



The Space Congress® Proceedings

1968 (5th) The Challenge of the 1970's

Apr 1st, 8:00 AM

Measurement of Space Environmental Effects on Biological Specimens

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Areas of Experimental Interest

Experimentation with live biology in space flight is a relatively new field of endeavor. The recently acquired ability of man to place relatively large satellites into earth, solar, and interplanetary orbit has opened to the field of biological research a great new area of investigation.

Bioscience experiments have already been flown on the Biosatellite and some of the Gemini flights. Many more are proposed. Some of the major areas of interest are as follows:

a. Studies of "zero-gravity" effects in living organisms. Reduction of the g vector to extremely low values for periods of time of more than a few minutes has, of course, been impossible until recently. The effects of the reduction in the gravitational force to essentially zero on the growth and reproduction of cells and on whole organisms is an area of great interest.

b. Studies of combined effects of reduced gravity and radiation:

Of obvious interest to proposed human space travelers is the question of whether the combination of "zero-g" and radiation is synergistic or antagonistic.

c. Periodicity studies in environments isolated from earth influence:

Earth orbital and solar orbital experiments are proposed to investigate the effects of extraterrestrial environment on the inherent periodicity of many physiological functions of biological organisms.

d. Studies of the performance and behavior of animals under stress:

Included in this area are studies of the effects of launch noise and vibration, accelerations, isolation, and reductions and changes in the gravity vector.

Support of Biology in Space

The support of live biological experiments in space involves problem areas which are not common to most physical science experiments.

Perishability

The inherent perishability of live biological specimens introduces a serious restriction on launch holds and delays. Experiments must be installed in the spacecraft such that access is available at the last possible moment in the countdown. Extended countdowns and built-in holds are virtually impossible for flights involving live specimens. Any unscheduled delays or holds would most likely cause degradation of experiments.

Hard Environments

Biological specimens are generally highly susceptible to the so-called "hard environments" of launch. These environments are usually considered to be shock, vibration and acceleration. Acoustic noise may also affect the scientific validity of the experiment. Specimens must be protected from these environments through state-of-the-art methods of packaging and mounting. In addition, ground control experiments are often required to isolate or "control out" the effects of these environments on the specimen. Often controls must be run concurrent with the flight, but delayed enough in time so that real-time or near real-time spacecraft data describing the experiment environment can be analyzed and applied to the controls. The need for concurrent controls is often dictated by the requirement to use specimens of exactly the same strain or subjected to exactly the same pre-launch history as the flight specimens. In any case, it becomes evident that it is most important to not only attempt to control hard environments to protect the specimens, but to measure and record the levels and transmit these to ground stations as rapidly as possible.

Environmental Control

Environmental control of the space occupied by the experiment is another critical problem. Most experimenters wish to maintain a sea-level atmosphere of oxygen and nitrogen. Trace contaminants may in some cases be more toxic to the biological specimen than to man and, therefore, must be rigidly controlled. The complexity of provision and control of a two-gas atmosphere is often complicated further by requirements to measure metabolic processes such as oxygen consumption or CO_2 production. Temperature must be maintained over relatively narrow ranges and wide excursions during reentry

would no doubt be detrimental to the more sensitive organisms.

Life Support

The complexity of life support systems is, of course, dependent on the biological specimen under consideration. Primitive organisms may be packaged in completely self-contained life-supporting packages and may require only the environmental control mentioned above. More sophisticated biological systems such as the larger mammals, including sub-human primates, may require extensive automated life support systems which dispense food and water; and remove and store feces and urine. Requirements to measure factors affecting metabolism may make measurement of water and food intake and waste production necessary.

Instrumentation and

Operation of Biosatellite Experiments Biosatellite 3-Day Mission

The 3-Day Biosatellite contained a payload of thirteen experiments selected to determine the effects of the space environment on various life processes. The experiments involved the investigation of effects of weightlessness on the entire organism and on the structure and growth of the cell and its basic biochemistry. Some of the specimens were exposed to measured doses of gamma radiation from an on-board source to determine the effects of a combination of weightless and radiation. A duplicate set of the irradiated experiments was flown in a location in the spacecraft shielded from the radiation source to serve as an on-board control exposed to weightlessness alone.

The Biosatellite 3-Day experiments are listed below:

1. Liminal Angle of a Plageotropic Organ Under Weightlessness (P-1017) by Dr. S. P. Johnson and Dr. T. Tibbitts of North American Aviation, Inc.
2. The Effect of Weightlessness on Growth of the Wheat Coleoptile (P-1020) by Dr. S. W. Gray and Dr. E. F. Edwards of Emory University.
3. Nutrition and Growth of *Pelomyxa Carolinensis* During Weightlessness (P-1035) by Dr. R. W. Price of Colorado State University and Dr. E. E. Ekberg of General Electric Company.
4. Mutagenic Effectiveness of Known Doses of Gamma Irradiation (P-1037) by Dr. F. J. De Serres and Dr. B. B. Webber of Oak Ridge National Laboratory.

5. Synergistic Factors Influencing Embryonic Differentiation and Development in the Space Environment (P-1039) by Dr. J. V. Slater of the University of California, Berkeley

6. Effects of Sub-Gravity on Cellular Phenomena of Developing Frog Eggs (P-1047) by Dr. R. S. Young of NASA Headquarters and Dr. J. W. Tremor of Ames Research Center

7. Mutagenic Effectiveness of Known Doses of Gamma Radiation in Combination with Weightlessness on *Habrobracon* (P-1079) by Drs. R. C. von Borstel, R. H. Smith, A. R. Whiting, of Oak Ridge National Laboratory, and Dr. D. S. Goshak of North Carolina State University and Dr. R. L. Amy of Southwestern University

8. Emergence of Seedlings with Zero Gravity (P-1096) by Dr. C. J. Lyon of Dartmouth College

9. Determination of Influence of Zero Gravity on Mutation Process Using Controlled Gamma Ray Exposure (P-1123) by Dr. A. H. Sparrow and Mr. L. A. Schairer of Brookhaven National Laboratory

10. Induction of Lysogenic Bacteria in the Space Environment (P-1135) by Dr. R.H.T. Mattoni of NUS Corporation, Dr. W. R. Romig and Dr. W. T. Ebersold of University of California, L.A., Dr. E. C. Keller of NUS Corporation, and Dr. F. A. Eislering of University of California, L.A.

11. Effects of Weightlessness on the Orientation of Root and Shoot of Corn (P-1138) by Dr. H. M. Conrad of Resources Planning and Control Corporation and Dr. S. P. Johnson of North American Aviation, Inc.

12. Effects of Zero-Gravity on Radiation-Induced Mutations in Mature Reproductive Cells (P-1159) by Dr. E. Altenburg and Dr. L. S. Browning of Rice University

13. Possible Effects of Zero-Gravity on Radiation-Induced Somatic Damage (P-1160) by Dr. I. I. Oster of Bowling Green State University.

The primary means of obtaining experimental data was the actual physical examination of the biological material after its return from orbit. The pepper plant package employed a system of lights and mirrors and a camera which was actuated by pulses from the spacecraft timer in order to obtain time-lapse pictures of the growth and development of the plant. Pictures were taken every 10 minutes; however, they were not telemetered; and for all experiments, recovery was essential for successful data retrieval.

The real-time and near real-time flight data consisted of spacecraft housekeeping data and data describing the environment seen by the experiments during flight. Experiment package

temperatures were telemetered as were capsule relative humidity, total pressure, and partial pressure of CO₂. The launch and reentry vibration and acoustic noise were recorded on an on-board Gemini-type biomedical tape recorder. A sample of the capsule atmosphere was taken immediately before launch and as soon as possible after recovery. This sample was analyzed for all gas constituents.

Certain of the experiments contained the same biological specimens in separated compartments. At pre-programmed intervals throughout the flight, fixatives such as formaldehyde or glutaraldehyde were injected into selected compartments in order to preserve the specimens in a condition which could be related to length of time in orbit. The specimens then could be examined after recovery as to their condition at various stages of the flight. In general, fixing was accomplished by the firing of small, squib-actuated valves, initiated by the on-board timer; however, fixing by ground command was possible in some cases.

Spacecraft accelerations were measured and controlled by the attitude control subsystem, and real-time accelerations were telemetered during each station pass (approximately every 90 minutes). The design requirement for accelerations in orbit was a maximum of $10 \frac{1}{4}$ g for 95% of the time and less than $10 \frac{1}{4}$ g for the remaining period.

It should be noted here that the two Biosatellite 3-Day missions have been flown. The first could not be returned from orbit. The second mission was successfully completed in September 1967, and experiment data is presently being studied.

Biosatellite 30-Day Mission

The 30-Day Biosatellite involves considerably more sophisticated instrumentation than the 3-Day (Biosatellite I and II). The payload consists of an instrumented restrained primate, *Maccaca nemestrina*, weighing approximately 15 pounds.

The general objectives of the flight are to study the effects of weightlessness on the central nervous system, cardiovascular system and metabolic processes in the muscular and skeletal system.

The following in-flight physiological measurements are made:

EKG - electrocardiogram
EEG - electroencephalogram
ZPG - impedance pneumogram (respiration)

EOG - electro-oculogram

- Blood Pressure
GSR - Galvanic Skin Resistance
EMG - electromyogram
- core temperature

The primate is held in a restraint suit and couch for the duration of the flight and because of his general immobilization, conventional measurement techniques can be used. Electrical measurements are sensed by implanted electrodes and are brought out by shielded wires or cables to amplifiers (signal conditioners) and the amplified signals are run to the spacecraft telemetry system or tape recorder as appropriate.

Blood pressure from 4 points in the body is obtained through the use of in-dwelling catheters which are run to externally mounted strain-gauge type pressure transducers. The catheters are kept patent by the periodic injection of heparin solution throughout the mission. Heparin is injected by small, positive displacement pumps which provide about 5 micro-liters per minute to each catheter throughout the 30 days.

A psychomotor tester is installed in the spacecraft and the primate is trained to perform discriminatory and visuomotor tasks at regular intervals throughout the mission. Success or failure in these tasks is recorded and during task performance the primate is photographed by an on-board camera. The primate is also photographed during launch, powered flight, and de-orbit, at 4 frames per second.

The number of food pellets dispensed is monitored and read out on telemetry as is the amount of water dispensed. Feces is stored for analysis after recovery. Urine output is measured through the use of a piston-actuated reservoir.

The urine is obtained through a surgically implanted catheter and is pumped by a peristaltic pump through the urine volume measuring device and thence through a urine analysis device to a storage tank in the spacecraft adapter which is not recovered.

Installed in the adapter is the in-flight automated urine analysis device. This device removes an aliquot of urine which is, in volume, 10% of the amount passed through the urine transport system every six hours. The analyzer then divides the aliquot further and by mixing with the proper reagents, chemically analyzes the urine for calcium, creatine and creatinine. Calcium is analyzed by mixing the urine with a calcein solution which causes the urine sample to fluoresce. The sample is compared with a reference, and a value corresponding to calcium concentration is stored in a data handling system. Creatine and creatinine are mixed with reagents and the optical density of the resulting mixture is compared with a reference. The values for concentration of these constituents are read out from the data storage through the spacecraft telemetry at station passes.

While most of the biological instrumentation except the urine analysis device is conventional in concept, it is required to be miniaturized and packaged for space-flight and must be capable of operation for 30 days unattended. The techniques for implanting the electrodes and the blood and urinary catheters for a 30-day flight required development and refinement over conventional surgical practice.

In addition to the biological measurements, the usual environmental measurements of temperature, humidity, pO_2 , pO , acceleration, vibration and noise are made.

The following is a listing of the experiments and experimenters for the Biosatellite 30-Day flight:

1. Monitoring and Brain Functions and Performance in the Primate Under Prolonged Weightlessness (P-1001) by Dr. W. R. Adey and Dr. A. Cockett of the University of California, L.A.

2. Monitoring Cardiovascular Function in the Primate Under Prolonged Weightlessness (P-1001) by Dr. J. P. Meehan of the University of Southern California

3. Primate Metabolic Study During Weightlessness (P-1001) by Dr. N. Pace of the University of California, Berkeley, and Dr. J. Rho of the Jet Propulsion Laboratory

4. An Investigation of Bone Density Changes in Various Sites of the Skeletal Anatomy of a Primate (P-1062) by Dr. P. M. Mack of Texas Woman's University

Biosatellite 21-Day Mission

The 21-Day mission payload contains experiments investigating (a) the effect of weightlessness on the gross body composition of the rat, (b) the effect of space environment on the rhythmicity of the rat's biological system, (c) plant physiology and morphology under weightlessness, and (d) the effect of weightlessness on isolated human cells.

The plant experiment consists of 5 growing Arabidopsis plants mounted such that stereoptic photographs may be taken at intervals throughout the flight to record the growth and development. A module containing Arabidopsis seeds will also be flown and the seeds will be irrigated in orbit by a pyrotechnic water release system.

The human tissue experiment contains a living culture of liver tissue, a media pump and the appropriate heaters and thermostats to maintain the culture at 98.6°F . A camera, microscope and lighting system photographs the cells at intervals.

The rat experiment consists of a group of 8 pie-shaped cages housing 8 unrestrained white rats. The cage lighting is controlled such that the rats are exposed to a regimen of 12 hours' light, 12 hours' dark. This regimen can be altered in-orbit by ground command to shift the beginning of a light or dark period by a number of hours, or to provide continuous light. Rats are provided with liquid diet by individual feeders in each cage and frequency of feeding is monitored.

Each rat is implanted with a completely self-contained telemetry transmitter. These transmitters which are approximately 3/4 inch diameter by 1/4 inch thick are surgically implanted in the abdominal cavity of the rat. The telemeters transmit to an antenna system mounted in the walls of the cage. The signal transmitted consists of pulses of approximately 10-50 microseconds duration; the repetition frequency being proportional to the rat's core temperature. Pulse repetition frequencies range from approximately 100-600 Hertz.

The telemeters are powered by mercury batteries and the entire telemeter assembly is encapsulated in paraffin. Lifetime of these telemeters averages about 6 months.

Through the system of 3 orthogonal antennas, the signal from the telemeters is used to derive a gross indication of activity of the rats.

Number of feeder operations, temperature data, body movement data and lighting status information is stored in a core memory device for readout at each station pass.

Each rat feeder is calibrated so that the amount of food dispensed can be monitored and upon recovery, the rats will be extensively examined for changes in body composition.

It is presently planned that during the flight the rat lighting regime will be given at least one six-hour phase shift and toward the latter part of the flight will be changed to continuous light in order to investigate the free-running characteristics of the circadian rhythm of the rat after a period of weightlessness.

The following is a listing of experiments for the 21-Day flight:

1. Plant Morphogenesis Under weightlessness for the Purpose of Defining and Verifying Experiment Suitable for Use in a BIOSATELLITE (P-1003) by Dr. A. H. Brown and Dr. A. O. Dahl of the University of Pennsylvania.

2. Influence of Zero-Gravity on Isolated Human Cells (P-1084) by Dr. P. O'B. Montgomery, Jr., of the University of Texas.

3. Metabolic Rhythms as a Temporal Gauge of Mammalian Performance (P-1093) by Dr. F. Halberg of the University of Minnesota.

4. Effect of Weightlessness on Gross Body Composition in the Rat (P-1145) by Dr. G. C. Pitts of the University of Virginia.

Future Bioscience Experimentation

Experiments are now proposed which require periods of 6 months and longer in orbit for both restrained and unrestrained animal subjects. In addition to the types of measurements made on the Biosatellites, such measurements as blood flow, oxygen consumption, and biological system heat and fluid balance are contemplated.

Implantable Measuring and Transmitting Devices

A primary requirement of biological instrumentation is that the subject be maintained in as natural and undisturbed state as possible. Implantable telemeters of small size provided the means of obtaining reliable physiological information without the requirement of restraint or restriction of motion of the animal subject. In addition, they appear to have little effect on the general well-being of the animal.

Implantable devices to measure EKG, body temperature and blood pressure are under development by a number of groups including those at Ames Research Center, Franklin Institute and Northrop Corporation. Pressure transducers small enough to be inserted into blood vessels through hypodermic needles have been developed and are under test at Ames. Transducers 0.043 inch in diameter have been used reading pressure up to 100 mm of mercury with frequency responses of zero to several thousand cycles per second. Developments in miniaturized circuitry and batteries will make possible multiple channel transmitters.

Waste Management

The use of unrestrained animals will require the development of new methods of waste management. Methods under consideration include the use of forced air circulation into traps and/or absorbing devices; however, the control of bacteria may prove to be a major problem in this area.

Operational Considerations

Operational methods for long-term spaceflight are still in the developmental

stage and long-term experimentation with live biological material will necessitate development of new procedures in launch, flight, and recovery operations. The use of astronauts to assist in the in-flight operation or maintenance of biological experiments may result in a reduction of technical complexity in some types of experimentation, but will most certainly result in increased operational complexity in such missions.

Summary

Techniques for experimentation with live biology in space are in the early developmental stage. While the instrumentation and operational procedures for the Biosatellite series are adequate, they represent only a first step into a field of scientific endeavor which is of great interest to the scientific community. Plans for long-term space biology laboratories have brought about the need for major developments in instrumentation, environmental control, life support and operational techniques. The need for rapid forward steps in these areas presents a great challenge to both the biological and engineering communities.