SPACE SCIENCE BOARD
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SPACE SCIENCE SUMMER STUDY

Working Group on
Fundamental Biology and Exobiology

A. Extraterrestrial life

1. Earth studies on origin of life

(a) Systematic investigation of the effects of electron microbeam and ion beam irradiation on the viability of bacterial spores, certain micro-organisms, and induced mutations. Since it is now possible by means of low-temperature electron microscopy to simulate many of the conditions encountered in Allen belt regions, these studies on the viability of spores and certain micro-organisms may prove to be of special significance (e.g. in connection with the "Panspermia" hypothesis). With improved instrumental and preparation techniques the electron microscope can now be used as a powerful tool both for the controlled production and direct observation of radiation damage in preselected macromolecular regions of hydrated biological systems.

2. Radiation aspects

(a) Exobiological aspects of radiation. The radiation environment, including UV, of ostensible exobiota should be characterized and the bearing of space radiations on the plausibility of panspermia investigated. Experiments should be undertaken regarding the capability of standard preparations of microbiota to survive the space environment, both without shielding and under various shielding conditions. Studies on the survival of terrestrial microorganisms trapped in hard deposits are also relevant. Simulated environmental studies should include the radiation environment. Consideration should be given to the use of radiation in combination with other methods for sterilization of equipment to be sent into space, in the hope of finding procedures less injurious to components than is heating alone.

The general subject of exobiology is regarded by all members of the subcommittee as being of paramount importance among all biological programs in the space sciences. Radiation aspects of this subject are listed below the foregoing two priorities only because of the specialized character of radiation in relation to exobiology.

3. Theoretical approaches

In all of these investigations it should be borne in mind that the detection of life on Mars would be only the first step. Equally important is its characterization from a chemical and biological point of view. We want to know whether Martian life--if such exists--originated independently from terrestrial life. This knowledge can be obtained only from detailed studies. If Martian organisms are fundamentally similar to our own, the possibility of their common origin with terrestrial organisms, and of transfer of living matter from one planet to another, will have to be considered.

Terrestrial studies related to exobiology should be supported. These include work on organic syntheses under simulated primitive earth conditions and investigation of organisms living under extreme conditions of temperature, pressure, humidity, etc. Many of the approaches proposed by other groups appear to be distinctly earth-centered. We recommend (a) a characterization, on the basis of our knowledge of Mars, of a possible organism on that planet, and (b) theoretical consideration of possible kinds of "life" other than that known on earth.

4. Expectations for specific regions in space

(a) Moon. The moon's surface is not considered to be habitable, but the possibility that life of some kind occurs beneath the surface should be considered.

(b) Venus. Although apparently inhospitable to life because of its high surface temperature, Venus should not be abandoned as a possible biological objective until definitive evidence is obtained regarding conditions on this planet. The possibility that life exists in the cloud layer should be considered, even if the surface is uninhabitable.

(c) Mars. Mars is the most important arena for exobiological studies, since evidence already at hand suggests that the planet is the abode of life. Despite the extreme dryness of the Martian environment, we believe that life as we know it--i.e., life based on nucleic acis and proteins--can exist there.

(d) Interplanetary space. The biological exploration of interplanetary space should be a major NASA program. Attempts should be made to collect micrometeorites under conditions which will preserve any living matter they may contain. Biological studies of such material would provide a test of the panspermia hypothesis. In addition, systematic attempts to obtain carbonaceous chondrites by tracking and by purchase from museums should be supported.

5. Detectors to be sent into space

(a) From a distance. Spectroscopic studies in the IR and UV from balloons, observatories, and flybys. Listening for radio signals from outer space.

(b) From landing spacecraft

(1) Organic chemical and atmospheric analysis, with special regard to biologically important substances such as water, oxygen, carbon dioxide, ammonia, methane, and hydrogen sulfide. Water of hydration in soil should not be neglected. Other ecological parameters such as temperature, pressure, UV flux, etc. are also biologically important and should have high priority.

(2) Direct life-detection experiments
Metabolic methods--e.g., as proposed in the Gulliver
Biochemical investigations--e.g., detection of enzymes, as proposed in the multivator.
Microscopy
TV observations
Microphone
motion —

6. Sample retrieval

In order to obtain all the information we require, it may be necessary to retrieve samples of Martian soil for study in terrestrial laboratories. Many of us believe that this should be recognized as the ultimate objective of exobiological missions. Sample retrieval may well require a manned expedition and will involve the most advanced engineering and life-support systems. One-way expeditions of colonists to Mars were discussed. The colonists could study Martian life in situ and transmit the information back to earth. No recommendation on this point.

7. Biochemical detectors

Use of metabolic criteria to detect living organisms. Gulliver, as we understand, is designed to pick up heterotrophs, place them in appropriate labelled substrate, and detect labelled CO_2 which may be released.

It seemed to us that the alternative of designing equipment to detect possible autotrophs might be considered. The bulk of the biomass on earth consists of autotrophs; the same might be true of another living system. Thinking of photosynthetic forms, collected samples might be presented with labelled CO_2, and search be made for fixed CO_2. This might have the advantages of (a) a greater probability of finding organisms, (b) less dependence on the nature of the substrate and (c) the possibility of carrying a dark control on the experiment.

8. Optical Detectors

(a) Spectroscopic examination of light reflected from a planet. Reflection absorption spectra of the surface of a planet might be used to detect possible biological pigments. The use of narrow band lasers for this purpose was suggested.

(b) Direct investigation of the chemistry, molecular and submolecular organization of extraterrestrial matter in the solar system (interplanetary dust, meteorites, interstellar matter (solid particles, submicroscopic grains of ca. 10 to 1000 A.U., including gas hydrates, etc.) as revealed by appropriate electron-optical and related analytical techniques at low temperatures and ultra-high vacuum. Electron microscopy and related electron-optical techniques are of unique operational value as basic tools for space (biology) investigations by virtue of:

(1) The requisite conditions for electron microscopy: high vacuum, electron beam and ion irradiation, thin speciments, and recently introduced low-temperature electron microscopy and electron diffraction, are very similar to conditions encountered in outer space.

(2) The special techniques that have already been developed for electron microscopy are readily applicable to examination of extraterrestrial matter. Thus, the use of thin graphite or single-crystal stable coherent films, replication, shadow-casting, etc., are suitable for this purpose. For example: By coating satellites and space probes with these thin films, and then stripping them off for direct examination by electron microscopy, it should be possible to obtain adequate samples from space, lunar and planetary probes. It should be pointed out that most of this material

would be well below the resolving power of light- and x-ray microscopes, and that electron-optical techniques would permit us to examine the structure and chemistry of these specimens (i.e. by electron microscopy, electron diffraction, electron microprobe analysis, etc.) in a way that can not be achieved by any other known technique.

(c) With present advances in the generation of stable superconducting electromagnetic fields, it is conceivable that the contemplated design of new types of high-resolution "Cryo-electron microscopes" operating at liquid helium temperatures and using "in situ" examination of lunar and planetary matter by electron-optical techniques. In view of the available high-vacuum and low-temperature conditions already prevalent in outer space such a cryo-electron microscope could be miniaturized. By being of smaller size and invested with a far greater resolution, of the order of 200 to 1000 x, than a light microscope, as well as the fact that the magnified images can be directly transmitted by modified television techniques, such a cryo-electron microscope would permit a far greater range of applications, and thus supplement the present contemplated light microscope systems.

(d) Successful application and development of these techniques requires special training and bio-instrumentation facilities. It is therefore necessary, that after working out the optimum conditions for application of these electron-optical techniques to existing space vehicles, thorough training of specialized technicians in the different NASA Space Research Centers be carried out. In view of the key operational significance of electron microscopy for future developments in biochemistry, biophysics, and the biomedical sciences in general, intensive development and research work is currently under way in specially organized centers in Russia, England, France, Germany, Holland, Japan, etc. Most of these electron microscopy centers are equipped and supported on an unusually large scale and on a long-term basis which reflects distinct awareness of the vast scientific and technological potential of this field and related electron-optical disciplines.

B. Radiation

1. Thorough characterization of space radiation by appropriate physical measurements. This characterization should include not only the energy distribution and flux of the various particles and other radiations, but also the temporal and spatical changes during solar flares. This knowledge is basic to all of the biological problems involving radiation in space.

   If opportunities should arise, the use of tissue-equivalent dosimeters is advocated as a stop-gap measure to secure a preliminary guide to problems of shielding, but this technique does not provide the information ultimately required, and is not regarded as having an independently high priority.

2. Theoretical radiation biology. Our accumulated knowledge in radiation biology and recent advances in molecular and cellular biology, together with an increased understanding of the in vitro action of radiation on biological macromolecules, suggest that it is time to attempt a theoretical analysis of radiation action on micro-organisms, animal and plant cells and possibly also on multicellular organisms.

   It is specifically suggested that several groups be set up in universities or National Laboratories to develop theories to explain and/or predict radiobiological responses at the molecular, cellular and organismal level. A conference should be held at least annually among the participants to discuss results, and proceedings should be published. It is estimated that about $300,000 annually would support the cost of the conference as well as the research in such a program. Interagency cooperation may be desirable.

3. Heavy Particle Experiments. Serious doubts were expressed in the subcommittee of the practicability of studying the biological effects of heavy primaries (Z greater than 2) in the space environment. The information provided the committee on the flux of these particles suggest that it is generally too small to permit reliable, interpretable biological experiments within the practicable flight times of recoverable capsules. Simulation by means of available facilities such as HILAC and other particle beams was regarded as a useful approach, especially when combined with theoretical studies on the role of LET in biological radiation effects.

4. Radiation Studies. If ~~experiments on the RBE of the radiations available in space are contemplated,~~ The advantages of using certain plant materials should be considered. Advantages of high sensitivity, and relative ease in the scoring of somatic mutations by proper choice of experimental material.

5. Chemical protection against radiation. The question of chemical protection against radiation was discussed. It was recognized that the known protection agents are much less effective against radiations of high LET, as expected in space, than against low LET radiations. The committee concluded that this approach would have a high priority only if a new idea is proposed and that it is a subject that may be profitably considered in a theoretical study of radiation biology.

C. Weightlessness

1. Sensing aspects

(a) Low gravity environments. "Weightlessness" or free-fall conditions are unique to space environments. Sensory deprivation in animals of the following inputs needs to be examined: vestibular and statocyst, kinesthetic, cardiovascular and microcirculatory. Threshold differences may be specific for different organisms. (a) For the circulatory categories fluid distribution, particularly in venous return in mammals, may well be altered in the low-gravity environment. (b) Much is known regarding crustaceans, fish and mammals which have been deprived of their equilibrium receptors but it is not possible surgically to remove all gravity input. (c) Long-term experience in the low-gravity state may lead to unique behavioral changes, particularly in mammals; short-term exposure may be less significant.

(b) Statolith response, function, and development as affected by gravity was proposed. Ranging from the "labyrinth" inner ear of the mammal to the unknown statolith of the plant, they appear well suited to satellite experiments, provided that adequate telemetering of elicited responses and modifications can be obtained. In view of the reported balance disturbances in astronauts, experiments of the above type might indicate correlations on the effects of sub-g on the CNS. The nature and biophysics of the plant sensors could be stressed in ground studies.

(c) Pertaining to some of the "reaction" topics, the suggestion was unchallenged that balloon flights were not a sufficient departure in gravitational field, and were of environmental characteristics that could be approximated sufficiently in ground-based setups, so that morphogenic studies in such airborne devices did not appear too exciting.

(d) The growth and development of differentiated plant organs in vehicle environments should be considered. Preliminary experiments in compensated fields indicate a complete loss of orientation with respect to spatial coordinates.

2. Growth and differentiation

(a) Considering capabilities in shielding and controlled environments, the only single environmental factor that could be unique is the gravitational. It was felt that studies of the role of gravity in morphogenesis could justifiably be a major emphasis in space oriented efforts, hither and yon. With this would be compatible directed support of ground-based investigations of the biophysics of sensor nature and action, particularly in plants, physiological sequelae of sensor activation, and threshold phenomena.

(b) There was the comment that the important questions in morphogenesis would be suggested and defined by the char-

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acteristics of the biological material that came back from sub-g environments wherein <u>de novo</u> development took place. As a result we should consider strongly such initial experiments that would permit multiplicity of observation at various levels of organization to determine qualitative and quantitative differences from the norm.

(c) A case can be made for the thesis that in some organisms, the polarization prerequisite for cyto- and histogenesis is modifiable and hence definable by gravity. One inferrable consequence of development in a sub-threshold field would be morphogenic departures ranging from altered form, structure, and function to acellular accretion. Hence, appreciable consideration was given to an experiment wherein undifferentiated, totipotent, embryonic tissues in supportive media is permitted to develop in a low-g field. Collaborating talents would then look for alterations of form and structure at gross, histological, cytological and molecular levels of complexity, for alterations of physiological function, and for biochemical and metabolic variations. The egg cell immediately subsequent to fertilization may also be considered in the above context of recoverable packages.

(d) Studies on the influence of gravitational field on the genesis of microspheres, both protein and non-protein, could well be stressed. The developmental and evolutionary implications of even minor departures induced by field differences are exciting.

(e) Leaf mesophyll development has an appreciable background in detailed morphogenetic studies; this material might prove well suited for a study of development in flight by virtue of its ready culture.

(f) Algal cell size and patterns of division in orbiting vehicles may be of particular interest in view of considered algal function in closed systems.

(g) Free-fall conditions. The terrestrial gravitational field is clearly important in <u>orienting</u> the growth of plants, and perhaps in <u>determining</u> a variety of morphogenetic processes in plants. Since the only methods available on earth to alter the gravitational forces on plants, are those using the clinostat and centrifuge, it seems clear to the group that experiments on plant development in an orbiting vehicle are in order. Pronounced effects on the gross morphology of plants would be expected, in conditions of free fall. It might even be anticipated that the gravitational field may be important in determining patterns of ultrastructure and of physiological mechanisms. (e.g. effects on grana structure, and on the photosynthetic mechanism.) Exploratory experiments should be designed with these possibilities in mind.

3. Low Gravity combined with other stimuli

(a) Influence on the space environment on biological effects of radiation. The possibility that radiation effects might be different in altered gravitational field was considered. It was thought that experiments designed to discover such differences must satisfy either of two criteria before they are actually performed. One criterion is that the experiment be sufficiently well planned so that a negative result will be conclusive to within a high degree of confidence. This type of experiment might be performed on systems in which there is no special reason to suspect that the gravity vector affects the organism. A second criterion is that the biological system under study be one that is already known or can reasonably be expected to be responsive to the gravity vector.

(b) Well-defined chromosome markers occur in Drosophila. Their support and mating in a satellite environment could contribute to the question of whether normal gametes are produced, and what environmental effects there are on the patterns of segregation and development.

D. Temperature extremes

1. Cryobiology is a challenging area of environmental physiology applicable to space but which can be studied on earth. Survival in the frozen state is common in Arctic plants and poikilothermic animals. Many cysts and spores withstand long periods at very low temperatures. In animals and plants, profound but unknown changes precede the entry into dormancy. There is evidence that up to 90% of the water in dormant organisms can be frozen. The state of the remaining and critical (presumably bound) water is not understood. It is possible that cryobiology may lead not only to appreciation of some of life's problems on Mars but also might be used in preservation of organisms for prolonged space flights. Related is the study of hibernation in homeotherms. Much remains to be learned concerning tissue function in hibernants at low temperatures.

2. Cryobiology and related low-temperature (electron microscopy) studies of biological specimens and cell constituents. The problem of establishing the ability of organisms to survive almost indefinitely at sufficiently low temperatures (i.e. liquid helium temperatures) is of major importance for future possibilities of prolonged space travel of sentient organisms under conditions of "suspended animation".

~~3. High temperatures (Venus~~?)

E. Exotic environmental stimuli

1. Magnetic fields

2. Radiofrequency

3. Electrostatics

4. Isolation

(a) Poorly-known sensory inputs fall under the cognizance of NASA. Sufficient reports are available to justify investigations on radio-frequency waves, electrostatic and magnetic fields and on whatever the stimuli are in bicoordinate navigation of birds. Use should be made of a series of special facilities for reducing and for increasing the first three of these stimuli. The reported effects are largely at the cellular level, to a lesser degree on behavior of intact animals.

(b) Isolation studies in man should be part of the space program. Psychologists who are familiar with isolation should be consulted. There are known syndromes which result from reduction in total sensory input. Systematic deprivation of specific modalities in various combinations can be done in earth studies.

F. Periodism-Circadian rhythms

1. <u>Relation of circidian or other cycles to radiation effects</u>. Since cyclic phenomena in organisms in the terrestrial environment may be subject to drastic alterations in space, the relation between such cycles and radiation effects may be instructive.

2. Altered periodism and phenomena of biological time. One limit to earth-bound organisms including man in space may be the capacity of the biological clocks to adapt to drastically altered periodism. On earth the clocks are normally set by photic stimuli but once set they maintain their periodicity which can be little altered. There is some question as to whether in the absence of photic triggers, exotic environmental factors such as daily variation in magnetic and electrostatic fields might be used to set the biological clocks. This is an important question which should be investigated by observation of rhythms in organisms in space environments. Organisms in the following orbiting conditions should be compared for their rhythms: (a) polar and equatorial orbits at about 150 miles, (b) orbits at less than, equal to, and greater than 22,000 miles. These critical tests in space should be prefaced by observations in controlled environments on earth. Rhythms of temperature in mammals, activity in mammals and crustaceans, oxygen consumption by incubating chick eggs could be examined.
3. Rhythmic processes
   The possibility that plants might present advantages for study of the mechanism of biological rhythms was considered. It was felt that the photosynthetic requirement of green plants for light, together with the general damping of rhythmic processes by continuous light, suggest that green plants offer no unique advantages for the study of rhythmic processes in an orbiting vehicle.

G. Life support

1. Component organisms in support systems. At such time as multibiotic life support systems are designed, the radiation responses of the component organisms may bear investigation.

2. Development of life support systems. It was observed that the effort to develop closed ecological systems, which depend on photosynthesis by algae, currently is being done with competent advice from plant physiologists. The effects of radiation on such systems should be considered. As manned space journeys become more extended in time, it may be desirable to gain experience with the growing of other plants than algae in spacecraft, stations, or their possible use in colonizing other planets, and experimentation with higher plants may be justified on these grounds. Earth-bound experiments directed toward use of higher plants in closed ecological systems should be considered.

H. Sterilization

The sterilization of spacecraft likely to impact on Mars is considered absolutely essential until further knowledge demonstrates that this is unnecessary. Procedures should be worked out that minimize the chance of contamination of the landing capsule--i.e., the entire capsule should be sterilized after assembly. No attempt to set up standards of sterility was made, but it was agreed that these should be as high as possible. Various methods of determining the actual degree of sterility of spacecraft were discussed. Only two methods seem feasible: (a) spot-checking of a number of selected areas, and (b) estimates based on survival studies of microorganisms exposed to the same treatment. It was recommended that studies be carried out to determine the effectiveness of cycles of freezing and thawing on the killing of microorganisms, since it may be presumed that terrestrial organisms deposited on Mars will be exposed to this treatment. This may significantly reduce the chance of survival of terrestrial contaminants.

I. Back-contamination

Back-contamination of the earth by spacecraft returning from the planets has been a source of concern to some scientists. It appears at the present time that this represents a negligible risk, since in all known cases pathogens have evolved in association with their hosts or with closely related species. Furthermore, we are not defenseless. By anticipating danger, we can minimize it by proper handling of returned samples and spacecraft. Obviously, many unmanned one-way missions to Mars will be carried out before a round-trip becomes feasible, and information regarding possible hazards will presumably be obtained in these missions. This question should be kept under constant review as our knowledge of Mars increases. At the present time, however, it appears to us that back-contamination is ~~not~~ a large enough hazard to cause us to modify our belief that the return of samples of Martian soil should be regarded as a prime objective of exobiological missions.

We invite the cooperation of other nations in this great enterprise.

J. Communication and training

1. Importance of inviting active participation and enlisting long-term cooperation of scientists from other countries (particularly European, Latin American, Eurasiatic scientists) in both the planning and instrumentation of the Space Biology (i.e. Bio-satellite etc.) programs and related studies. Key advantages and desirability of combining these programs with envisaged broad cooperation and scientific exchange programs with Latin American countries, and decisive role of NASA sponsored efforts in these areas of cooperative research and training.

2. Suggestion of "NASA Space Biology International Fellowships"--Space Biology Journal. Advantages of combining these cooperative research and training programs with "International Space Biology and Biomedicine Symposia" and related scientific Meetings of the type organized by IBRO: International Brain Research Organization, International Union of Biological Sciences, UNESCO, etc. Enlightened Scientific Publication Policy, and participation in both national and international Meetings in order to more fully acquaint the international scientific community with the "Space Biology Programs" of NASA. This and other determined efforts should be made to "win back the respect of the scientific community in the Space Biology effort" which has suffered as a result of inadequate planning and communication of research work in this area in its present incipient stage.

3. Integrated Research and Training Program in the fields of "Space Biology", Exobiology, and "Space Molecular Biology", with particular reference to correlated biophysical and biochemical studies, applications of electron microscopy and related electron-optical techniques, light microscopy, x-ray diffraction etc., and of cryobiology in the study of:

   (a) Molecular organization and function of biological systems under controlled conditions simulating space-environment, and in unusual terrestrial and extraterrestrial environments.

   (b) The study of artificial and biosynthetic forms of life at different levels of organization down to the molecular and submolecular levels.

(c) Critical studies of the effects of ionizing radiation, low temperatures and pressures, abnormal gravity conditions and related parameters of extraterrestrial environments at critical stages in the development of the nervous system and other systems of vertebrate and invertebrate embryos. In general fundamental research of morphogenesis, developmental and environmental biology at all levels of organization in selected biological systems.

(d) It was also felt that more satisfactory foundations of ground-based experiments in developmental morphology were necessary. One contribution would be the stimulation of interest of recognizably capable biologists. Some devices could be the invitation of active participation of scientists in Europe, Latin America and the East in the planning and experimentation of biosatellites and related programs; of open international symposia to acquaint the scientific community further with space biology; of persuasive invitation to such symposia of scientists and teachers unconnected with space biology; of sponsorship by NASA of international fellowships in space biology to stimulate training and research in this field.