal region is the site of recent memory.

The hippocampal region is of interest in connection with memory processes for a number of other reasons. Adey (1963) and his group observed a transient fall in electrical impedance in this region when cats learned to perform in a T-maze in response to a visual cue. It was supposed that the electrodes were situated within glial cells of the dendritic zone of the hippocampal pyramidal cell layer. Extinction of the learned habit abolished the briefly evoked impedance changes, which subsequently reappeared with retraining. Heath and his associates at the Tulane Medical School have found significant changes in the electrical activity of the hippocampal region when taraxein is injected. Taraxein is obtained from the serum of humans with schizophrenia, and in certain circumstances is able to induce schizophrenic-like behavior in both humans and monkeys.

A number of other studies more or less indirectly implicate RNA in the learning process. For instance, in retinal cells of rabbits raised in darkness, there was virtually no ribonucleo-protein as compared with normal amounts in the cells of animals raised in light (Brattgard, 1952). Further, maintenance of normal electrical activity of isolated perfused cat brains is highly dependent upon the presence of the ribonucleic acid precursors uridin and cytidin in the perfusate (Geiger, 1958) and severe derangements occur if any of a variety of pyrimidine nucleic acid antagonists are added (Geiger and Richter (ed.), 1957). Brief electrical stimulation of cat cortical tissue causes an increase in nucleic acid cytidin and adenine, thus indicating a synthesis of altered polynucleotides. Finally, injections of RNA in animals have shown interesting effects. When given at a dose of 116 mg/kg daily for one month, rats showed an enhanced response and greater resistance to extinction in a shock-motivated behavioral response. It has been shown by another group that injections of RNA enhance the ability of young animals to learn various tasks.

Planaria have been used in a variety of studies which seem to bear on the problem of memory. Quite recent evidence by Bennett, Calvin, and their associates have cast somewhat of a pall over the studies; nevertheless, the work may have some validity. Interest in the use of flatworms, particularly planaria, for study of memory began with a demonstration by McConnell that these simple animals could undergo conditioning (Thompson and McConnell, 1955). Subsequently, it was found that some conditioning was retained when the animal was transected and allowed to regenerate. The retention of training was found in both new animals, although the very simple brain, really only two ganglia, was in the head section (McConnell et al., 1959).

Apparently, some diffusely distributed component of the animal was responsible for retention of learning. Evidence has accumulated to indicate that this material is RNA. Among this evidence is the following:

1. The two halves of a trained planaria were allowed to regenerate in a solution containing RNA-destroying enzymes. Whereas the head ends retained some training, no retention was observed in the animals derived from the tail end (Corning and John, 1961).

2. When pieces of trained planaria were fed to untrained animals, the untrained cannibal required a shorter time to become trained to a critera. It would appear that the digestive system of planaria is so simple that the material responsible for the transfer of the information was not broken down.

3. When RNA, obtained from trained planaria, is injected into the digestive tract of untrained animals, there is a transfer of information.

Neurophysiology*

Neurophysiological studies concern the functions of the nervous system – in particular the central nervous system (CNS) – under normal, simulated and actual flight conditions. Of paramount importance is the maintenance of equilibrium and orientation in three-dimensional space. The ability of man and his close relatives among the vertebrates to maintain these functions depends on an integrated sensory input from the vestibular organ, the eyes, the interoceptors of the muscles, tendons, joints and viscera, and the exteroceptors of the skin.

Certain parameters of the environmental and space flight conditions drastically affect man's ability to maintain equilibrium and spatial orientation. Centrifugal forces modify or reverse the directional vector of gravity. Linear acceleration may increase enormously, as may

*An excerpt from Chapter 34, Physiological and Behavioral Sciences, Proceedings of the NASA-University Conference on the Science and Technology of Space Exploration, Vol. 1, November 1-3, 1962, by Siegfried J. Gerathewohl and Bo E. Gernandt.

angular stimulation. The sensory organs listed above are unreliable under such conditions. The very organ which is designed specifically to furnish information on spatial orientation may malfunction in man while he is in flight. Thus, with respect to sensory orientation, these labyrinthine organs are by no means precision instruments.

The use of classical histological methods and the observation of equilibrium disturbances resulting from operative interference with the internal ear have in the past been the two principal sources of knowledge concerning the structure and function of the labyrinth, but the answers given to various questions vary considerably in their value. The development of electrophysiological techniques and the refinement in recent years of the ultrastructural analysis by means of the electron microscope may allow more precise experimental studies of the correlation of function and structure.

Before considering vestibular impulses in their bulbar and descending spinal pathways, a recent study concerning the generation of impulses in the labyrinth must be mentioned. Von Bekesy's (1951) finding of the direct current potentials in the cochlea aroused speculation about the existence of similar labyrinthine potentials. Such DC potentials were also detected in the semicircular canal of the guinea pig by Trincker (1957), who measured the potential changes in the endolymph, surface of the cupula, or side of the crista during cupular deflection. It seems likely, however, that the effects do not represent the physicochemical changes in the cupula but the electrical potentials in the nerve and nerve endings of the crista. Attempts at differentiating these effects have failed so far. Great expectations are brought by the advances of microchemistry, microphysiology, and physical chemistry with regard to the excitatory processes, the generation of the nerve impulse. Quite apart from a need to understand vestibular nerve discharges and patterns more adequately in such terms, the analysis of the vestibular system has in the past revealed general biological principles which were not readily discernible through the examination of other issues (Livingston, 1960).

The neural connections of the vestibular organ consist of numerous chains of neurons, reciprocally linked in many ways and having their synapses in various anatomical nuclei. All the chains work in intimate collaboration, and the final pattern of reflex responses is attributable largely to the highly complex integrating activity of the center. The labyrinthine function is automatic, carried out in a reflex fashion; in other words, mostly below the level of consciousness. The brain centers through which the labyrinth elicits the various appropriate muscular reactions of the head, body, limbs, and eyes – the righting, the postural and the ocular reflexes – represent an intricate mechanism. Before we can hope for a satisfactory understanding of their functional organization we will have to know their anatomy in more detail. Thus, we are confronted with a fruitful field for the exploration of basic mechanisms of neuronal activity. Major advances during the last years have provided us with new information about the neuroanatomy of the vestibular system (Brodal, 1960a, b; Carpenter, 1960; Dow and Morruzzi, 1958).

Vestibular impulses invading the brainstem ascend and descend the neuroaxis and cross the midline. It was previously implied that the vestibular apparatus had only subcortical projections. Recently, however, it has been established by means of electrophysiological methods that the organ is represented by a projection area in the cerebral cortex of some animals (Kempinsky, 1951; Mickle and Ades, 1952; Walzl and Mountcastle, 1949; Anderson and Gernandt, 1954). The use of brief electrical stimulation of the vestibular nerve in order to elicit a cortical response has been of great value for the mapping of these areas.

Among a great variety of sensory receptors, the vestibular ones are capable of evoking the most widespread somatovisceral effects throughout the body. Moreover, vestibular effects seem to be relatively more imperious and less dependent upon the state of readiness of the nervous system. As a consequence of the extensive distribution of vestibular effects, there are many opportunities for central integration. From the intricate compensatory motor performance following activation of the vestibular system, it can be asserted that vestibular activity is influenced in a delicate and purposeful manner. Proprioceptive and vestibular systems are both known to be active in posture and locomotion; streams of impulses arising from the receptors in each of these systems must converge to influence the activity of the final common path. The state of the motor centers of the spinal cord, as affected by vestibular stimulation, has been tested by dorsal root and other sensory input interventions. These experiments have provided us with insight into the mechanisms concerned with the vestibular control of spinal reflexes (Gernandt et al., 1953, 1955, 1957, 1960a, b).

It has long been known that the vestibular apparatus is essential for the development of motion sickness. Commonplace subjective experience of nausea relates to visceral changes mediated through autonomic efferent pathways and may ultimately involve rhythmic somatic nerve discharges to skeletal muscles responsible for retching and vomiting. However, very little is known about the central nervous mechanisms responsible for elaboration of the whole syndrome. Since the maintenance of vestibular bombardment for some length of time seems essential for the development of motion sickness, one would presume this to be an instance of slow temporal summation. Experimental findings demonstrate a powerful effect of temporal summation upon somatic motor outflow during vestibular stimulation (Akert and Gernandt, 1962), and not upon parasympathetic outflow.

The practical implication of these studies is closely related to physiological effects of weightlessness. Based on experimental evidence from short weightless periods obtained in aircraft it was concluded that "when the exposure becomes longer, there may develop minor physiologic disturbances which, if cumulative or irritating, may cause or enhance psychiatric symptoms" (Gerathewohl and Ward, 1960). Many of the early theories were repudiated or verified by controlled experiments and casual observations. Although the zero-g condition per se does not cause spatial disorientation if visual cues are provided, the astronauts reported a temporary loss of orientation during the orbital flight while they were engaged in activities which diverted their attention. However, no disturbing sensory inputs were observed during the weightless period. Violent head maneuvers within the limited mobility of the helmet were performed in every direction without symptoms of illusions or vertigo. The subjective sensations of "tumbling forward" after sustainer engine cutoff reported by the Mercury astronauts, and Titov's motion sickness attacks, which were particularly dismaying during head movements, were well within the entire range of psychosomatic experiences already obtained during aerodynamic trajectories (Gerathewohl, 1956). Interestingly enough it now appears that the otolithic output in mammals and man is the differential quotient of linear acceleration, but unaffected by zero-g.

Of pertinent interest in this connection are the problems which may be encountered during and following long-term exposure to weightlessness. Although there is no evidence of adverse effects on operative behavior, the possibility of biological disturbances on a cellular or sub-cellular level, which may cause a deterioration of the somatic basis, has been repeatedly stressed. Whether effects of this sort will occur or whether the organism will be able to adapt is still an open question. Since motion sensitivity based on vestibular stimulation is highly different among individuals, the selection of astronauts is not only necessary but may also solve the problem of agravic vestibular disturbance. Reports from the MA-8 (Sigma 7) and Vostok III and IV flights seem to support this assumption. Moreover, experiments are being made in the slow rotation room at the Naval School of Aviation Medicine to study the Coriolis effects which arise when "artificial gravity" is produced by angular acceleration. Since man can adapt to wave motion on shipboard within a few days, a similar process may be expected to occur in case of long-term weightlessness (Henry et al., 1962).

V. MOLECULAR BIOLOGY AND BIOINSTRUMENTATION

In order to support biological investigations in space and to accumulate baseline data needed for the manned space flight, NASA has supported a program in laboratory research and theory. A multidisciplinary approach has included such fields as ecology, physiology, organic and biological chemistry, engineering, electronics, and optics. Qualitative and theoretical research has been emphasized rather than purely descriptive research. Studies have emphasized the investigation of fundamental biological phenomena at all levels--from the molecular to the total life form.

MOLECULAR BIOLOGY

Research in molecular biology has included chemical, physical, biological, and theoretical investigations of prebiological conditions on Earth and, possibly, on other planets; studies of cellular inclusions; genetic material (DNA and RNA) and coding, as well as energy transfer between biological materials.

The understanding of prebiological conditions on the Earth--and possible conditions on other planets--depends upon the nature of the complex chemical species which might be encountered. It is accepted by many scientists that biologically interesting compounds, such as amino acids, can be generated in a primitive atmosphere by the application of an electrical discharge, ultraviolet radiation, or heat to a gaseous mixture. Detectable biologically interesting compounds can be removed from such a system by condensation or absorption. However, in the limited time and space available in such experiments, many compounds are not produced in sufficient quantity to be measured in the laboratory.

In an attempt to analyze compounds similar to these, the National Biomedical Research Foundation and the National Bureau of Standards are conducting an investigation starting with molecular species. The distribution of molecular species at equilibrium is independent of the way in which that equilibrium was reached. It depends only on pressure, temperature, and elemental composition. Many of the conditions which might have arisen naturally can be approximated by thermodynamic equilibrium. Compounds which can be formed at equilibrium need no special mechanism to explain their presence. However, special mechanisms have to be sought for those compounds which could not be so produced and which would have been required for the structure and nutrition of the first living things.

In the absence of a precise theory of the composition of the primitive and planetary (other than Earth) atmospheres, equilibrium concentrations with a wide range of temperatures, pressures and elemental compositions are being explored by the above-mentioned organizations. These investigators have postulated that the maximum atmospheric pressure may have approached 100 atmospheres, if the primitive Earth was sufficiently hot and if an appreciable portion of the water on the Earth's surface today was present on the primitive Earth. (If the present oceans were to evaporate, the surface pressure would be approximately 300 atmospheres.) Low pressures of 10^{-6} atmospheres are being used to approximate upper atmospheres. Temperatures between 500° and 1000° absolute are being used.

A large range of N, O, C and H compositions are being explored for interesting and plausible combinations of factors. In these calculations an IBM 7090 computer is being used to obtain data on a very large number of combinations of chemicals. Other chemical species will be added as the research continues. Some results of this study give an insight into the variety of biologically significant chemicals which might possibly have existed during the Earth's primitive prebiological condition or may now exist on other planets' surfaces and atmospheres. (Dayhoff, et al., 1964; Armstrong, et al., 1964; White, et al., 1958.) The general method described by White, et al. (1958) which minimizes the free energy of the system was used. The solution was approached by an iterative process, starting with an initial guess of concentrations of the compounds. At each step, M + 1 linear equations are solved where M is the number of elements in the system.

In addition to listings of concentrations of all compounds included in each problem, it has been found convenient to express the results of many problems on a triangular composition diagram. A coarse grid of 60 points is used to survey all elemental compositions. Finer grids are used in regions of interest. The resultant concentrations of the compounds at each composition are stored and, finally, a series of triangular diagrams is printed out, each showing the concentrations of as many as four compounds at the grid points.

Figure 2 shows the results obtained in the systems C, H, O. Organic compounds in concentration greater than 10^{-20} mole fraction are found everywhere except where free O_2 is present. Solid carbon theoretically becomes stable along the lower dashed line at $500^{\circ}K$. However, reactions producing it are very slow. The supersaturated region beyond the line of potential carbon formation was also investigated. A threshold was found where polynuclear aromatic compounds are sufficiently concentrated to form a liquid phase. This process may have been involved in the primordial formation of asphaltic petroleum.

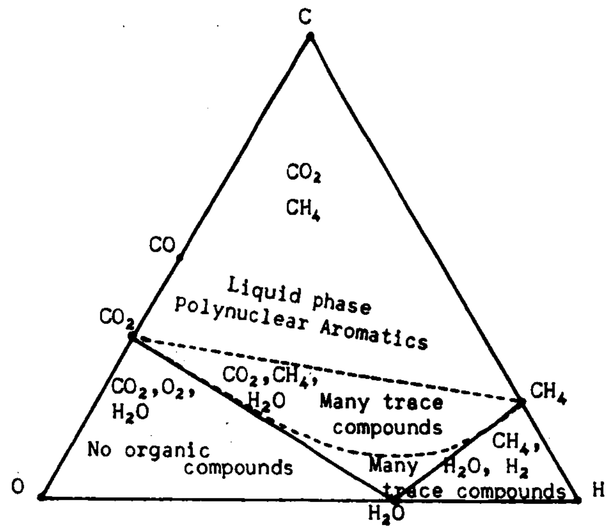

Figure 2. Thermodynamic Equilibrium in Atmospheres of Varying Elemental Proportions

Jukes and associates at University of California, Berkeley, have been investigating the coded structure of the amino acid sequences concerned in protein synthesis, the key by which the sequence of bases in DNA is translated into amino acids during the synthesis of proteins. The amino acid code was solely a matter of theory until 1961, when a crucial experiment was carried out by Nirenberg and Matthaei at the National Institutes of Health.

This experiment bridged the last remaining gap that had separated theoretical genetics and test-tube biochemistry. It now became experimentally possible to search for codes for the other twenty amino acids concerned in the synthesis of proteins.

Previously it had been concluded that the code did not consist of either one or two bases per amino acid, simply because there are twenty different amino acids and only four different bases. There are only sixteen ways of arranging A, C, G, and T in pairs. For this and other reasons, it is thought that a triplet of three consecutive bases is needed to code for each amino acid. It is known that the sequences of bases in either of the two strands of a DNA molecule are unrestricted with respect to the order in which they occur; apparently any one of the four bases can be next to any of the other four, although, of course, each base must be paired with the corresponding complementary base in the adjacent strand. The same lack of restriction is true of the amino acid sequences in the polypeptide chains of proteins. Any of the twenty amino acids can occur next to any other. Moreover, the sequences in DNA are subject to mutational changes in which one base replaces another, or bases are added to or deleted from the DNA. Such rearrangements plus the possibility of lengthening of DNA molecules can be numerous enough to account for all the evolution of living forms since the first appearance of life on Earth.

Most of our knowledge is due to experiments with synthetic RNA carried out with extracts of E. coli. The majority of the work has been at Nirenberg's laboratory at the National Institutes of Health and at Ochoa's laboratory at New York University. Various combinations of A, C, G, and U were used in preparing the synthetic RNA molecules that are used in experiments to explore the code. These molecules are made by incubating a mixture of ribonucleoside diphosphates with a specific enzyme, polynucleotide phosphorylase. An important property of this enzyme is that it arranges the bases into polynucleotide strands containing random sequences depending on the proportion of each base. For example, if the enzyme were furnished with a mixture of 5 parts of A and 1 part of C, it would make strands containing, on the average, 25 sequences of AAA, 5 of AAC, 5 of ACA, 5 of CAA, and 1 each of ACC, CAC, and CCA. The proportion of triplets within the strands of a polynucleotide is reflected in the proportion of amino acids in polypeptides that are obtained in the cell-free system. It is upon this concept that most of the present knowledge of the amino acid code is based. All the proposed codes that have been discovered by this experimental approach in which the synthetic RNA molecules are used as "artificial" messenger RNA. The list has now been completed by Dr. Jukes and his associates. (Jukes, 1964; Nirenberg and Matthaei, 1961; Ochoa, 1964.)

A project of interest, conducted at the Stanford Research Institute, is the investigation of the uses of an extremely sensitive method for the measurement of magnetic susceptibility is the possibility of detecting macroscopic quantum effects in macromolecules of biological interest. In the first fifteen months of a project devoted to the development and initial use of equipment specifically designed for this purpose, good progress has been made. A new superconducting circuit, together with superconducting magnetic shields and persistent current magnets, has been constructed. This apparatus is capable of measuring the magnetic susceptibility of small organic samples at temperatures between 1 and $300^{\circ}K$ in fields up to 40,000 gauss. It can detect flux changes of 10^{-7} gauss-cm^2, which is equivalent to the capability of detecting a change in specific susceptibility of 10^{-9} in a 100-milligram sample under an applied field of 10 kilogauss.

Several hundred preliminary measurements were made on samples of coronene. The most reliable of these were in agreement with published values of the magnetic susceptibility of coronene. Experience during these measurements led to changes which have resulted in an apparatus well suited to the measurements on macromolecules.

An improved version of the superconducting circuit now available shows promise of a further improvement in sensitivity by a factor of more than a thousand. (Deaver, et al., 1964.)

Representative of another class of activities in molecular biology is the examination of passive ion flux across axone membranes. This work is being done by Goldman at the National Naval Medical Center. The general question of stimulus transmission by nerve tissue is far from simple, and the ion concentration associated with nerve membranes is a significant part of the answer. Because the space environment may very well produce alterations in these ion potentials, an investigation of their natures and significance becomes extremely important. A working theory is now being developed as a result of this study.

Vital cell processes, chemical transformation, and mechanisms that provide energy for cell maintenance and activity have been studied by Kiesow (1963 - 1964) at the NMRI. The common objective of all phases of this project is elucidation of reaction-steps in which both energy and matter are transformed in living systems. Compared with photosynthetic organisms, chemosynthetic bacteria offer distinct advantages for the study of energy-assimilation. These have led to the following experimental findings: With the energy from oxidation of nitrite, NO_2^-, as an inorganic source, to nitrate, NO_3^-, and with added organic chemical energy from the hydrolysis of adenosinetriphosphate (ATP) to ADP and inorganic phosphate, chemosynthetic bacteria are capable of reducing diphosphopyridinenucleotide (DPN^+) to DPN·H, in a coupled oxidoreduction-diphosphorylation. Thus, in the crucial step of chemosynthesis, ATP is consumed, not produced. However, in simultaneously proceeding cell-respiration the energy-donor, DPN·H, is oxidized and generates more ATP than is required and re-used for DPN^+-reduction with the added energy of nitrite-oxidation. This "Breeder-Cycle" for DPN·H--with different ratios of cell-respiration and biosynthesis--results in a net-production of either excess DPN·H, or excess ATP, or both. Production of DPN·H in the cycle leads immediately to the assimilation of ^{14}C from $H^{14}CO_3^-$. Thereby the fact of elementary biosynthesis is explained without the classical hypotheses of either direct phosphorylation or direct CO_2^- reduction by inorganic-chemical or electromagnetic energy. The cycle transforms the free energy of nitrite oxidation into the free energy of the organic donors. Cell-respiration and elementary biosynthesis proceed through structure-bound enzyme systems in the same fraction of subcellular particles. Three components, two cytochromes and one flavoproteid, have been identified. A thermodynamic analysis of the DPN·H Breeder-Cycle appears to be attainable by measurements of redox-potentials and calorimetric determinations of heats of reaction.

Studies are also being conducted by Pollard and associates, Pennsylvania State University, in an attempt to formulate a theoretical basis for the description of the process of synthesis, growth, division and differentiation of a living cell. Such a theory would be basic to an understanding of very primitive life forms or prebiological material which might be found elsewhere in the universe. In addition, the information generated by this study will lead to an understanding of the effects of weightlessness and radiation on the cell and cellular processes. For these purposes, studies in macromolecular reproduction are being undertaken which differ from the studies involving cellular genetic material. Theories are being developed on the problem of replication of cellular structures and on information storage in two-dimensional (cellular) systems. Theories are also being developed about the function of mechanisms which control and regulate receptor and enzymatic activities within the cell.

One study involved the rate of mutation in cells and disposed of the suggestion that the process of mutation consists of a "tunneling" of a hydrogen nucleus from one base to another in DNA. Such a suggestion can no longer be advanced as a major explanation of mutations.

Work is also being conducted on the centrifugation of cells of E. coli. It has been shown that cells which are exposed to as low as 100 g actually have a modification in their behavior. This has been looked at from the point of view of thymine uptake, which would be concerned with the formation of DNA, and also from the point of view of the induction of an enzyme, which would correspond to the transcription of the DNA. Preliminary experiments in the latter case indicate considerable effect of centrifugation. The thymine uptake is affected, but not nearly as much. Further work is in progress in this area.

Progress has been made with the high intrinsic viscosity machine. This is now working and the viscosity preparations of DNA have been measured. It is hoped to use this to study the variation in viscosity in DNA as a function of the cell cycle.

An important piece of work has been completed on the cells of E.coli grown on maltose, which can be induced to produce betagalactosidase by the addition of thiomethyl galactoside (TMG). If cells are irradiated shortly after induction, the transcription of the DNA ceases and the enzyme produced by the messenger RNA is observed to reach a maximum. This enables the calculation of the half-life of unstable messenger RNA. The half-life for this decay is readily measurable, and values are given over a temperature range from 17°C (5.2 min.) to 45°C (0.56 min.). These agree very well with half-lives measured by others by inducing for short times and watching the course of enzyme formation. The rate of transcription is involved in the kinetics of cessation and values for the rate of transcription can be measured. Arrhenius plots for this rate and the rate of decay are given and the activation energies measured are about 16,000 cal/mole. The cessation of transcription is linked to the degradation, possibly of only one strand, of DNA.

It has been suggested that one important action of ionizing radiation is concerned with the transcription of the genetic message into RNA (Pollard, 1960). Clayton and Adler (1962) showed that induced catalase synthesis in Rhodopseudomonas spheroides is inhibited by low doses of X-rays, giving experimental support to the idea. Pollard and Vogler (1961), using cells in which the process of induction involved both permease and induction showed that there is some sensitivity to gamma radiation. Novelli, et al.(1961) found a reduced sensitivity as compared with colony formation, but it is still a considerable sensitivity.

The process of induction of an enzyme indicates that the transcription of the genetic message is repressed by something which can be acted on by a small molecule, the inducer, to remove repression and permit the formation of messenger RNA, which then acts to make the enzyme. The messenger RNA undergoes decay, by a process which is still not clear. Very elegant measurements by Kepes (1963) show that for the messenger RNA for betagalactosidase the half-life is 1.02 min. at 37°C and 2.05 min. at 25°C. The time of onset of the enzyme after induction was found to be about 3 minutes.

If the process of transcription is indeed sensitive to ionizing radiation, then the irradiation of cells which have just been induced should show development of the enzyme to the extent of formation of new messenger RNA within a few minutes plus the formation of the enzyme while the messenger RNA is decaying. This pattern was found by Clayton and Adler. The experiments conducted by Dr. Pollard and associates amplify and extend their work and also permit relatively accurate agreement with the work of Kepes (1963).

BIOINSTRUMENTATION

Fernandez-Moran (1964 a and b) (1965), at the University of Chicago, has devised a new electrostatic multi-electrode lens, which he has incorporated into an electron microscope. This necessitated the development of a novel high-voltage power source and voltage regulator of extreme stability and accuracy. Some promising work has now been done on superconducting lenses. In a series of experiments with a simple electron microscope without pole pieces, using high field superconducting niobium-zirconium solenoid lenses in an open air core liquid helium Dewar, electron microscopic images of test specimens have been recorded while operating at 32,200 kilogauss in a persistent current mode, with accelerating potentials of 4 to 8 kV. These preliminary experiments have demonstrated the exceptional stability of the images (both short-term and long-term) over a period of 4 to 8 hours, and the relatively high quality of the images at magnifications of 50 to 100 X.

An instrument is under development by Wald, University of Pittsburgh, to automatically analyze cytogenetic material and, thus, extend cytogenetic methodology as a research technique and as a biological monitoring procedure, using automatic electronic scanning and computer analysis of chromosomes. Chromosomal aberrations can thus be monitored under unusual and abnormal conditions such as weightlessness and radiation, since chromosomes are very sensitive to stress situations. In this device a sample will be prepared and automatically inserted under a microscope lens. The device will then scan, identify and photograph on 35mm film a predetermined number of mitotic cells and process the film. The data will be recorded under the direct control of a digital computer. The computer will perform a detailed quantitative analysis of the pictorial data.

Because of the necessity for obtaining physiological data, significant effort has been expended in the development of instrumentation for recording electrophysiological information. One such instrument, developed by the Franklin Institute, Philadelphia, Pennsylvania, is a temperature sensing microprobe. This microprobe is an implantable and remote broadcasting instrument. These developments are associated, in part, with training programs in order that competent individuals may be trained not only in electronics but also in the biological uses of the devices they construct.

Living organisms possess many unique processes and systems which are complex and poorly understood. The new theoretical approaches, combined with laboratory studies, are expected to result in advances which will expand both our scientific and technological horizons.

VI. FLIGHT PROGRAMS

BALLOONS

Biological and biomedical experiments carried out on balloon flights, both manned and unmanned, have a history that antedates the establishment of NASA. Aside from the early use of balloons in flights that could be called simply flight-survival studies, balloons have made important contributions to our present knowledge of the effects of cosmic radiation and to various questions of space travel.

The achievements of the Strato-Lab and Manhigh series by the U.S. Navy and the Air Force include a wealth of information on balloon travel and the survival of man at altitudes close to and above 100,000 feet. Generally, balloon launches of animals, which reached a maximum in the number of yearly launches in 1953 when 23 balloons were released, have established the feasibility of a program of extended manned balloon flights to high altitudes.

Atmospheric life studies outside the area of cosmic radiation effects have been comparatively few. Results from two manned flights, Strato-Lab I and II, indicate that the flights did produce pronounced changes in white blood cell count; however, the data suggest that these changes were due to psychological rather than physical stress. Exposure to altitudes above 90,000 feet for a total of 62 hours did not produce any general behavioral loss in two Java monkeys, according to other balloon flights. Many of these flights were effective in testing equipment, telemetering devices, and in pointing to directions for other flights.

Stratoscope I and II are projects, originally undertaken by the Office of Naval Research (ONR), involving various astronomical observations with the aid of a balloon-borne telescope, television and camera system. NASA cooperated with ONR on Stratoscope II (36" telescope compared with Stratoscope I's 12" telescope) which has already resulted in significant discoveries about the nature of the planets and stars. Water vapor has been identified in the atmosphere of cool red stars; and an analysis of the Martian spectra showed a greater abundance of carbon dioxide than had been believed characteristic of the planet's atmosphere. Since the balloon-borne telescope is carried beyond the Earth's obscuring atmosphere, the Stratoscope projects have yielded valuable photographs of the sun, stars and various planets.

ROCKETS AND SATELLITES

Historically, biological experiments on rockets and satellites have been limited to a "piggyback" and "noninterference" basis on military rockets. For the past few years, however, as the effort toward manned space flight leading to lunar and Martian landings gained impetus, more attention was devoted to experiments designed to show the effects of space on living systems. As in the balloon flight programs, the U.S. Army, Navy and Air Force played an important role, reaching what might be considered a high point with the successful launch and recovery of a ballistic rocket experiment with monkeys Able and Baker. Aerobee rockets as well as Thor IRBM's carried biological payloads consisting of mice and/or monkeys on six launches, contributing to our knowledge of the effects of weightlessness, radiation, etc., on higher animals.

Van der Wal and Young (1958) used Thor-Able combinations to serve as boosters for lifting a 20-pound biocapsule to a peak altitude of 1400 miles and over a distance of about 5300 miles from Cape Kennedy to the west coast of Africa. Weightlessness was attained for a period of almost forty minutes. During re-entry into the atmosphere, a peak acceleration of about 60g was reached. Each of the three capsules flown carried one mouse (Mouse-In-Able), and two of the mice were instrumented for the telemetry of heart rate. Although all three mice were lost, the two experiments with "Laska" and "Benji" yielded physiological results.

The experimenters designed effective instrumentation for registering the electrical activity of the mouse's heart through a single commutated telemetry channel. Records were obtained for both animals during various portions of the flight. The results indicate that both animals were alive when the nose cones hit the water.

Two South American squirrel monkeys ("Gordo" and "Baker") and a rhesus monkey ("Able") were launched into space from Cape Kennedy, Florida, in 1958 and 1959 by U.S. Army Jupiter missiles. The vehicles reached speeds of approximately 10,000 mph, altitudes of 300 miles on 1500-mile trajectories which lasted for about fifteen minutes.

Time courses of cardiac and respiratory rates (Graybiel, et al.,1959) of the two squirrel monkeys show that the noise of the engine at liftoff immediately produced an increase in their heart rates. Respiration also increased temporarily, but slowed later with increasing accelerations. Heart rates fluctuated considerably during launch accelerations, which reached about 15g at cutoff.

The period of free flight and weightlessness was characterized by pronounced fluctuations of heart activity in the post-acceleration phase. Thereafter, the heart rate of "Baker" remained relatively constant, whereas the cardiac activity of "Gordo" fluctuated markedly and decreased slowly almost to the end of his flight. Slight changes in the RST segment and T waves of the electrocardiogram were also noted, which were transient and not pathological in nature. "Gordo's" respiration was very shallow during maximum launch acceleration, when "Baker's" reached its highest value, only to be approximated again during re-entry when forces of about 35g were encountered.

"Able's" cardiac and respiratory rates indicated that, after an initial startle reaction, the heart rate dropped transiently and then increased steeply, reaching a maximum of 259 during the ten-second interval at peak acceleration. Respiration increased but slightly throughout the launching phase. There was a period of "tachycardia" during post-acceleration weightlessness, then the heart rate declined steadily and was disturbed only by several startling missile events. At the end of the sub-gravity phase, "Able's" cardiac rate was slightly below normal.

Although the periods of high g force and free flight were short, the extremes were considerable and the changes from one state to the next were rapid. In spite of this, the cardiovascular, hemodynamic, and electric phenomena were remarkably well maintained. Apparently the animals were not in serious plight at any time. That psychologic factors entered into the observed phenomena is clearly evident from the increase in cardiac rate associated with the noise of the engine prior to lift-off and also from the cinemographic record of facial expressions. Nevertheless, the integrated responses resulted in animals whose physiologic states remained sufficiently normal and sustained to ensure a safe flight.

LITTLE JOE FLIGHTS

The first step in an attempt at animal verification of the adequacy of the Mercury flight program was the development of two tests by NASA in collaboration with the U.S. Air Force School of Aviation Medicine in which there would be a biomedical evaluation of the accelerations expected during the abort of a Mercury flight at lift-off and shortly after lift-off. These flights were launched at the NASA Wallops Station with a "Little Joe" solid-fuel launch vehicle.

Two Little Joe launches were made, with activation of the escape rockets during the boost phase to secure maximum acceleration, and only a brief period of weightlessness was attained. The first launch was on December 4, 1959, and the other on January 21, 1960. A 36- by 18-inch sealed, 125-pound, cylindrical capsule containing the subject, an 8-pound Macaca mulatta, the necessary life support system and associated instrumentation was flown in a "boiler plate" model of the Mercury spacecraft. The rhesus monkeys were named "Sam" and "Miss Sam."

The flight profile included maximum accelerations of about 10 to 12g and weightless periods of about three minutes at ± 0.02g. The peak altitude obtained in the last flight was about 280,000 feet. The experimental capsule was pressurized at 1 atmosphere at 100 percent oxygen at the start of the experiment and reduced to about 350 mm Hg of oxygen due to breathing during flight. The capsule temperature was kept between 10°C and 20°C in both flights.

The measurements taken from the rhesus monkeys were the electrocardiogram, respiration, body temperature, eye movements, and bar pressing, but only partial results were obtained in the first flight. Oxygen tension, total pressure, capsule temperature, and relative humidity were recorded. Both animals were recovered alive and did not show pathologic alterations in their physiologic and psychologic reactions.

MERCURY ANIMAL TEST FLIGHTS

In the Mercury animal test program a Redstone missile carried a chimpanzee, "Ham," on a ballistic flight to a height of 155 miles to provide animal verification of the success with which the Mercury system could be applied to manned flight. The male chimpanzee was trained to perform a two-phased reaction task during the 16 minutes of flight. The chimpanzee, "Enos," was put into orbit for three hours and twenty minutes.

Results of the two flights show that:

1. Pulse and respiration rates during both the ballistic (MR-2) and the orbital (MA-5) flights remained within normal limits throughout the weightless state. Effectiveness of heart action, as evaluated from the electrocardiograms and pressure records, was also unaffected by the flights.

2. Blood pressures in both the systemic arterial tree and the low-pressure system were not significantly changed from preflight values during 3 hours of the weightless state.

3. Performance of a series of tasks involving continuous and discrete avoidance, fixed ratio responses for food reward, delayed response for a fluid reward, and solution of a simple oddity problem, was unaffected by the weightless state.

4. Animals trained in the laboratory to perform during the simulated acceleration, noise, and vibration of launch and re-entry were able to maintain performance throughout an actual flight.

From the results of the MR-2 and MA-5 flights, the following conclusions were drawn:

1. The numerous objectives of the Mercury animal test program were met. The MR-2 and MA-5 tests preceded the first ballistic and orbital manned flights, respectively, and provided valuable training in countdown procedures and range monitoring and recovery techniques. The bioinstrumentation was effectively tested and the adequacy of the environmental control system was demonstrated.

2. A 7-minute (MR-2) and a 3-hour (MA-5) exposure to the weightless state were experienced by the subjects in the context of an experimental design which left visual and tactile references unimpaired. There was no significant change in the physiological state or performance of the animals as measured during a series of tasks of graded motivation and difficulty.

3. Questions were answered concerning the physical and mental demands that the astronauts would encounter during space flight and it was shown that these demands would not be excessive.

4. It was also demonstrated that the young chimpanzee can be trained to be a highly reliable subject for space-flight studies.

The suborbital ballistic flight of the chimpanzee, "Ham," on January 31, 1961, was the prelude to Alan B. Shepard's suborbital space flight, while the orbital flight of "Enos" on November 29, 1961, preceded the orbital flight of Astronaut John H. Glenn.

The fact that we now categorize these events as belonging to the rather distant past, although they occurred only about 4 years ago, serves to emphasize the pace of development in the exploration of space. While the chimpanzee program may pale in the light of subsequent successes, its scientific and technological contribution should not be overlooked.

The significance of this project can be fully appreciated, and its contribution judged, only within the context of knowledge existing at the time of its conception. In addition to its essential training function, it verified the feasibility of manned space flight through operational tests of the Mercury life-support system. It demonstrated that complex behavioral processes and basic physiological functions remained essentially unperturbed during brief exposures to space flight. The

Mercury Chimpanzee Program marked the first time that physiological and behavioral assessment techniques were combined for evaluating the functional efficiency of the total organism in space.

Perhaps the ultimate contribution of this program was in providing the technological framework of knowledge upon which future scientific experiments on biological organisms, exposed to flights of extended durations, must be based. Biosatellite experiments designed to seek more subtle and elusive effects of prolonged space flight on biological functioning will require even more refined and difficult techniques, but will depend heavily on the technological groundwork laid in these early steps of Project Mercury.

A summary of the more important animal suborbital and orbital flights during the period 1957 to 1964 is presented in Table 9.

In another NASA-supported flight, NERV #1, various experiments were carried in a suborbital flight of 20 minutes. Neurospora molds showed a surprisingly high level of mutation, but the control molds also had high rates.

The Discoverer XVII and XVIII flights, to which the Air Force contributed, resulted in many interesting findings relative to the responses of living systems to space flight. On the Discoverer XVII flight Clostridium sporogenes showed inhibition of labilization as a result of space radiation; samples of human gamma globulin and rabbit antiserum specific for human gamma globulin showed an increase in reactivity with apparently no inhibition due to irradiation and samples of synovial and conjunctival cells showed no changes in their genetic characteristics.

Discoverer XVIII was launched during a massive solar flare which began and lasted for the first 13 hours of the 48 orbit, 3 day flight. Neurospora conidia, nerve tissue, algae, human bone marrow, eyelid tissue, gamma globulin and cancer cells were put in orbit. The results indicated that biological specimens may be able to withstand solar flares with a minimum of shielding and that aluminum shielding may be better than lead.

In 1949, the USSR began a systematic, uninterrupted, research program in biological space experimentation. They have studied the effects of physical stress; immune reactions; psychobiology and behavior; genetics; and responses to environmental factors such as temperature changes and ambient radiation. The organisms and biological materials included tobacco mosaic and influenza viruses; T-2 and T-4 bacteriophage; Bacillus aerogenes; Lysogenic bacteria; Clostridium butyricum; Escherichia coli; actinomycetes; yeasts; Chlorella pyrenoidosa; seeds of fir, pine, onion, corn, lettuce, wheat, cabbage, carrot, buckwheat, cucumber, beet, Euonymus, fennel, mustard, pea, broad bean, tomato, and nutmeg; Tradescantia paludosa plants; Ascaris ova; snail spawn; Drosophila melanogaster; loach roe; frog eggs and sperm; guinea pigs; mice; rats; hamsters; rabbits; dogs; monkeys; human and rabbit skin; HeLa tissue cultures and other tissues. (Sisakyan, 1962; Sisakyan and Yazdovskiy, 1964.)

TABLE 9. ORBITAL AND SUBORBITAL ANIMAL FLIGHTS 1957-1964

UNITED STATES

Year	Investigator(s)	Animal Subject	Flight Profile
1958	Van der Wal	Mice "Wickie," "Laska" and "Benji"	1400-mile. None of the three flights were recovered.
1958	Graybiel et al.	Squirrel Monkey "Old Reliable"	300-mile altitude over a 1300-mile distance via a Jupiter rocket. Not recovered.
1959	Graybiel et al.	Rhesus Monkeys "Able" and "Baker"	300-mile altitude over a 1500-mile distance via a Jupiter rocket. Recovered.
1959		Black Mice	500 seconds of weightlessness via a Thor-Able rocket in Discoverer III. The Discoverer vehicle did not go into orbit and animals were lost.
1959	Green et al.	Rhesus Monkey "Sam"	280,000-foot altitude in Little Joe. Recovered.
1960	Green et al.	Rhesus Monkey "Miss Sam"	49,000-foot altitude in Little Joe. Recovered.
1960	Clamann et al.	C-57 Black Mice	650-mile altitude over a 5000-mile range via Atlas RVX-2A. Recovered.
1961	NASA, Aeromedical Lab, Holloman AFB, N. Mex.	Chimpanzee "Ham"	156-mile altitude over a 414-mile range via a Redstone booster, Mercury capsule. Recovered.
1961	NASA, Aeromedical Lab, Holloman AFB, N. Mex.	Chimpanzee "Enos"	2 Earth orbits, 183 minutes of weightlessness at an apogee of 146 miles and a perigee of 99 miles. Atlas booster, Mercury capsule. Recovered.

SOVIET UNION			
Year	Investigator(s)	Animal Subject	Flight Profile
1958		Dogs "Belyanka" and "Pestraya"	450-kilometer altitude in hermetically sealed cabin. Recovered.
1959		Dogs "Otvazhnaya" and "--" and a rabbit	Over 100-mile altitude. Recovered.
1960		Dogs "Belka" and "Strelka," 21 black and 21 white mice	16 Earth orbits (24 hours) via Sputnik V. First successful recovery of living creature from orbital flight.
1960		Dogs "Pchelka" and "Mushka"	16 Earth orbits (24 hours). Spaceship destroyed during reentry.
1961		One dog, mice, guinea pigs and frogs	One Earth orbit at an apogee of 249 kilometers and a perigee of 183 kilometers. Recovered.
1961		Dog "Lvetzdochka"	One Earth orbit. Recovered.
FRANCE			
1961		Rat "Hector"	95-mile altitude in a capsule boosted by a Veronique rocket. Recovered.
1963		Cat "Felicette"	95-mile altitude in a capsule boosted by a Veronique rocket. Over 5 minutes of weightlessness. Recovered.

THE NASA BIOSATELLITE PROGRAM[1]

The space environment offers a unique opportunity to study the basic properties and nature of living Earth organisms with new tools previously unavailable, and opens up new areas of research for which biological theory fails to provide adequate predictions. Unique components of the space environment of biological importance are weightlessness or greatly decreased gravity, the imposition of an environment disconnected from the Earth's 24-hour rotation (particularly its effect on biorhythms), and cosmic radiation with energies and particle sizes unmatched by anything produced artificially on Earth.

As progress is made in the manned exploration of space, the biological effects of the unique environmental factors found only in space become of greater importance. It is essential to determine the effects of space environment on man's ability to perform physical and mental tasks. It is necessary to develop those systems that are required for his survival and for his physiological and psychological well being, both during the performance of his space mission duties and in his subsequent resumption of normal life patterns. Despite nearly a century of research and development in environmental physiology, there are a number of phenomena to be encountered in long-term manned space flight with which we have not had the experience that would enable us to predict the effects nor to develop the necessary protective or remedial measures (Pace, 1963).

Many of the experimental programs in the Bioscience area are being carried out or planned so that the deleterious effects of these phenomena may be determined, predicted, or avoided before they are encountered in manned flight.

Biological experimentation has been carried out in orbiting spacecraft by Russian and American scientists preparatory to manned space flight. These first-generation exploratory experiments had the following objectives: (a) to discover whether complex organisms could survive space conditions and to test life support systems; (b) to determine whether complex organisms (dogs and primates) could survive during launch, orbital space flight, reentry, and recovery; and (c) to determine the effects of ambient space radiation and any obvious effects of weightlessness on biological organisms. These biological studies indicated that manned space flight was practicable, and the various cosmonaut and astronaut flights have proven the validity of the results.

The National Academy of Sciences' Space Science Board summer study (1962) recommended that "NASA should exploit special features of the

[1]From Jenkins (1965).

space environment as unique situations for the general analysis of the organism-environment relationships including, especially, the role environmental inputs play in the establishment and maintenance of normal organization in the living system. NASA should support studies in ground-based and in orbiting laboratories (Biosatellites) on the biological effects of gravity fields both above and below normal. This should be considered a major responsibility of NASA in the area of environmental opportunities. NASA should support studies of biological rhythms in plants and animals including man as part of its effort in environmental biology. Investigate by observation of rhythms in organisms in space in (a) polar and equatorial low orbits; (b) orbits less than, equal to, and greater than 22,000 miles. Properly designed experiments should be conducted to explore the effects of different environmental factors when these impinge simultaneously on test organisms."

The Panel on Gravity of the Space Science Board (1964) stated that the major effects of low gravity would be expected in heterocellular organisms that develop in more or less fixed orientation with respect to terrestrial gravity, and which respond to changes in orientation with relatively long induction periods; these include the higher plants. On the other extreme are the complex primates which respond rapidly, but whole multiplicity of organs and correlative mechanisms make the occurrence of malfunction and disorganization probable, but not certain. The Panel recommended emphasis on early embryogenesis and histogenesis, particularly of plants during exposure to low gravity, and anatomical studies after low gravity. They stated that perturbations of the environment to which the experimental organism is exposed must be limited or controlled in order to reduce uncertainties in interpretation of results. At the same time, the introduction of known perturbations may assist in isolating the effects due solely to gravity. Ground-based clinostats and centrifuges should be used in conjunction with the experiments, and an attempt should be made to extrapolate effects of low gravity with the clinostat.

It is anticipated that study of the effects of unique or unknown space environmental factors will yield unexpected results which may drastically modify future technical approaches. The results from these Biosatellite studies will have broad application to longer-term, manned space flight including manned space stations, and lunar and planetary exploration.

The Biosatellite Program is a second-generation series of carefully planned and selected experiments, including some highly sophisticated experiments which have required several years of baseline study and equipment development. These orbiting recoverable Biosatellites will provide opportunities for critical testing of major biological hypotheses in the areas of genetics, evolution, and physiology.

The biological scientific community showed great interest in the Biosatellite Program; and scientists from universities, industry, and government have submitted 185 flight experiments involving primates and other mammals, vertebrate and invertebrate animals, microorganisms, and plants.

The selected Biosatellite experiments include studies at the cellular, tissue, and organism levels, including embryological development and growth experiments at the tissue level. The experiments on organisms involve physiological, behavioral, reproductive, and genetic studies.

The experiments are divided into six categories: (1) primates; (2) mammals (nonprimate); (3) animal, cellular, and egg; (4) plant morphogenesis, photosynthesis, and growth; (5) biorhythm; and (6) radiation.

Twenty experiments have been selected for flight to study the effects of weightlessness and decreased gravity during 3- to 30-day orbital periods. The experiments include a wide variety of plants and animals from single-celled organisms to higher plants and animals. The effects of weightlessness will be studied on the primate, especially the central nervous, the cardiovascular, and the skeletal systems during orbits of 30 days' duration.

Experiments have been selected to study the effects of weightlessness combined with a known source of radiation (Sr^{85}) to determine if there are any antagonistic or synergistic genetic or somatic effects on various organisms (Space Science Board, 1963).

Experiments are included to study the effects of the unique environment of the Earth-orbiting satellite and removal from the Earth's rotation in relation to biological rhythms of plants and animals.

Six Biosatellites are included in the presently approved program, with the first flight in 1966. They will be launched from Cape Kennedy by the improved two-stage, thrust-augmented Thor-Delta into a nearly equatorial circular orbit at an altitude of 180-200 miles for periods up to 30 days. Recovery will be by Air Force airplane during capsule/parachute descent. The spacecraft weigh 1000-1200 pounds, have a 280-pound recoverable capsule and, while in orbit, will not experience greater than 1/10,000 g of acceleration. The life support system will provide an environment at sea level pressure of 80 percent nitrogen, 20 percent oxygen, and no more than 0.5 percent carbon dioxide with a temperature of 75°F ± 5°F.

All experiments have been developed into the breadboard stage and flight test hardware is being constructed. The experiments and hardware are being subjected to preflight tests simulating launch and recovery stresses. Rhesus, pigtail, and squirrel monkeys have been subjected to the dynamic forces of the simulated flight under conditions of complete, partial, and no restraint. Three types of centrifuges have been utilized to simulate the flight profile. Primates were fully instrumented with deep brain electrode implants, implanted catheters, and other implanted sensors. During all phases of centrifugation, motion pictures were taken. These primates were semi-restrained in form-fitted couches which allowed movement of the body while facing the accelerative force in a ventrodorsal position (eyeballs in). In this series of tests, all primates were normal following the tests and exhibited no unusual behavior or effects. X-rays showed that implanted catheters and electrodes remained in place, and there were no movements causing tissue damage. However, when the primates were placed with their backs toward the accelerative force, dorsoventral (eyeballs out), the animals suffered visible damage. At 6 g there was no visible stress, but at 8 g swelling of the lower eyelids was noticeable. At 11 g both eyelids were swollen shut. In the Biosatellite Program, primates will be placed in the semi-restraint couches in a position facing accelerative forces, ventrodorsal (eyeballs in), to prevent these observed effects.

VII. MANNED SPACE FLIGHT

Bioregenerative life support systems

Flight of man into space requires a complete life support system capable of supplying all of the oxygen, food, and water, as well as removal of excess carbon dioxide, water vapor, and human body wastes. In addition, the oxygen, carbon dioxide, and barometric pressure must be maintained at a suitable level. Any accumulated toxic products and noxious odors must be removed.

In the spacecraft the human is confined in a restricted environment in which it is necessary to miniaturize in this small volume a completely balanced microcosm or closed ecological system. This is an enormous biological and bioengineering problem. Weight, size, simplicity of operation, and, in particular, reliability are important factors.

Food, oxygen, and water can be stored and made available as required; and the various waste products can also be stored on relatively short missions involving one or a few astronauts. In longer missions, particularly involving more than one astronaut, more efficient regenerative systems will be required. These can be either chemical or biological regenerative systems. Any regenerative system introduces a fixed cost in weight of processing equipment and, also, energy requirements to obtain a lower slope of increasing weight cost per day.

Chemical, or partially regenerative, methods for providing breathing oxygen by the regeneration of metabolic products such as water vapor and carbon dioxide include the thermal decomposition of water and CO_2, photolysis and radiolysis of water, electrolysis of carbonates and aqueous solutions, and the chemical reduction of CO_2 with H_2 followed by electrolysis. Chemical regenerative systems have been developed for removal of excess carbon dioxide and water vapor from the atmosphere. Non-biological regenerative systems are time limited to the amount of essential food, water, and oxygen that can be carried or recovered. These physical-chemical processes show great potential, but they also present many difficulties, including requirements for extremely high temperatures, considerable amounts of power, the formation of highly toxic materials, high susceptibility to inactivation, and insufficiency. None of the presently studied non-biological processes can function as completely as a closed bioregenerative system. All of these non-biological systems have unrealistic supply requirements and produce unusable wastes.

Consequently, for long planetary missions the bioregenerative systems, which also are beset with problems and difficulties, are potentially far superior to their physical and chemical counterparts.

The physiological requirements of man, shown in Table 10 (Del Duca et al., 1964), depicts the material balance in human metabolism. A man breathes about 10 cubic feet of air per minute, or 400,000 liters, daily. A suitable level of oxygen must be supplied at all times. The expired air contains about 4 percent carbon dioxide. Man normally breathes air containing 0.03 percent CO_2, but can withstand comfortably about 0.3 percent CO_2. Anything in excess of 1.5 percent will produce labored breathing, headaches, and, if greatly exceeded, death. A man exhales about 1.1 pounds of water per day. In addition to perspiration and other sources, excess water must be removed from the air.

TABLE 10. THE MATERIAL BALANCE IN HUMAN METABOLISM

Inputs	Lbs. per man day	Outputs	Lbs./day
Oxygen consumption.	2.0	Water vapor generation. . .	2.20
Food requirement (dry basis)	1.32	Carbon dioxide generation .	2.25
Carbon in food converted into CO_2.	0.615	Metabolic water generation.	0.41
		Urine production.	3.00
Hydrogen in food converted into H_2O.	0.046	Fecal output.	0.33
H_2O derived from food . . .	0.99	Fecal water (at 75 percent)	0.25
Net water intake for drinking and food preparation	4.46	Solid waste from food . . .	0.08
		Total H_2O output.	5.45

Two types of biological regenerative systems have been proposed. The photosynthetic closed ecological system was proposed as early as 1951. This involves the use of single-celled algae and higher plants, including floating aquatic and terrestrial plants; and requires the introduction of light energy with CO_2 and H_2O to produce O_2, cells, and water. Another system proposed in 1961 involves electrolysis of water by electricity into oxygen and hydrogen, and the concurrent use of

Hydrogenomonas bacteria which take up hydrogen, carbon dioxide, and urine and provide oxygen and bacterial cells.

The difference between the two biosynthetic processes, photosynthesis and chemosynthesis ("dark" synthesis), is not the synthesis as such, but the way by which the hydrogen is made available from water to reduce the hydrogen carrier. For photosynthesis, light energy is utilized to split the water (photolysis). If only artificial light is available, a heavy loss (about 80-90 percent) has to be taken into account in the conversion of electricity into visible light. The generation of hydrogen by electrolysis is much more economical (60 to 85 percent efficiency). A comparison of both efficiencies in the conversion of electricity reveals a sevenfold gain with electrolysis.

Moreover, chemosynthesis is considered a process which can manage the hydrogen more efficiently in the conversion of carbon dioxide. There are indications (Packer, et al., 1963) that the conversion of carbon dioxide in chemosynthesis would be 30-50 percent better than in photosynthesis. Both the efficient generation and the efficient utilization of hydrogen would allow a power consumption in chemosynthesis of only one-tenth of the power required for an artificially illuminated photosynthetic system.

The present designs of regenerative systems make use of the process of photosynthesis in green plants for oxygen generation from water, the removal of carbon dioxide from human respiration, and, sequentially, the production of food. Light is the energy source which supports the life cycle in the system. Under conditions where sunlight is abundant and conveniently available on a constant basis, this exchanger may yield good service.

The values given in Table 11 indicate relative weights, powers, and volumes required to provide the gaseous environment for manned space cabins. If one considers operating temperatures and hazards, other systems may offer advantages which offset the weight, volume, and power advantages of the hydrogen reduction or LiOH system.

Research is being conducted by NASA on life support systems technology applicable to missions planned for twenty years in the future. Life support systems include the requirements for supplying breathing gases, control of contaminants in the cabin atmosphere, water reclamation, oxygen regeneration and food supply, and personal hygiene. The disciplines involved in such systems include biology and microbiology, cryogenic fluid handling in zero-g, heat transfer, and thermal integration with other systems, such as power. The physiological, psychological, and sociological problems of man are also being considered.

TABLE 11. REQUIREMENTS FOR REGENERATIVE LIFE SUPPORT SYSTEMS

System	REQUIREMENTS/MAN[A]			3 MAN[B] (270 MAN-DAY MISSION)	
	Wt. (Lbs.)	Vol. Ft.	Power Kw	Wt. (Lbs.)	Power Kw
Partial-Chemoregenerative				730[C]	1.75
LiOH	275	0.7	1.4		
NaOH	340	0.9	7.68		
CO_2-H_2	75	1.3	0.36		
Full Bioregenerative					
Algae Artif. Illumination	255	6.15	5.5	1300	25.0
Algae Solar Illumination	227	9.45	1.7	784	0.6
Electrolysis Hydrogenomonas	110-130	2.3	0.35	285	2.6

[A] After Del Duca, et al. (1964).

[B] After Bongers and Kok (1964).

[C] Includes instrumentation and food storage.

Photosynthetic System

Green plants contain chlorophyll, which captures light energy thermodynamically required to convert carbon dioxide and water into carbohydrate-like materials or other foods such as protein and fat. During this process carbon dioxide is consumed, and approximately equal amounts of free oxygen gas are liberated. As a first approximation, photosynthesis is the reverse of the oxidative metabolism of animal life:

$$C_6H_{12}O_6 + 6O_2 \xrightarrow{\text{oxidation}} 6CO_2 + 6H_2O + \text{heat}$$

light

$$6CO_2 + 6H_2O \xrightarrow{\text{photosynthesis}} C_6H_{12}O_6 + 6O_2$$

The photosynthetic process in plants has been studied intensively, and the respiration process has been studied during photosynthesis and several metabolic pathways have been elucidated. Mechanisms are being studied to explain the inhibitory effects of strong visible light on this process. This program may lead to the use of chloroplasts or chlorophyll without cells in future photosynthetic bioregenerative systems for long-term space travel.

One of the prime considerations of a closed ecological system is that the environmental gases shall remain physiologically tolerable to all of the ecologic components. Ideally, a photosynthetic gas exchange organism should possess the highest gas/total mass exchange ratio (considering all equipment and material incidental to growth, harvesting, processing, and final utilization); and a controllable assimilatory quotient to maintain steady state gas composition. It should also be (1) amenable to confining quarters which may be imposed by inflexibility of rocket and/or space station design; (2) genetically and physiologically stable and highly resistant to all anticipated stresses; (3) edible and capable of supplying most of all of human nutritional requirements; (4) capable of utilizing the end products of raw or appropriately treated organic wastes; and (5) amenable to water recycling as demanded by other components of the ecosystem.

Higher Plants

Efforts to utilize multicellular plants as photosynthetic gas exchangers have been somewhat neglected since it has been assumed by many that algae would be more efficient. Small aquatic plants of the family Lemnaceae (duckweeds) are primitive plants with a minimum of tissue differentiation. Practically all of the cells of the plant contain chlorophyll and apparently are capable of photosynthetic activity. They reproduce principally by asexual budding of parent leaf-like fronds. They can be grown readily on watered moist surfaces (Ney, 1960) on almost any media suitable for the growth of autotrophic plants. With duckweeds the problems of gaseous exchange and harvesting are simplified and the media volume can be greatly decreased as compared with algae.

Ney (1960) obtained a very high gas exchange rate with duckweeds. Using small cultures under controlled optimal conditions of temperature, light (600-1000 fc), and CO_2 concentration, he estimated that 25 ft^2 of frondal surface of duckweed, at a gas exchange rate of one $L/ft^2/hr$, would provide sufficient gas exchange for one man. This would produce about 25 grams of dry plant material per hour.

In duckweed studies by Wilks (1962), the exchange rate was over $0.1 L/ft^2/hr$, but the data are not comparable with algal data because of great differences in the light intensity (500 vs 8000 fc) as well as the concentration of CO_2 (1 percent vs 2-5 percent). A comparison of the efficiency of light utilization in the two systems is as follows:

$$\text{Efficiency of duckweed system} = \frac{\text{wt. dry duckweed} \times 3.5 \text{ cal in mg/ft candle} \times 100}{\text{hrs. to produce} \times \text{illumination} \times \text{caloric value of a foot-candle/hr./Cm}}$$

$$\frac{25{,}000 \times 3.5 \times 100}{1 \times 1{,}000 \times 23{,}200 \times 2.8 \times 10^{-3}} = 135\% \qquad \text{Nèy (1960)}$$

$$\frac{19{,}000 \times 3.5}{24 \times 500 \times 10{,}000 \times 2.8 \times 10^{-3}} \times 100 = 19\% \qquad \text{(Wilks, 1962)}$$

Algal efficiency (Ward, et al. 1963)

$$\frac{25{,}000 \times 3.5}{1 \times \frac{8{,}000}{2} \times 83{,}000 \times 2.8 \times 10^{-3}} \times 100 = 11.4\%$$

A few nutritional studies have been carried out with duckweeds. Nakamura (1960) considered Wolffia as a possible source of food for space travel and found that it contains 25-60 percent carbohydrates, protein 8-10 percent, fat 18-20 percent, minerals 6-8 percent, B_2, B_6 and C, with C the most abundant.

One of the desirable features of a duckweed system is that the gas exchange is direct between the atmosphere and the plant and does not require an elaborate system of dissolving the respiratory gases in a bulky fluid system which introduces special engineering difficulties in zero or sub-gravity conditions.

In the design of equipment for closed ecological system studies, careful consideration should be given to the material used in the construction of the unit. Most plastic materials are subject to photo-oxidative degeneration, with CO as one of the products. When air is recirculated through plastic tubing and transparent rigid plastics in the presence of light, considerable quantities of CO were given off. With the high intensity illumination such as sunlight, a CO buildup of several hundred parts per million is not uncommon. Also, plant pigments such as the carotenoids and chlorophylls will react similarly when exposed to light of high intensity. If the plants die, then the CO is released quite rapidly.

At Colorado State University the responses of plants to high-energy radiation (ultraviolet to infrared) are being studied. Plants from high mountain tops exposed to greater ultraviolet light are being studied for specialized adaptations. The effect of temperature on photosynthesis is being explored. Various plants are also being studied under germ-free conditions.

Screening of various higher plants for possible use in bioregenerative systems at Connecticut Agriculture Experiment Station resulted in selection of corn, sugar cane, and sunflower. Under optimal conditions it has been shown that from 100 to 130 ft^2 of leaf surface are required to support an astronaut.

Plants considered as a possible source of food include soybeans, peanuts, rice, and tomatoes, which can be combined with algae to give a well-balanced and reasonably varied diet. Hydroponic systems use large quantities of water, but progress is being made in reducing this.

The possibility and practicality of the use of animals in the closed ecological system is open to question, particularly in the absence of gravity; and much work remains to be done utilizing plant materials or products from wastes. Animals which have been considered are crustaceans, fish, chickens, rabbits, and goats.

Algae

Algae have the fastest growth rate and are among the most efficient plants for oxygen and food production. It has been amply demonstrated by Myers (1954) and other workers that Chlorella can be used in a closed ecological system to maintain animals such as mice and a monkey. The use of algae for supplying O_2, food and water, and removing CO_2, water vapor, and odors has been considered by many authors for use in spacecraft, space platforms, and for establishing bases on the Moon or Mars.

Estimates of total efficiency are based on extrapolation from laboratory data and vary widely, since many different types of data have been used as a basis for these estimates.

The respired air containing about 4-5 percent CO_2 is bubbled into the Chlorella culture, either at atmospheric or increased pressure of several atmospheres. Air containing a high percentage of oxygen released from the algal system would be saturated with moisture.

The use of algae for several purposes might necessitate from one to three separate algal systems. For food production, Chlorella produces 50 percent protein and 50 percent fat in the presence of high fixed nitrogen content media. In reduced nitrogen content media, it produces 85 percent lipids. Proper choice of Chlorella strains and media will produce not only the necessary calories but also the necessary specific nutrients required. Certain strains are more effective in O_2 production, and others in utilization of urine and other wastes.

Some of the early estimates, using Chlorella grown at 25°C, for supplying these requirements for a single man in space include the following: 168 kg of algal suspension (Myers, 1954); 200 kg of algal suspension and 50 kg of equipment including pumps (Bowman, 1953); and 100 kg of algal suspension and 50 cubic feet for equipment and gas exchange (Bassham, 1955). Using the blue-green alga Synechocystis, 600 kg of algal suspension would be required (Gafford and Craft, 1958). These estimates are based on preliminary studies and are quite high and not of real practical value in their present state.

Other studies have indicated an extremely efficient algal system which offers a real potential for a practical and effective gas exchanger (Burk, et al., 1958). A high-temperature thermophilic strain of Chlorella with an optimum temperature of 39°C and an optimum temperature for photosynthesis at about 40°C can multiply 10,000 times per day. Operating at one-half maximum efficiency, this alga produces by photosynthesis 100 times the cell volume of oxygen/hour. Burk et al.(1958) state: "Future engineering development should lead to a space requirement, per adult person, of no more than 3 to 5 cubic feet of algal culture, equipment, and instrumentation for adequate purification of air." The requirements for this system would necessitate addition of energy in the form of light and of small amounts of nitrogenous and mineral material for the algae. The light source used by Burk, et al.(1958) is a tungsten filament quartz lamp the size of a pencil, which has a long life, is five to ten times brighter than sunlight and operates at a 10-12 percent light efficiency.

Research is being carried out on algal regenerative systems by about 40 or 50 laboratories in the United States. NASA is supporting several basic studies on photosynthesis, the physiology of algae, and engineering pilot plant development. Much of the research on algae is being supported by the Air Force.

Most algal studies have been carried out in small units and the data obtained have been used as a basis for extrapolating logistic values relative to the use of these organisms as photosynthetic gas exchangers in manned space vehicles. Myers (1954) has shown that the quantity of algae necessary to support man (with an assumed O_2 requirement of 625 liters per day) would be in the order of 600-700 g dry weight per day. If algal growth in mass cultures could be maintained in a steady-state concentration of 2.5 g dry weight/liter with such a growth rate as to yield 10 g weight/liter/day, the volume of algal culture would be 60-65 liters and the total mass of the algal culture would approximate 200-250 pounds.

Using an 8-liter system (Ward, et al. (1963) has produced algal concentrations of 5-7 g/dry algae/liter with a high temperature algal strain. The maximum growth rate observed with the culture was 0.375 g dry wt./liter/hr. or 9 g dry wt./liter/day. This was accomplished by using 1 cm. layers of culture and a light intensity of 8,000 fc. The culture system consisted of a rectangular plastic chamber 0.5 M^2, illuminated on each side to an intensity of 4,000 fc (cool-white). To produce 25 liters O_2/hr., an area of 8-1/3 M^2 (85 ft^2) would be required.

The major problem in large-scale production of algae is that of illumination. Conversion of electricity to light has an efficiency of only 10 to 20 percent. In addition, the maximum thermodynamic efficiency of light utilization by <u>Chlorella</u> algae lies in the range of 18-22 percent. This results in a maximum efficiency of only 4 percent for photosynthetic systems. Another problem involved in conversion of electricity to light is the production of heat which has to be eliminated even with thermophilic algae. With a human demand of 600 liters of oxygen per day, the minimum electrical requirement becomes 4 kw. No large-scale culture has yet been managed at anything close to this minimum figure.

A difficult major problem is the lack of penetration of light into concentrated cultures of algae. This necessitates construction of large, but thin, tanks on the order of 1/4-inch thickness. This results frequently in fouling of the surfaces of the tank by algae and makes the removal of the excess algae difficult. Production of one liter of oxygen results in the production of one gram dry weight of algae. Although a small amount of CO is produced by some algae, it can probably be removed by catalytic oxidation. Other problems include mutation and genetic drift of the algae and the necessity for maintaining bacteria-free cultures. There are also difficulties in maintaining a sterile culture if urine is to be utilized as a nitrogenous source in the regenerative system. While there is a potential of using algae as food, more research is required before it can be determined what quantity and methods of processing can be used. Research and development being supported on algae is much greater than on both higher plants and the electrolysis <u>Hydrogenomonas</u> systems.

Electrolysis-Hydrogenomonas Bacterial System

Hydrogen bacteria obtain the energy needed for the assimilation of CO_2 by oxidizing gaseous hydrogen. This is advantageous since hydrogen and oxygen gases can be generated by electrolysis with an efficiency of 60-80 percent. Conversion of electricity to light has an efficiency of 10-20 percent. This process appears to hold real promise due to the efficient energy utilization (electrical energy to food energy) and the lower weight and volume which is more compatible with space operations.

Electrolysis provides the oxygen for human respiration. The simultaneously evolved hydrogen and the exhaled CO_2 are converted into cell material (carbohydrates, fats, proteins, etc.), utilizing energy supplied by additional water electrolysis. The principle of operation of this life support system is based on the coupling of electrolysis of water with biosynthesis by hydrogen bacteria.

An electrolytic cell is a closed unit containing an alkaline solution with an anode and a cathode used for the commercial production of pure hydrogen and oxygen gas. These produce a maximum yield (60-80 percent or more) in gas production per unit of power consumption. According to Dole and Tamplin (1960), a unit capable of producing enough oxygen to sustain one man would be highly reliable, weigh approximately 40 pounds, and require a power input of 0.25 kw. Simultaneously with the O_2, however, twice its volume of H_2 is generated, which can be utilized in the removal of CO_2 and in the production of food. The two gases, H_2 and CO_2, are converted in the second unit, which contains the biological suspension.

Inorganic energy sources other than light are all chemical. Chemoautotrophic bacteria carry out chemosynthesis rather than photosynthesis. The energy liberated in the combustion of practically any naturally occurring chemical can be used by some microorganism to build and maintain itself with CO_2 as the sole carbon source. The chemosynthetic conversion in the combustion of molecular hydrogen carried out by Hydrogenomonas bacteria makes available a considerable amount of energy. An enzyme, hydrogenase, mediates the conversion.

In the chemosynthetic gas exchange system, water is split directly by electrolysis rather than by light. Oxygen is evolved (available for human respiration); and the hydrogen, aided by adenosine triphosphate (ATP) formed in a partial recombustion, reduced CO_2.

The reaction carried out by Hydrogenomonas is the following:

$$6\ H_2 + 2\ O_2 + CO_2 \rightarrow (CH_2O) + 5\ H_2O,$$

the expression in parentheses representing carbohydrate. When combined with the electrolysis reaction for six moles of water,

$$6\ H_2O \rightarrow 6\ H_2 + 3\ O_2,$$

a net production of oxygen and carbohydrate from water and carbon dioxide is the result:

$$H_2O + CO_2 \rightarrow (CH_2O) + O_2.$$

Hydrogenomonas bacteria are characterized by their ability to grow and multiply in a strictly inorganic medium, similar to that of algae. Laboratory scale experiments have been carried out with the objective of defining culture conditions with respect to volume (weight) and power requirements. The volume of suspension required per man will be determined by the conversion capacity of a unit volume. This capacity is a function of the cell concentration; hence, the more cells that can be packed in a unit volume of suspension (and adequately provided with H_2, O_2, and CO_2), the less the volume of suspension required.

The results of experiments by Bongers (1964) on conversion capacity-density relationships are summarized in Table 12. The rate of CO_2 conversion obtained with suspensions up to approximately 10 grams, dry weight, per liter shows a linear relation with density. This indicates that the supply of H_2, O_2, and CO_2 is adequate. Upon a further increase in cell concentration, an increased conversion rate is still effected--but to a lower degree. The highest amount of CO_2 taken up per liter of suspension was approximately 2 liters per hour. At these excessively high cell concentrations, the relationship between rate of conversion and density is no longer linear. Apparently, due to energy limitations, not all organisms participate fully in the process. This is demonstrated when the conversion rate is calculated per unit cell weight instead of per unit suspension volume. The rate per gram, dry weight, decreases from 146 to 68 milliliters of CO_2 per hour (Table 12). The rate of gas diffusion from the gas into the liquid phase, most probably hydrogen and oxygen, seems to limit the conversion capacity.

If the results, as obtained with cultures of small volume, can be duplicated with cultures of practical size, then the suspension volume involved in the recycling of the CO_2 output of a man can be estimated.

With a suspension at a density of approximately 10 grams, the conversion of 1.1 liters of CO_2 per liter and per hour is obtained. At a CO_2 output of 22 liters per man per hour, 20 liters of suspension would be sufficient to balance the metabolic needs of a man.

At higher cell concentrations, less volume of suspension would suffice, on the condition that gas equilibration can be maintained at the higher consumption rates to avoid anaerobic conditions. These could lead to fermentation. The technological problem of gas transfer from the gas to the liquid phase determines the optimal cell concentration and, therefore, the required suspension volume.

The amount of power involved in a chemosynthetic regeneration was estimated by Bongers (1964) on the basis of the biological efficiency. While this depends upon a number of variables, with suspensions of medium density (10 g/liter), on the average, a combustion of 4 moles of H_2 would suffice for the conversion of 1 mole of CO_2--the hourly production of a man. The removal of this amount would require the cleavage of 4 moles of water on a basis of 4 H_2 combustions per mole of CO_2. In addition, the human respiratory oxygen demand of 1 mole of O_2 per hour requires the cleavage of 2 moles of water. Therefore, the chemosynthetic regeneration and human respiration together require--on the average--the splitting of 6 moles of water per hour. The wattage required for electrolysis at 60-80 percent efficiency would range from 0.7 to 0.9 kw. This means that a man can be maintained in a closed ecological system at a power input of five to six times his caloric need, utilizing two efficient energy conversion processes: electrolysis and chemosynthesis.

TABLE 12. EXPECTED POWER AND VOLUME REQUIREMENTS OF A CHEMOSYNTHETIC LIFE SUPPORT SYSTEM

	ml. CO_2 Uptake/Hr.			
Cell Concentration Gram/Liter (dry)	Per Liter Suspension	Per Gram Dry	Liters Per Man	Power (kw)
3	440	146	50	1 or 1
9	1100	122	20	1 or 1
25	1700	68	13	1 or 1

The effects of temperatures ranging from 20 to 42.5°C on the growth rates of Hydrogenomonas eutropha were studied by Bongers (1964), and the optimal temperature was found to be 35°C. Experiments at 25° and 35°C indicated that the efficiency of energy conversion was essentially identical at both temperatures. Hydrogenomonas requires, as part of its substrate, a mixture of three gases--hydrogen, oxygen, and carbon dioxide. Experiments were performed by Bongers (1964) to determine the toleration limits of ratios of the gases in the mixtures; nitrogen was used as a balance. Identical growth was found when hydrogen varied from 5-80 percent. Nearly identical growth was obtained when CO_2 partial pressures were 5-60 percent, being slightly lower at higher partial pressures. The organism was highly sensitive to oxygen. Solvated oxygen concentrations above 0.13 mM were found to inhibit cell division. Energy utilization at 0.2 mM oxygen concentration was found to be approximately twice as high as at 0.05 mM oxygen.

While CO_2 and water from breath and perspiration are the most abundant by-products or wastes arising from man's occupancy of a closed system, less abundant, though no less important, are urine and feces. The urine is important for the content of nitrogen compounds and other products of man's metabolism. Urine serves as a very effective substrate for cultivation of hydrogen bacteria. Maximum closure of the system necessitates utilization of the urea in urine for this nitrogen source. The inorganic ion and trace element requirements for growth of the hydrogen bacteria are supplied by urine, especially if iron compounds are added.

Continuous Culture of the Hydrogen Bacteria

Growth of Hydrogenomonas as a batch culture, after an initial period of adjustment, becomes steady and rapid during the exponential growth phase. This steady state of growth is temporary, however, and ceases when nutrient substrate or gas concentrations drop to limiting values. For the maintenance of steady state for periods of long duration, conditions must be provided for the continual supply of fresh medium and the simultaneous removal of an equal volume of growing culture maintained at constant volume. Growth then occurs under steady state conditions for prolonged periods; and such variable factors as nutrient concentration, oxygen, pH, and metabolic products, which eventually change during the growth cycle in batch culture, are all maintained constant in continuous culture.

There are two main methods for the control of continuous culture. The Turbidostat attains the steady state through regulation of nutrient controlled by optically sensing turbidity of the culture. Thus, the dilution rate varies with the population density at the culture and fluctuates about a mean, maintaining the density within a narrow range. The microbial concentration is held at a fixed value and the dilution rate is allowed to find its own levels. In the internal control system (Turbidostat), it is necessary to operate in or near the exponential phase of growth where small changes in dilution result in large changes in concentration of organisms. Growth rate is always maximal and the organisms grow at the maximum rate characteristic of the particular choice of physical and chemical conditions. Although the concentration of organisms can be selected over relatively wide limits, the growth rate cannot be changed except by modifying the nutrient medium, gas concentrations, or incubation temperature. A disadvantage of the Turbidostat is that all nutrient concentrations in the culture chamber are necessarily higher than the minimum, resulting in poor efficiency of utilizing nutrients. Also, it has been found that the accuracy of turbidity measurements are severely hampered by the growth of cells on the glass surfaces in the path of the meter's light beam. Various procedures have been used in an attempt to overcome this difficulty, including addition of surfactants to the culture and the use of "windshield" wipers.

The second means for obtaining steady-state growth, the Chemostat, utilizes the principle that growth of an organism is limited by nutrient concentration below certain values. A constant feed of medium with one nutrient in limiting concentration with constant removal of culture at the same rate is used to achieve steady state. This continuous flow system permits the selection of a desired population density by regulation of a limiting concentration of nutrient flowing into the microbial culture and the selection of a desired growth rate by regulation of the flow rate. In the Chemostat, the dilution rate is set at an arbitrary value and the microbial population is allowed to find its own level; by appropriate setting of the dilution rate, the growth rate may be held at any desired value from slightly below the maximum possible to nearly zero.

In the Chemostat the cell population also grows exponentially, but the steady state is maintained constant at a value somewhat less than the maximum growth rate by virtue of employing a limiting concentration of nutrient. This, in effect, constitutes a self-regulating system and allows selection of a desired growth rate. A wide variety of substances can serve to limit or control growth.

Both approaches are being studied with NASA support. The Turbidostat offers the greatest potential for greatest efficiency in weight and volume, but is less efficient in use of nutrient materials and has greater engineering complexity. The Chemostat would appear to be less efficient in weight and volume, but has greater simplicity and reliability.

The Turbidostat system developed by Battelle Memorial Institute (Foster and Litchfield, 1964) includes electrolysis of water in a unit, and the H_2 and O_2 are alternately fed into the bacterial substrate in the Turbidostat to prevent explosion. An alternate method studied by Magna Corporation maintained the hydrogen-producing electrode of an electrolysis cell in the bacterial culture. Resting cells of Hydrogenomonas eutropha were shown to consume hydrogen produced at a cathodically polarized electrolysis cell. A specially constructed Warburg electrolysis cell was employed. Attempts to immobilize Hydrogenomonas cells on a porous conductor were partially successful. The potential of the bioelectrochemical approach is that interactions of the bacteria with the hydrogen-producing electrode will actually lower requirements compared to those for the isolated subsystems and that this interaction may also allow the utilization of smaller volumes (and therefore weights) than are required by the sum of isolated subsystems.

While the discovery of the electrolysis-Hydrogenomonas system is relatively new, NASA is supporting a well rounded program involving several universities and industrial corporations. Initial studies carried out by Dr. Bongers at Martin Marietta Company, Baltimore, Maryland, with Hydrogenomonas eutropha have been continued by Dr. Bongers at RIAS. Basic studies on physiology of growth substrate requirements and optimal growth conditions are being studied by Dr. Bongers and also by Dr. Tischer, Mississippi State University. Additional studies are being carried out to determine whether any toxic products accumulate during culture. Magna Corporation, in an initial contract with the U.S. Air Force, made a preliminary screening of various hydrogen-fixing organisms and independently selected Hydrogenomonas eutropha. Dr. Goldner studied the potential use of the Chemostat in continuous culture of Hydrogenomonas. Present studies involve utilization of urine as the major substrate for growth of Hydrogenomonas. Chemostat studies are also being carried out by Dr. Tsuchiya, University of Minnesota, and Dr. De Cicco, Catholic University. The Battelle Memorial Institute has developed a Turbidostat for continuous culture of Hydrogenomonas (Foster and Litchfield, 1964). Bacteria produced during culture studies of Hydrogenomonas are being sent to Dr. Calloway, University of California (Berkeley), to carry out nutritional studies feeding the organisms to both animals and human volunteers.

Although research has been productive and progress has been rapid, several major problems exist in cyclic mass culture of Hydrogenomonas. These include possible change of Hydrogenomonas bacteria from autotrophic to heterotrophic growth in the presence of some organic material in the urine substrate. During heterotrophic growth, hydrogen is not taken up and utilized. Heterotrophism can result from mutation of the stock strain of Hydrogenomonas. Both genetic studies and utilization of C_{14} are being used to study this problem. Genetic studies are being carried out to determine the genetic stability and mutation rates of Hydrogenomonas. The effects of radiation will be studied in the future. Another major problem includes the potential destruction of cultures by phage, which has been isolated on four occasions from Hydrogenomonas facilis. Methods for control of lysogenic phage contamination are being studied at Catholic University. Grantees and contractors supported by NASA meet regularly to discuss results and problems and circulate reports to each other. As soon as sufficient data are available from continuous culture using both the Chemostat and Turbidostat, the most efficient method will be selected for development into a pilot plant scale.

Cabin Atmospheres[1]

In the first U.S. manned space flight program--Mercury--and in the face of very severe weight limitations, a cabin atmosphere of pure oxygen at 1/3 atmospheric pressure was adopted. This choice probably represented the greatest simplification which could be achieved readily and, at the same time, it provided protection against some of the risks of rapid decompression. Although the breathing of pure oxygen at higher pressures was known to be attended by some undesirable physiological effects, the short duration of the flights to be undertaken, and the low pressure employed, suggested that no harmful results would accrue in this case. That these expectations were generally borne out is now history. Preparations for space flights of longer duration--many weeks or months--present similar problems and require special attention to phenomena which may be either undetectable or of trivial significance on a time scale of a few days.

Principal considerations which appear most likely to be of significance in the selection of the cabin atmosphere for flights of a few weeks or longer are reviewed. While effects on respiratory physiology receive the major emphasis, other factors, such as thermal properties and risk of fire, are important enough to merit attention.

Physiological Criteria in the Choice of Cabin Atmosphere

If maintenance of normal respiratory physiological function without regard to external circumstances were the only consideration, provision of a cabin atmosphere of about sea level composition and pressure might

[1] This report includes part of the Summary of the Working Group on Gaseous Environment for Manned Spacecraft, Space Science Board, NAS/NRC (1963), and results of research sponsored by the Bioscience Programs, NASA.

represent an ideal and straightforward choice for manned spacecraft. This atmosphere has, in fact, been used in the manned space flights conducted by the Soviet Union. No other atmosphere has been shown to be more satisfactory from the physiological point of view and the tedious respiratory studies which should accompany the use of other atmospheres could then be avoided. Nevertheless, the formidable problems of spacecraft design, and the necessity to take all prudent steps to safeguard the crew from accident, compel consideration of other atmospheric compositions and pressures. For, if a cabin at one atmosphere pressure of ordinary air were decompressed to space suit pressure (0.3 atm.), the occupants would develop decompression sickness, i.e., "bends."

Several engineering considerations would seem to argue for low cabin pressures and for a single gas--pure oxygen--composition. Among these are structural design and complexity, weight of atmospheric gas storage and control equipment, and the difficulty of contriving pressure suits which allow operation at pressure nearer one atmosphere. Such departures from the normal human gaseous environment, however, require the demonstration of an acceptable level of physiological performance.

The limits between which the composition and pressure of acceptable cabin atmospheres must be sought are then set by: (i) a pure oxygen atmosphere at a pressure which will provide an alveolar oxygen partial pressure equal to that at sea level, and (ii) a mixed gas (oxygen and inert gas) atmosphere of such a pressure and composition as will allow decompression to the highest acceptable pressure suit setting without risk of bends. A numerical value for the lower limit (i) is, approximately, 0.2 atmosphere of pure oxygen. The upper limit (ii) is determined by the operating pressure and composition of the space suit atmosphere and may be of the order of 0.5 atmosphere for a cabin atmosphere of 50 percent oxygen. Within these bounds it is necessary to ascertain the extent of physiological tolerance under the conditions, and for the durations, of intended use, not only for survival but also for performance of the necessary tasks. Only in this way is it possible to establish with reliability the fitness of any given atmosphere or the limits within which it must be controlled.

Atelectasis and Pulmonary Edema

Localized or diffuse collapse of alveoli in the lungs may, if the condition persists, lead to arterial hypoxia which, under the stresses of space flight, may be extremely undesirable. Biophysical considerations lead to the conclusion that the alveoli are probably unstable and when breathing pure oxygen and, especially, at low pressures they tend to collapse if there is blockage of the connecting airways. This collapse tends to occur because each of the gases present in the alveoli (oxygen, water vapor, and carbon dioxide) is subject to prompt and complete absorption from the alveoli by the blood.

The alveoli are normally stabilized against collapse by the presence of an abundant proportion of inert and relatively insoluble gas (nitrogen) and an internal coating of lipoprotein substances with low surface tension.

Theoretical and experimental results strongly suggest the desirability of using oxygen-inert gas atmospheres for long missions in order to avoid atelectasis and other gas absorption phenomena, such as retraction of the ear drum, but further experimental evidence is required both to confirm this point and to establish the upper limit of suitability of pure oxygen atmospheres from this point of view.

At Ohio State University, bioscientists in 1962 studied the effect on young rats exposed for 27 days to 100 percent oxygen, at reduced pressure, equivalent to 33,000 feet altitude, with no nitrogen. The rats showed no difference in growth rate, oxygen consumption, food and water intake, or behavior from control rats in normal air.

Oxygen Toxicity

It has long been known that breathing pure oxygen at normal atmospheric pressure often produces pulmonary irritation and other "toxic" effects both in man and animals. This knowledge has occasioned concern over the use of pure oxygen atmospheres in spacecraft.

The effect of 100 percent oxygen at a simulated altitude of 26,000 ft. for 6 weeks was studied using rats at Oklahoma City University under a NASA grant. Radioactive carbon-14 injected in rats revealed an average reduction of total metabolism of 15 percent in the 100 percent oxygen-exposed rats, compared with rats in normal air. There was a 20 percent decrease in lipid metabolism in the liver compared with controls but no decrease in heart metabolism. There was no gross change in body weight.

The White Leghorn chick between the ages of two and seven weeks is markedly resistant to the toxic effects of 1 atmosphere of O_2. Continuous exposure (Ohio State University) for as long as four weeks caused neither deaths nor obvious morbidity nor any signs of pulmonary damage on gross autopsy. Nevertheless the hyperoxia had some adverse effects, primarily reducing the growth rate to 3/4 to 1/4 of normal, reducing feed intake per unit body weight to 3/4 of normal, slowing respiratory rate by 31 percent, decreasing erythrocytes, hemoglobin, and hematocrit by 9-12 percent, and causing reversible histological changes in the lungs. Arterial O_2 tensions were elevated over 300 mm Hg, but arterial PCO_2 and blood pH were unaffected. No residual effects were noted upon return to air breathing. It is possible that the anatomical peculiarities of the avian lung play some role in the chicks resistance to hyperoxia, but it is also possible that this resistance is a function of age, similar to the tolerance shown by the young rat, but not the adult.

Carbon Dioxide Tolerance

Studies of CO_2 tolerance in submarine crews indicate that no loss of performance is involved if the concentration in air at normal pressure does not exceed 1.5 percent with exposures of 30-40 days. Biochemical adaptive changes were observed, however, at this concentration.

Inert Gas Components

If other investigations establish the need for an inert gas in manned spacecraft atmospheres, gases other than nitrogen may be considered. In particular, the physical properties of helium and neon offer advantages, compared with nitrogen, with respect to solubility in body fluids, storage weight, thermal properties, etc.

Studies at Ohio State University, under a NASA grant in 1964, showed that helium substituted for nitrogen in a closed container causes humans to feel "cold" at a normally comfortable temperature. Studies with animals have shown that in a helium atmosphere there is greater heat loss due to the increased conducting capacity and probably greater evaporative capacity. In six days at 21 percent oxygen and 79 percent helium at normal air pressure, young rats grew at the same rate as controls, but drank more water, excreted more urine, and had a higher rate of food and oxygen consumption than controls in normal air. Men are being tested on a bicycle ergometer in saturated and low relative humidity helium atmospheres to study heat balance.

Mice were exposed to 80 percent argon and 20 percent oxygen continuously at normal atmospheric pressure for 35 days at Oklahoma City University. Carbon-14, injected into the mice to study metabolic effects, showed a slight slowing of metabolism with a two to three times increase in the amount of fat deposition.

Bends

Decompression, whether accidental (due to damage of the spacecraft) or intentional (as in the use of the pressure suit outside the capsule), carries the risk of bends if the inert gases dissolved in the tissues and body fluids come out of solution. The magnitude of this risk is determined to a very considerable extent by:

(a) individual susceptibility;

(b) the extent to which the nitrogen (or other inert gas) concentrations of tissues and body fluids have been reduced; and

(c) the magnitude and rate of the inert gas partial pressure change on decompression.

These effects can be mitigated by:

(a) selection of bends-resistant individuals;

(b) thorough denitrogenation before flight;

(c) limitation of decompressive pressure changes by appropriate choice of cabin atmosphere pressure and composition; and

(d) space suit pressure setting.

In some cases, further improvements might be obtained by using, in the cabin atmosphere, an inert gas component which has a lower solubility in tissue and body fluids or less tendency than nitrogen to form bubbles.

Heat Exchange

Some spacecraft atmospheres of interest differ significantly from air in the thermal properties which affect heat transfer under forced convection. This in turn will affect the power required to control the crew's heat balance and to cool internal equipment. While such considerations may not be of very great physiological importance in the selection of the atmosphere, they must be taken into account in the final engineering design.

Fire Hazard

Experience indicates that fires in pure oxygen atmospheres even at low pressures (e.g., 1/3 atmosphere) are extremely difficult to extinguish. While this phenomenon has nothing to do with respiratory physiology, the risk on flights of long duration may be so serious as to demand special measures. Unless effective counter-measures can be devised, the existence of this risk may argue very strongly against the use of such atmospheres in the future. Further experimental investigation is required.

Acceleration Effects on the Lungs and Pulmonary Circulation

Forces produced by high acceleration overdistend one part and compress another part of the lungs. Blood flow diminishes in some parts and increases elsewhere in the lungs. Fluid leaks from the blood into the tissues and into the air sacs in parts of the lungs. These effects cause difficulty in breathing, low arterial oxygen saturation, and impaired consciousness during high sustained acceleration and, to a lesser extent, after its cessation. They enter into a consideration of the best gas to be breathed in that a high partial pressure of oxygen is favorable for consciousness, but a low inert gas concentration during acceleration is unfavorable for rapid lung recovery afterward.

At Indiana University, studies were undertaken in 1963 and 1964 to measure the ability of human volunteers on a treadmill to work beyond the body capacity for supplying oxygen. Arterial and venous blood was sampled continuously by use of catheters during exercise on the treadmills. Men on the treadmill accumulated an oxygen debt in 3 minutes and could continue 8 minutes, but the debt did not continue for 15-40 minutes after stopping exercise, as reported in another laboratory.

Physiological Problems

Study of the manned space flights which have occurred to date and consideration of relevant laboratory observations suggest that when long periods of weightlessness are involved, some physiological difficulties may arise which, if not adequately compensated, may produce serious effects on human performance. Although recent experience gives no grounds for expecting insuperable difficulties, neither the quantity nor quality of the available observations permits the conclusion that long-term exposure to weightlessness will not have serious consequences. The critical role to be played by the astronaut demands that every effort be made to identify, in advance, those phenomena which may affect performance and to ascertain the relevant qualitative and quantitative dependences so that proper actions can be taken.

Lawton (1962), in reviewing the literature on prolonged weightlessness, found few instances in which physiological function was truly gravity dependent. It was further stated that the physiological systems likely to be most affected by weightlessness were the musculoskeletal system, the cardiovascular system, and the equilibrium senses. Subsequent experience proved this to be the case. McCally and Lawton (1963) analyzed the data from experiments since 1961 and concluded that much more basic work is necessary in the laboratory. Studies dealing with bed rest, immobilization, immersion, and cabin-confinement techniques were recommended approaches toward simulating weightlessness.

Much of the difficulty in obtaining precise information of anticipated problems arises from a prevailing lack of knowledge concerning a wide range of normal mammalian physiological functions. Many of these deficiencies can be remedied satisfactorily in the laboratory. Where space flight development is concerned, however, two distinct investigational approaches can be adopted. The first of these may be characterized as empirical and incremental; that is, the capabilities of the astronaut are explored in successive flights involving relatively modest increases in difficulty or severity of the environmental conditions. In this way it is hoped to ascertain the human limitations without running too great a risk. The second approach can be described as fundamental, where one would seek to find, by a series of carefully controlled experiments (or observations), the effects of exposure to space flight conditions upon comparative mammalian physiology with emphasis on man. A fundamental understanding of the observed effects would be sought so that predictions for new situations (and possible ways to control them) could be made with confidence.

It is not now possible to predict for flights of 14 days (or more):

(a) the effects of sudden reimposition of reentry accelerations and terrestrial gravity;

(b) changes in body fluid distribution and composition;

(c) the effects of violent physical effort on respiratory and cardiovascular systems in prolonged weightlessness; or

(d) central nervous system functions with respect to coordination, skilled motor performance, judgment, and sleep-wakefulness cycles.

NASA has emphasized that planning for manned space programs involves a systematic extension from physiological observations in animals to the management of comparable problems in man, and finally, with the establishment of man as part of the man-vehicle system design. Moreover, these studies require the separate and combined evaluation of central nervous, cardiovascular, respiratory, gastrointestinal and other systems, to be considered as a matrix in mutual interdependence. Particular interest hinges on the effects of weightlessness in flights exceeding 14 days.

Mammalian flights of the order of 30 days' duration also merit early and close attention, including the development of satisfactory solutions to problems in life support and other systems which must precede such a program. Development of facilities equipped for biological experiments may well be an important requirement for studies in anticipation of manned flights of longer duration than Apollo. Unless the biological satellite programs of the type mentioned above are successful in providing the necessary data, such a manned laboratory may also be important in studies of shorter range.

Evaluation of Integrated Physiological Organization in the Weightless Mammal; General Studies of Biological Rhythmicity

The effects of weightlessness on the organism as a whole may be manifested by important changes in integrated behavioral patterns having an inherently rhythmic character. Modifications in basic behavioral patterns and performance may occur as disruptions of rhythmic physiological phenomena which are themselves the end product of interrelated functional activity in a number of physiological systems, such as the neuroendocrine, cardiovascular, and central nervous system.

Measurements of interdependent components of biological rhythmicity lend themselves readily to analysis by methods well established in physics--including correlation and spectral analyses, and sensitive techniques revealing phase modulation and variance in rhythmic processes. A wide variety of physiological functions can themselves be treated as periodic variables in the analysis, including rhythmicities in cardiac output and blood pressure, respiration, brain waves, and the slower tides of appetite, sleep-wakefulness, etc. The importance of such investigations argues for their inclusion in forthcoming flight programs. Their relative simplicity from an experimental point of view is an additional advantage. Biorhythms have been discussed in more detail in the section on Environmental Biology.

Effects of Weightlessness on the Cardiovascular System

In the cardiovascular system, there is evidence that orbital flight in animals and man may be associated with hypertension involving both systemic and pulmonary circulations, and that this persists at least into the initial hours of weightlessness. Many highly significant circulatory parameters are inaccessible to any techniques of surface monitoring currently available. In view of the inconvenience of using deeply implanted transducers in man, the desired information can be secured only by comprehensive implantations of experimental animals (primates) for collection of data suitable for extrapolation to the human.

Ear lobe oximetry, indirect measurements of blood flow and of blood pressure by finger plethysmography or impedance plethysmography, and ballistocardiographic techniques have potential value to warrant consideration of their ultimate application to manned space flight.

Further, highly significant baseline data should be sought from animal experiments in the laboratory aimed at determining threshold conditions for irreversible cardiovascular changes associated with high environmental temperatures in conditions of pre-existing debilities, such as dehydration.

Adaptation to prolonged exposure to weightlessness or to lunar gravity may cause difficulties when the astronaut is exposed again to reentry forces and terrestrial gravity. It is possible that these adaptive changes may thus produce unacceptable effects on performance or cause risk to life. It is important to obtain experimental evidence on this subject.

It is common knowledge that following a stay in bed, dizziness, faintness, and weakness characterize arising from bed, and that a general feeling of weakness may persist for several days. The phenomenon has been investigated in a number of laboratories. One approach has been to put healthy young subjects to bed and even in extensive casts for periods of two or three weeks or more. Two major findings have emerged from these kinds of studies. First, it is clear that a substantial adjustment in the blood circulatory system occurs, the end result of which is termed the hypodynamic state. Second, it is equally clear that there is a large decrease in the skeletal and muscle mass of the body.

There are two kinds of evidence for the hypodynamic state: measurement of parameters of circulatory function, and measurement of the response of the individuals to a quantitatively imposed mild gravitational load. After three weeks in bed, otherwise healthy persons exhibit an increase of more than 20 percent in heart rate, a reduction of 10 percent in total blood volume, primarily as a result of reduction of plasma volume, and a decrease in heart size of about 8 percent. Coupled with these circulatory changes is a reduction of 9 percent in the basal metabolic rate. It appears as though the circulation and metabolism are reset to a lower functional level commensurate with the reduced demands placed on the whole organism.

After three weeks of bed rest the response of the subjects to a gravitational stress was measured by using the tilt-table technique. In this procedure, the subject lies passively on a platform which can be tilted to the near-vertical--70° from the horizontal--and measurements of heart rate and blood pressure are made before and after tilting. The characteristic response of a normal individual is an increase of 10 to 15 beats per minute in the heart rate and a fall of 1 to 2 mm Hg in systolic blood pressure.

Also, after the three weeks of bed rest, all of the subjects tested showed pronounced orthostatic hypotension. After tilting, the heart rate on the average increased by 37 beats per minute and the systolic blood pressure fell some 12 mm Hg; and some of the subjects fainted. The measurements were continued for 16 days after the bed rest period, and it was found that recovery was not quite complete when the experiment was terminated.

Trained Navy SCUBA divers remained under water continuously for 18 hours to simulate weightlessness, and then were tested for blackout threshold on a human centrifuge. All of the subjects exhibited a reduced tolerance for acceleration, again reflecting some decrement in cardiovascular function. Astronaut Walter Schirra exhibited orthostatic hypotension for about a day after his six-orbit flight. There appears to be little question that in prolonged exposures to the weightless state, there is a fair probability of extensive circulatory adjustments whose practical seriousness cannot be foretold on the basis of present information. While it is likely that the astronauts will adapt successfully to long periods of weightlessness at some new circulatory functional level, the remote possibility exists that the circulatory changes may be progressive to the point of ultimate failure.

Metabolic Effects of Weightlessness

Without metabolic information, accurate planning of environmental systems for long flights is difficult and likely to be wasteful. Importance is also attached to early evaluation of weightlessness effects on body fluid equilibria. The results of Earth orbital flights and of terrestrial water-immersion experiments suggest the occurrence of undesirable changes--although no effects leading to operational incapacity have yet arisen in the relatively short durations so far encountered. In both situations, a profound diuresis of sufficient severity to produce hemoconcentration with a facial plethora and polydipsia has been described.

In both recumbency and immersion, a similarly significant redistribution and release of body fluids occur. It has been suggested that recumbency may affect an extracellular fluid volume receptor mechanism which, by decreasing aldosterone secretion by the adrenal gland, would decrease sodium reabsorption by the renal tubules. Aldosterone excretion decreases during recumbency and during standing in water but increases while standing in air. There is also evidence for cardiac atrial volume receptor mechanisms, with reflex inhibition of release of the pituitary antidiuretic hormone (ADH) by increased filling of the left atrium with a resulting diuresis (Henry-Gauer reflex).

Available evidence indicates that the profound diuresis incurred in the weightless condition may be far from transient, and that adaptive mechanisms, if any, may arise relatively slowly. The condition itself and appropriate combative measures can only be evaluated by careful mammalian investigations in the space environment, and possibly only in man and in adult primates characterized by an erect posture in the terrestrial environment.

Altered fluid equilibria in buoyant states are accompanied by shifts in intra- and extracellular electrolyte distribution, including sodium and potassium. Evidence from recumbency studies indicates a strong correlation between loss of erect posture or weight bearing and excretion of calcium stores in bone.

When man lives under weightlessness conditions, loss of muscle tone and mass, as well as loss of calcium from the skeleton, is anticipated. During periods of bed rest, patients lose rather large quantities of bone mineral.

A bone X-ray densitometry method has been developed by Mack (1965), Texas Woman's University, for accurately determining the loss of bone mineral (± 2 percent accuracy) in humans and animals. The heel bone and spine are X-rayed using a calibrated aluminum wedge for a baseline comparison. This technique will be utilized for pre- and post-flight analysis of the Primate being flown in the 30-day Biosatellite. It has been recommended as well that the X-ray bone densitometry method be used on astronauts before and after flights in the Gemini and Apollo programs. Such comparative appraisal of bone mineral behavior will be invaluable for future flight missions.

Bed rest and immobilization studies at Texas Woman's University (Mack, 1965) have shown loss of skeletal mineral and increased loss of calcium in the urine and excreta. Four bed rest studies, each extending for two weeks, compared different levels of calcium intake. Four men were used in each study and the men served as their own controls during extended ambulatory periods. During two-week periods, up to 10 percent of calcium mineral was lost from the heel bone. The calcium loss was also determined in the urine and feces. In other studies, isometric exercises have helped prevent some loss of bone mineral during bed rest.

Excretion of calcium in the urine is accompanied by risk of its deposition as calculi or "kidney stones" in the urinary tract. Currently, changes in calcium metabolism resulting from weightlessness over periods up to two weeks is not considered a hazard requiring precautionary measures.

Flights in excess of two weeks, however, constitute a more serious problem of sufficient significance to warrant obtaining comprehensive data in the planned 14-day orbital flights and the 30-day Biosatellite primate mission. Therapeutic immobilization, post-poliomyelitis immobility, and experimental restraint in normal subjects clearly lead to a negative calcium balance, with some hypercalcuria. The hypercalcuria accompanying this negative calcium balance raise questions of possible renal damage or renal calculi or both.

Long exposure to low gravity environments, as on the Moon, and to weightlessness is likely to produce muscle wasting unless compensating measures can be applied.

Central Nervous System Functions in Weightlessness

Exposure to the weightless environment will powerfully affect integrative nervous mechanisms, probably to a greater degree than any other physiological system.

The wide range of individual tolerance to disruptive effects of vestibular stimulation has emphasized the importance of this factor in astronaut selection. At the same time, vestibular functions require consideration jointly with visual task performance, since both have special significance in vehicle docking procedures. Vestibular functions in the weightless state remain almost completely unknown. Limited evidence from animal and manned space flights suggests that head turning, through the associated vestibular stimulation, may seriously interfere with concurrent visuo-motor performance, but that these disturbances show significant differences in individual suceptibility, and that at least partial adaptation occurs relatively quickly.

Research is proceeding in behavioral testing of animals sensitive to small increments in g forces, both to establish thresholds necessary to induce behavioral change, and to give animals an opportunity to select preferred g levels. Animal experiments also offer an opportunity for exploration of selective, temporary, pharmacological interference with functions of either semicircular canals or vestibular organs on a reversible basis.

NASA is currently collecting extensive baseline electroencephalogram data under controlled conditions in a form suitable for mathematical analysis. Data are being taken from about 200 subjects in major national and overseas centers. It is intended that this study will assist in astronaut selection, and subject monitoring in the space environment.

Many effects of weightlessness on nervous functions require monitoring of the autonomic nervous system, including gastrointestinal activity, secretory functions, lacrimation, salivation, sweating, and the central control of respiration. Urinary estimations of catecholamines and aromatic substances, such as 5-hydroxyindoleacetic acid, would provide important data, if collected in appropriate fractions and assessed in relation to appropriate pre- and post-flight controls.

Major areas have been outlined in which prolonged weightlessness may be expected to interfere with performance, judgment, and ultimately, chances of survival. These include cardiovascular, metabolic, central nervous, psychophysiological and biorhythmic effects. They have been dealt with separately and in sequence, but it is not intended that they should be viewed as hierarchic. The relative scarcity of control data necessarily precludes such an evaluation.

Soviet experience with zero gravity and weightlessness has given impetus to their emphasis on this spaceflight factor and was an important topic at the May 1964 COSPAR meeting. Discussion of the post-flight medical status of Bykovsky (5 days) and Tereshkova (3 days) revealed a genuine concern for the significance of prolonged weightlessness and the presence of post-flight physical debility and fatigue of varying degrees following each of their Vostok 3 through 6 flights. These changes have required a number of days to return to normal values. Among the physiological conditions singled out for mention are:

(1) Body fluids--Cosmonauts have shown a loss of weight from 1.9 to 2.4 kg post-flight. It appears to reflect a redistribution of body fluid initially upon exposure to weightlessness due to elimination of the hydrostatic pressure gradients of Earth gravity. There is the suggestion that this redistribution and equilibrium are complete within the first 24 hours of flight. Titov is reported to have been dehydrated post-flight with early hemoconcentration and evidence of reduced circulatory blood volume. These findings directly support predictions made from ground-based research.

(2) Cardiovascular--Post-flight orthostatic tachycardia is reported for Titov as long as 23 hours after recovery; at 48 hours there was significant residual intolerance to the upright posture. Cosmonauts have demonstrated a 20-35 percent increase in oxygen consumption during the standard post-flight exercise test which is interpreted as a reflection of decreased muscle reserves. These relate to the deconditioning effects of prolonged weightlessness.

In both of these areas, normality returned within the post-flight reporting period. The Soviets have continued their biologic experiments in space through the Vostok/Voshkod series in a further attempt to identify the effects of space flight factors on living organisms. The fixing of biologic specimens in flight by Bykovsky demonstrated a critical role for man and made possible an expanded experimental program. Biopackages have become more complex with each succeeding flight.

With the exception of post-flight orthostatic intolerance after the third and fourth Mercury flight, changes as a result of exposure in a zero gravity environment have not been noted by U.S. investigations in space. Ground-based research proceeds here at an advanced pace and is supported in large measure by both the USAF and NASA. An evaluation of the relationships among renal and systemic hemodynamics, neurohumoral cardiovascular regulation, and detailed renal excretory function in various subjects positioned differently is under way, as are studies related to orthostatic and acceleration tolerance.

Depressed Metabolism

In anticipation of prolonged manned space flight in the future, NASA sponsored research related to depression of metabolism. The daily food requirements, for example, of astronauts during a voyage of several months can constitute a major portion of the weight and storage capability of the man-carrying spacecraft. Thus, a most promising and fundamental approach to this problem is the reduction of daily metabolic requirements of the astronaut. It has been suggested that astronauts on prolonged space missions be put in a state of suspended animation until their destination is reached. Of course, this sounds fantastic; but ten years ago no normal cell had been frozen to temperatures compatible with prolonged storage. Today this is common-place in that tissues can be frozen, stored at low temperatures, thawed and maintain their viability and functional activity.

The depression of animal metabolism may be accomplished by a reduction in body temperature, as through the processes of hibernation and hypothermia. Other means by which metabolism can be lowered include various drugs and electronarcosis. Aestivation is a state of depressed metabolism induced by exposure to hot and dry conditions and occurs in desert organisms.

Hibernation is a non-stressful modality and results in a great decrease in metabolism. However, human beings are not hibernators; and much research needs to be accomplished before the chemistry of hibernation will be completely elucidated.

Hypothermia reduces the rate of cellular metabolism. Today, extracorporeal systems combined with cooling are in routine use in most medical centers throughout the world. Hypothermia is not impeccable, however, for general body hypothermia is a stressful condition.

Suspended animation or cooling at extremely low temperatures, although not commonplace in depressing metabolism of animals, is a method of great potential.

Pharmacologic induction of hypothermia can be accomplished by such drugs as chlorpromazine and harbamil. Other drugs can be used to depress metabolism; yet, these are not without some disadvantage.

In recent years there has been a growing interest in electronarcosis, the induction of sleep by an electric current. Although potentially valuable, this method is far from routine application.

Outstanding strides have been made in the suppression of metabolism. Recent progress in the biochemistry and physiology of hibernation, hypothermia, and low-temperature biology have evolved fundamental concepts whereby it is possible to reduce the oxygen requirements of individual mammals, organs, and tissues. When the chemical composition of the blood and the cardiac output are sufficient to meet cellular requirements, regulatory mechanisms remain effective and animal survival is assured. In contrast, when the rate of oxygen transport is interrupted, a reduction in cellular activity occurs, and regulation is impaired. In induced hypothermia, the low temperature slows the rates of all processes and modifies the action of metabolites and other substances. This in itself is not harmful, as shown by the true hibernating animal--e.g., ground squirrel--but will become disastrous as soon as anoxia and chemical imbalance begin to develop. Thus, the basic physicochemical considerations in hypothermia relate to the laws governing the dependence of cellular activities and their enzymatic reactions on temperature, ions, metabolites, and drugs.

Hypothermia by definition means a state of subnormal temperature in a warm-blooded animal such as man.

The phenomenon of natural hibernation is being investigated in the laboratory in the hope that the unusual tolerance of hibernating animals to low body temperature may some day be transferrable to ordinary laboratory animals and man. Experiments with the ground squirrel, a typical hibernator, show that the artificially cooled ground squirrel does not tolerate such long periods of low body temperature as during natural hibernation.

Other studies of the brown adipose (fat) tissue that is present in most hibernating mammals is believed to be an endocrine gland intimately concerned with hibernation and essential to that process. Indications that brown fat has a thermogenic role in rats exposed to cold suggested that certain comparable functions may occur in true hibernators (Smith and Hock, 1963). It appears that arousal of the hibernating animals by cold is induced by sympathetically activated thermogenesis in areas of brown fat so located, relative to the vasculature, that heat is transferred to those areas of the body intimately associated with normal metabolism, thereby inducing arousal.

A comparison of the various influences of increased resistance to acceleration shows deep winter hibernation to be most effective, followed by deep hypothermia, and least effective in drug narcosis (USSR).

Experimental evidence is accumulating to show that hibernation and hypothermia afford some protection of animals against radiation. With regard to hypothermia, clinical studies in the irradiation of patients with cancer indicates that decreasing the oxygen requirements by lowering the body temperature reduces cellular metabolism and thus decreases tissue sensitivity to gamma radiation. (Storer and Hempelman, 1952.)

The use of prolonged hypothermia, the application of the principles of hibernation, drugs and electronarcosis appear to hold great potential for the reduction of daily metabolic requirements of astronauts. If one or more of these methods become practical, a real advantage would be offered for drastically reducing human requirements for food and oxygen with a concomitant reduction on the amount of waste production and subsequent elimination. Simultaneously, there is the possibility that any of these methods may afford protection against exposure to gamma and cosmic radiation as well as a tolerance to the forces of increased acceleration.

Nutrition in Space[1]

The human body is capable of utilizing stored metabolites so that the actual nutritional requirements of the individual can be subverted for a short time. This will vary widely among individuals and each individual may exhibit characteristic patterns of nutritional behavior. Requirements in short-range flights can be materially different from those for long-range flights. Muscular efficiency may not change significantly over a period of four to six days. Unfortunately, mental activity begins to decline after 24 hours. Feeding requirements can be divided into two categories: short-term (for missions of less than 21 days) and long-term. Dehydration can occur under adverse conditions in a matter of hours and so water requirements must be considered as a special case.

Water Requirements

Water requirements are extremely critical and the amount supplied should not, under any circumstances, be kept to a minimum, but rather a large margin of safety should be included. There is a wide variation

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[1]This report includes part of the Summary of the Working Group on Nutrition and Feeding Problems of the Man in Space Committee, Space Science Board, NAS/NRC (April 1963), the results of research sponsored by Bioscience Programs, NASA. See Conference on Nutrition in Space and Related Waste Problems (1964), NASA SP 70, p. 400.

in individual requirements based on data from a limited number of individuals. It is extremely important that additional data be obtained on a number of individuals under similar conditions as rapidly as possible.

The environmental conditions for which water requirements are now known and data on the relation of water requirements to the distribution of temperature and evaporation do not approximate those which will be encountered in space flight.

Present water requirements show a very strong dependence upon suit inlet temperatures. In the absence of accurately controlled suit temperatures, water requirements can easily double. If this occurs, the mission would probably have to be aborted, since it is very doubtful if proper electrolyte balance would be maintained at such high rates of water loss. Normal or even extreme conditions of the terrestrial environment usually include diurnal variations in temperature. These conditions will not obtain in the spacecraft. There are real dangers inherent in present uncertainties. In addition to ground-based experiments, measurements of water intake should be made under actual flight conditions. Data from short-term flights should be used for extrapolation to longer missions.

Formula Diets

The tacit assumption which now prevails, "astronauts even on short-term missions require a diet of great variety," is apparently not supported by experimental evidence. In many parts of the world, the people subsist on a monotonous diet consisting of only a few types of food with no apparent ill effects, provided their basic nutritional requirements are satisfied. Experimental evidence from many sources (e.g., the Army Medical Research and Nutrition Laboratory) shows that individuals can be kept on a single disagreeable formula diet for as long as 60 to 90 days without suffering ill effects. Since highly motivated individuals are chosen for the initial space flights, it is unlikely that they would object to the monotony of a formula diet and would probably prefer its simplicity. Also, there are definite possibilities for the development of a formula diet of greater acceptability than those which have been used. There is no reason to anticipate adverse effects from the use of formula diets in short-term flights.

Formula diets would be extremely desirable for short-term flights. A formula diet (a rehydrated liquid formula could be used) would considerably reduce the number of manipulations and the time required for in-flight preparation when compared to a varied diet. These two considerations could contribute materially to the safety of a flight since (a) the astronauts would not be preoccupied with food preparation for so long a period, and (b) the food could be dispensed without the removal of suit components, such as gloves. Storage requirements could be significantly simplified with this type of diet. Weight, however, would not be lowered without the development of more refined formulas than those now available. It is possible to develop a diet which will materially decrease fecal production since disposal is apt to be a problem. Formula diets could readily be adapted to the determined metabolic requirements of the individual astronaut. Investigation of nutrition under the combined stresses of space flight appears extremely important. Packaging problems will be simplified by the use of formula diets. Formula diets can be varied with respect to flavor and color with no difficulty.

Waste

The problem of waste production is intimately related to the nutrition and feeding of the astronaut and can be solved or simplified by taking advantage of effects brought about by dietary changes. Any diet should be adjusted, even on a short range basis, for the minimum production of solid waste materials.

It is apparent that water will be sequestered by net accumulation in the feces. The net loss would approximate 40 to 60 grams per man per day under normal conditions.

Astronauts can be fed low residue diets prior to flight in order to minimize problems during flights.

The effect of flatus can be serious; and, in time, considerable concentrations of toxic gases may accumulate. The purification of the recirculated atmosphere must take this problem into consideration; but, in any event, the diet should be planned to minimize the problem.

The production of urine and its storage is of importance, particularly on short-term flights. The desired analyses necessitate the individual packaging and labelling of urine specimens.

Metabolism

An accurately measured intake of nutrients, calories, and water is a requisite for determining metabolic demands imposed in any space flight. Insufficient knowledge is at hand to predict total metabolic requirements under the numerous stresses which can be anticipated. Simulator studies are of great importance even for short duration flights.

The two most important variables to be considered in establishing the minimal but adequate diet are protein and energy requirements. NASA is supporting research at the University of California (Berkeley) to determine these requirements and to estimate their individual variance in healthy young men. The possibility of minimizing need through biological adaptation is being explored.

The estimation of the minimal protein requirement of adult man is difficult. The generally accepted criterion of minimal adequate protein nutrition in the adult is the maintenance of nitrogen balance at minimal intake. The minimum protein requirements are related to endogenous nitrogen loss. Analysis of the relatively few data available indicates that 2 mg of nitrogen per kilocalorie of basal energy expenditure seems to be the best estimate. However, this figure is higher than that noted in experiments in some human subjects.

After having established minimal nitrogen requirements and minimal amino acid requirements, studies will be directed towards investigation of caloric restriction and adaptation to restriction of calories. It has been suggested that caloric restriction in animals and man results in apparent decreased energy need for the same activity. This apparent paradox has never been explained. It has been shown that there is adaptation to repeated episodes of caloric restriction both in animals and man so that subsequent periods of caloric restriction result in decreased rate of weight loss, nitrogen loss, and to longer periods of survival.

Additional experiments are urgently required to determine the metabolic demands for minerals--in particular, the electrolyte balance of calcium, potassium, sodium, and phosphorus. Under conditions of high water utilization, large mineral losses are to be expected. Failure to replace these can cause an imbalance which will impair the efficiency of the individual to the extent of endangering the flight.

Analysis of samples taken in flight--both of urine and fecal matter--should be made. Respiratory quotients can be determined in flight, blood samples should be taken before and immediately after flight for the analysis of selected components (in simulator studies these could be taken periodically), and nutritional intakes (which would be facilitated by formula diets) must be measured and analyzed. The relation of nutrient, excretion, and other biochemical parameters to food intake and work periods under flight conditions should also be determined.

Short Range Technology

There are many practical difficulties in providing for food storage and accessibility in spacecraft under design.

The packaging of food materials, both dehydrated and liquid, has proceeded satisfactorily under the supervision of the Food and Container

Institute. If packaging materials are to be designed to withstand very high relative humidity and large variations in temperature, additional technological investigations are required since containers which will meet these demands are not yet available. It is imperative in packaging that serious consideration must be given to the ease with which the food may be reached and eaten.

If dehydrated formula foods are fed in short-term missions, additional work is required on the rehydration of such formulas. Present methods of water measurement under weightless conditions are not satisfactory, and better methods will have to be contrived.

Long-Term Nutritional Problems

There is a dearth of metabolic information, even for short duration flights, and without this information it is impossible to extrapolate changes in metabolic patterns to longer flights. However, using scattered information, it is possible to hypothesize certain changes which may be encountered. Decalcification of bone, with attendant formation of calculi, and changes in water holding capacity of the body may be anticipated. It is also possible that changes in proportion of fat to lean body mass could be experienced and should be considered in nutritional planning. Nutritional requirements are related to size, particularly lean body mass, to sex, to physiological state, and to individual metabolic rates. Therefore, individuals for space flight should be screened with these factors in mind if it is desirable to minimize food intake in long flights.

The factors which influence the total nutritional requirements of the individual also influence his mental and physical responses to stress.

A satisfactory basis for extrapolation of nutritional requirements is badly needed. Trials in simulators and short space flights provide a basis for extrapolation but minimize the number of new variables introduced as the complexity and duration of flights are raised.

Synthetic Foods

The development of food materials other than those derived directly from animal or vegetable origin is of interest. Advantages of such diets may be low residue per unit mass, ease of storage, ease of rehydration, and ease of manipulation. Since the molecular configuration of physiologically active amino acids and fatty acids is highly restricted, the economic separation of these from synthetic mixtures is questionable. Experiments with chemically defined synthetic diet for humans have been carried out by Medical Sciences Research Foundation, San Mateo, California.

The complete liquid diet is composed of required amino acids, fat, carbohydrate, vitamins, and minerals. A cubic foot of the diet (in 50 percent H_2O) supplies 2500 calories per day for one month, and has been flavored with a variety of artificial flavors.

The synthetic diet has been fed to human volunteers in a pilot study at the California Medical Facility, Vacaville, California, for a six-month period; and the results are being reviewed. The feeding has been completed but analyses of specimens and data interpretation must be completed before determining future research requirements. Schwarz Bio-research, Inc. is studying the storage, stability, and packaging of chemically refined synthetic diets for human and animal flights.

Food Production in Space

Long-term feeding in space depends upon the ability to provide a payload of stored food unless food is produced during flight. If sufficient propulsive energy is available, the duration of missions which can be provisioned with stored food may be quite long. However, in emergencies in which a mission lasts longer than planned, survival may depend on the ability to produce food extraterrestrially. Eventually it will be desirable or necessary to produce food beyond the confines of Earth.

Good nutrition can be adequately maintained for a certain period through the use of a properly constituted formula diet.

The primary objective of a feeding program on long flights is to supply a diet to the individual which is adequate in terms of nutritional balance. The nutritional requirements of the crew will be influenced by activity, physical and psychological stresses, individual size of the members, individual metabolic rates, etc. The food intake will then have to be adjusted to meet these requirements. The use of drugs, low temperatures, and other external means to alter metabolic patterns should not be contemplated until there is sufficient physiological information on their effects to warrant consideration. The depression of metabolic state is discussed elsewhere in this report. It is necessary to know the nutritional requirements of each astronaut and the way in which these are altered by the conditions of space flight in order to estimate the needs in long missions. Without this information, the food supplied for the longer flights may be too much, too little, or improperly balanced with regard to essential nutrients. Where dependence would not be on stored food alone, but on food produced en route, more exact information on requirements is needed to determine the size and capacity of food production units.

Regardless of the source of energy, it must be delivered in a form suitable for the food production process.

In the discussion of bioregenerative systems (Section VII) suggestions were made for the production of food materials employing photosynthetic organisms and tissue cultures (e.g., bacteria, algae, duckweed, and other higher plants), nonphotosynthetic organisms and cultures (e.g., Hydrogenomonas), and higher plants. In contrast to the production of living tissues, reprocessing of waste materials by chemical treatment or the actual synthesis of high energy chemical compounds have been suggested. No system has yet been demonstrated as workable in the economical production of food in space; and the systems considered produce materials which may be converted to food, but are not food as such.

Algal cultures have had the most extensive investigation as food in space, but the technological problems of using this material as a food source have not yet been solved. It is apparent from the investigations to date that algae will require treatment before they can be used directly as a food material. In limited trials, difficulties have been experienced with amino acid deficiencies, digestibility, high residues, and gastric distress; also a large proportion of the protoplasm is not available to the human body. Processing methods which would be applicable in space travel and the possibility of a secondary conversion by other animals or plants should be systematically investigated, with appropriate biological advice.

IX. SIGNIFICANCE OF THE ACHIEVEMENTS

SIGNIFICANCE TO SCIENCE

One of the major and most critical research areas of the space program is that of bioscience. Of both practical and philosophical significance in exploring the origins of life and the possibilities of life on other planets, it also promises much in medical aspects. Space offers to biologists completely new environmental factors such as the effects of zero gravity and of removal from the Earth's rotation. These effects have been studied in attempts to advance understanding of the basic mechanisms of physiology, and rhythmic fluctuations of biological organisms. These studies can be of great value in dealing with problems of disease and metabolic disorders.

Biological research is fundamental to the problem of successfully maintaining man in space, adequately protected and sustained in the peculiar and hostile hazards of space environment. Understanding human requirements and the variations in their response to various environmental factors and conditions offers value in medical research for human survival and comfort. The many technological discoveries and advancements in electronic and engineering equipment greatly enhance medical diagnosis, treatment of disease, and the extension of human life; also, many new practical contributions can be expected.

The life sciences, encompassing biology and medicine, are fundamental to the success of manned exploration of space, and this fact marks a unique and significant development in the long history of man's conquest of new frontiers. Those who pioneered across other frontiers on land and sea and in the air were not forced to await biological and medical research. Even the pioneers of aerial flight began their efforts without first seeking biomedical data. The search for such data followed flight experience and, indeed, was made only after problems arose.

Project Mercury, NASA's first program for manned space flight, stimulated immediate and extensive studies in the life sciences to sustain man in space. Before a vehicle could be designed to carry an astronaut into space, anticipated biomedical problems associated with space flight were studied. Life support systems were designed to offer adequate protection from environmental stresses peculiar to space, such as zero gravity, removal from the Earth's rotation, and high energy cosmic radiation. These life support systems utilized knowledge already gained from research for manned space flight by the United States Air Force.

Our entry into space has put us at the threshold of important fundamental and far-reaching discoveries in the biological realm which have profound implications for other areas of human thought and endeavor. As man goes farther into space, the hazards increase; but past (and present) accomplishments indicate that the road ahead holds more promise than peril and that the vistas of knowledge that may be foreseen are as vast as space itself.

It can be predicted as confidently for space biology as for other space sciences that the economic costs will be amply repaid in the long run by applications of space-oriented biotechnology to other fields of biology and medicine. There are inevitable substantial, though indirect, contributions of NASA's continuing efforts in space biology.

Almost everything which now can be said about the effects of extraterrestrial environments and about life on the Moon or the planets lies in the realm of pure speculation. There is one prediction, however, that can be made with considerable certainty by reason of historical precedent: the presentation of an opportunity to investigate a wholly new area, such as is offered by space exploration, is certain to produce a burst of scientific interests as soon as the path is charted by a few pioneers. Over the next few decades a progressively larger proportion of biological interests will turn to space. We may well expect that the discoveries made here will revolutionize some of our concepts of biology.

It should be fully realized that the accumulation and dissemination of biological and other scientific information is not only of great value to science and humanity but is of tremendous import to the prestige of this nation as it is to any nation.

SIGNIFICANCE FOR PRACTICAL APPLICATIONS

NASA-supported biological research has many practical applications and "spin-offs" which contribute to the fields of health and medicine, to food and agriculture and to industry and manufacturing. Some of these are presented to show the range and value of applications which have resulted from the basic and applied biological research. In addition to those listed are many others from the Biosatellite Program, particularly in the fields of bioengineering and miniaturization.

Health and Medicine

Solar panels, which tap the Sun's energy to operate space systems, are now being employed as a source of power for studies on brain function.

A miniaturized solar panel developed by General Electric provides enough power, under ordinary house lights, to stimulate an animal's brain and to telemeter respiratory, cardiovascular and brain wave data while the animal is allowed to move about freely. Such a system is now in use by the National Institute of Mental Health Laboratory at Bethesda, Maryland.

Scientists at the Ames Research Center have devised a new technique for detecting the source of organic compounds, i.e., synthesized in the laboratory or produced by a living system. This involves a property of living matter called "optical activity." The current method of testing for optical activity has been plagued by its low sensitivity. The new method is many times more sensitive and represents a genuine contribution to modern analytical instrumentation.

Studies on calcium metabolism and bed rest simulating weightlessness is providing valuable medical data on prevention of demineralization of the skeleton; treatment of Paget's disease and osteoporosis; prevention of muscular atrophy; the cause and treatment of renal calculi (kidney stones); optimal calcium for the human diet; and the factors influencing calcium absorption, metabolism, and excretion. The results will have great importance in bone healing and repair; care and treatment of fracture cases; treatment of paraplegics; and treatment of polio patients and similar cases. These grant studies at Texas Woman's University have also proven that the X-ray bone densitometry method can accurately detect changes in the skeleton.

A primary objective of the planetary exploration program is the detection of possible extraterrestrial life. The study of the fundamental properties of living things on Earth is restricted to the sort of life which has been evolved and survived here. Life which has been exposed to totally different environmental conditions must have markedly different physiological characteristics. The impact of the new information obtainable from the study of extraterrestrial life upon the sciences of medicine and biology will unquestionably be of fundamental and far-reaching importance. Advancement in the treatment of disease and the problems of aging are among the many possible consequences.

New developments such as ultraviolet spectrometry, biochemical polarimetry, and gas chromatography are under way for the detection of biochemicals for use in hospitals and in toxicological and pathological laboratories. These will also be useful in studies of atmospheric pollutants such as smog.

Studies of the chemistry of living systems, molecular biology, and biophysics of cellular processes will create a better view of the basic mechanisms of life, leading to an understanding of both inherited and acquired disease processes, especially neoplastic conditions and chemical upsets incident to mental health breakdown.

The University of Pittsburgh is conducting a study to increase the usefulness of biological methodology as a research technique and monitoring procedure by developing an automatic electronic scanning device utilizing computer analysis for recording, counting, and sorting chromosomes. Anatomical changes in chromosomes and blood cells can indicate degree of radiation damage as well as damage resulting from various environmental stresses. Accordingly, this instrument, when developed, can be used as a radiation dosimeter in civil defense by swiftly detecting the degree and type of chromosomal aberrations (or blood cell changes). Thus, casualties in nuclear attack could be quickly detected and separated. This system would also be useful for nuclear industrial plants and for military maneuvers. In medicine, various disease trends could be monitored. (Chromosomes exhibit anomalies in leukemia and mental retardation as well as other states.) In space exploration and experimentation the device can "spot monitor" radiation dose level as well as changes resulting from any of the environmental stresses experienced in space. This apparatus can be modified for use as an extraterrestrial life detecting instrument by scanning growth of cells (or cellular inclusions), computing rates and telemetering changes to the researcher.

Investigations of rhythmic phenomena of various physiological systems can result in knowledge of the utmost importance to medicine. Rhythmic phenomena are found in the cardiovascular system of normal humans. Disruption of these rhythms has the potential of foretelling abnormalities (heart disease, arteriosclerosis, coronaries) before outward signs are manifested, allowing for earlier diagnosis, treatment, and control or cure.

The spacecraft sterilization program requires the use of rooms having the lowest attainable level of bacterial contamination. The rate of dissemination of bacteria from the humans in the room is basic to the problem. Data on this matter are being obtained through support of the Communicable Disease Center of the U.S. Public Health Service. The findings are having an effect on the measures used in surgical practice to lower infection rates.

Studies on physiological factors of hibernation in mammals are important in order to understand temperature regulation and the mechanism for survival at low body temperatures. Potential applications of this type of research include the understanding of utilizing reduced metabolic activity in astronauts on future extended space flight. Other applications are in studies of the mechanisms of injury in that freezing biological organisms which will lead to better techniques in hypothermic surgery, pathology, and preservation of tissue for human grafting.

An electromechanical device, the picture Brailler, capable of reproducing line drawings in Braille has been designed, developed and evaluated at MIT under a project sponsored by NASA and other federal agencies. This is believed to be the first attempt at mechanized reproduction of ordinary pictures in Braille, which had hitherto been laboriously done by hand. In principle of operation the picture Brailler is similar to an electric typewriter. This device was demonstrated to teachers of the blind at a recent conference held at Perkins Institute for the Blind in Watertown, Massachusetts.

Food and Agriculture

Gathering agricultural information by remote sensing of the Earth's surface from elevated platforms such as aircraft, balloons and satellites is a potentially useful area for research and development. Current needs for data gathered in this way include crop and livestock surveys for marketing planning; soil mapping; crop disease, insect and weed surveys; soil conservation management and research; and crop acreage control programs. As population and world trade increase, the needs for regularly scheduled synoptic surveys of the world's agricultural lands for crop plantings and harvests, condition of crops as affected by drought, disease or insect outbreaks and studies of the lands suitable for agricultural development in underdeveloped countries will become even more intense. The only way that worldwide synoptic surveys can be made is by using orbiting platforms.

The NASA nutrition program for developing diets for prolonged manned and animal space flight lends itself to usefulness for civil defense purposes; military maneuvers where space and weight are prime considerations; polar and desert exploration; reducing hunger in underdeveloped countries; and for detecting metabolic diseases as well as diseases of infancy and old age. For space research a diet such as this can be used on prolonged manned space flights; animal experiments in space; manned orbiting laboratories; and space and planetary stations. Studies on the packaging and stability of foods under various conditions of humidity, temperature, and radiation will lead to better processing and storage of comestibles for human consumption.

How microbial spores are transported by the air is important to biology, agriculture and medicine. Besides spreading crop destruction, microbial spores produce allergic responses in some human beings. To ascertain the facts one needs to know not only the biology of microorganisms but also the weather factors that induce the flight of mature spores. Thus, both biological and meteorological problems are involved.

Data from a NASA contract with the General Mills Electronic Division (now part of Litton Industries) indicate that spores of fungi are present in low numbers in the stratosphere. A reservoir of spores exists which cannot be brought down by the normal scrubbing mechanisms of rainfall and other meteorological disturbances present in the troposphere. This finding has important implications for reducing the spread of agricultural crop diseases; for protecting persons suffering from allergies; and for possible dissemination of biological warfare agents. This project has indicated the necessity for designing novel biological samplers to be used in the stratosphere. Such samplers will aid in determining various pollutants of the atmosphere.

Our program to develop sterile spacecraft for the biological exploration of Mars will contribute improved methods of sterilization that can be applied to the canning industry.

In preparation for missions to search for extraterrestrial life, research on the psychrophilic or cold bacteria, halophytic or salt bacteria, and on specialized bacteria and other organisms growing in extreme environments, is defining the ranges of conditions under which life can exist. Increased knowledge about organisms that can grow in or on refrigerated, dried or salted foods and other materials should have practical applications for food storage and preservation. Research on psychrophilic bacteria is supported at Whirlpool Corporation and the NASA Ames Research Center.

Work on the synthesis of new anti-metabolites will result in new techniques for killing disease or pest forms of life.

Studies of theoretical Martian life involve investigations of plant and bacterial spores. Many of these forms are spoilage organisms, some of which produce lethal toxins. This work has potential ramifications for food processing and for obtaining more precise knowledge of how wounds become infected.

The program for investigating possible forms of life on Mars includes a thorough study of anaerobic microorganisms. This research has led to the discovery of new types of nitrogen-fixing bacteria other than the more familiar types usually found in the root nodules of leguminous plants. Thus, it may be possible to utilize these microorganisms, or the principles involved, in the incorporation of vital atmospheric nitrogen into terrestrial soils which are now unproductive.

Studies on sterilization at low temperatures for long periods of time are being supported by NASA at the Massachusetts Institute of Technology, the Communicable Disease Center and the Sanitary Engineering Center of the Public Health Service. The developing capability is making possible the heat sterilization of products that never before could be thoroughly sterilized.

Industry and Manufacturing

Batteries that have been developed in the space program to endure high sterilization temperatures for extended times will have greatly increased shelf life at normal storage temperatures and will be serviceable after many hours' baking at high temperatures.

Currently, the highest quality tape recorders are subject to imperfect reproduction simply because the tapes they use are heat labile, i.e., they soften and stretch when warm. The development of valid, highest quality sound-track tapes for space data recorders is an outgrowth of the materials developed to meet spacecraft sterilization requirements. These improved tapes will be useful for all types of recording--industry, automation controls, home, studio, etc.

OUTLOOK FOR BIOSCIENCES--MAJOR PROBLEMS

The problems which have been undertaken to solve are among the most challenging - if, indeed, not the most challenging - man faces on the space frontier. These include the quest for the origin of life; the explanation of life and life processes; the elucidation of the role of environment in establishing and maintaining normal organization in living organisms; and, finally, the possibility of extraterrestrial life on other planets - the concern of exobiology. The greatest promise for their solution lies in advances in biological theory rather than other avenues of research; and it is, therefore, fortunate that the need to solve them has come at a time when developments in experimental biology are at a high level. In addition, technological developments in electronics and engineering are providing new and wonderful instruments for this great exploration into the sources and meaning of life. Many of these have had practical application which has made possible important advances in medical diagnosis and treatment.

The broad national space goals initially charted by NASA have gone beyond space flight in near-Earth orbit to lunar and interplanetary exploration by man and machines. For such missions, more intensive and comprehensive research in the life sciences is needed. Before manned voyages for extended periods into deep space will be possible, solutions must be found for problems such as the development of bioregenerative life support systems, the prediction of evolutionary patterns in extraterrestrial environments, communication with nonhuman species, and the development of unorthodox methods of transferring knowledge to the human brain over new dimensions of space and time.

The problems are all of the type which could, perhaps, be solved by truly great advances in biological theory, and probably not by any other avenue.

SUMMARY

A broad research and flight mission program in space biology has been developed and supported by NASA. This includes (a) the development of life detection experiments and a concept for an automated biological laboratory for planetary exploration for life; (b) the development of a Biosatellite Program including scientific experiments to study the biological effects of weightlessness which can be studied only in space, radiation combined with weightlessness, and removal of organisms from periodicities associated with the Earth's rotation; (c) the development of a recoverable Biosatellite spacecraft system; and (d) all of the supporting research and technology required for these flight missions as well as biological support for manned space flight.

Research in exobiology has advanced our knowledge concerning abiogenic synthesis of life-related compounds and possible origin of life, the organic constituents of meteorites, methods and concepts for life detection, and methods for spacecraft sterilization.

Various Earth organisms have been subjected to simulated planetary environmental conditions and the growth or survival established. A detailed program on the exact definition of the physical and chemical environmental extremes for growth and reproduction of Earth organisms has helped establish extreme environmental parameters of life which can indicate the potential existence of life on other planets.

Research in environmental biology has increased our knowledge of the effects of space environmental factors on living organisms, and the effects of the dynamic profile of space flight including launch and reentry forces. The effects of weightlessness are of special significance and have received greatest attention. Sophisticated experiments on the effects of weightlessness on the cardiovascular and neurological systems of primates have been developed. Radiation is of major significance and much research has been completed on the effects of proton, high energy heavy particle, and other simulated space radiation. A series of experiments on the effects of weightlessness and combined radiation from a known source have been prepared for orbital flight.

Research in behavioral biology has elucidated some of the effects of the space environment on behavior, and experiments involving weightlessness have been developed. Advances have been made in neurobiology related to space factors including the vestibular system in relation to gravity and rotation. The knowledge of the process of memory storage and retention on a molecular basis has been advanced. Experiments on biorhythms have been developed with various organisms to define the effects of removal of animals and plants from the normal circadian 24-hour rhythms associated with the daily periodicities of the Earth's rotation.

Some biological flight programs have been conducted by NASA in flying both monkeys and chimpanzees in suborbital and orbital flights to test systems and to demonstrate safety of space flight before the astronauts' flights. A sophisticated Biosatellite Program has involved detailed research and development, as mentioned above. The Biosatellite will be placed in orbit during the 1966 and 1967 time period.

A new bioregenerative life support system has been developed which appears to be a technological breakthrough. Water is split into hydrogen and oxygen by electrolysis, and Hydrogenomonas bacteria use the hydrogen and waste products from the astronaut. A broad research and development program of NASA indicates a very efficient system as compared with other regenerative systems. Research on algal and higher plant regenerative systems is continuing with primary support by the Air Force. Various cabin atmospheres, including pure oxygen, and substitution of helium or argon for nitrogen have been studied with different animals.

The effects of bed rest simulation of weightlessness have been studied, including research on calcium loss using X-ray bone densitometry, and cardiovascular studies. These techniques are being used with astronauts and orbiting primates. There are also studies on reduced metabolism and hibernation related to prolonged manned missions. Research has been quite promising using a chemically defined liquid diet with human volunteers.

The basic and supporting research program has been carried out in universities, private research institutes and foundations, by industry, and in NASA research centers. It is expected that the program will continue to produce valuable and needed results in the future. The research program is carefully coordinated with research programs of other government agencies.

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